REGULATION OF CELL-CELL ADHESION AND ACTIN CYTOSKELETON IN NON-TRANSFORMED AND TRANSFORMED EPITHELIAL CELLS

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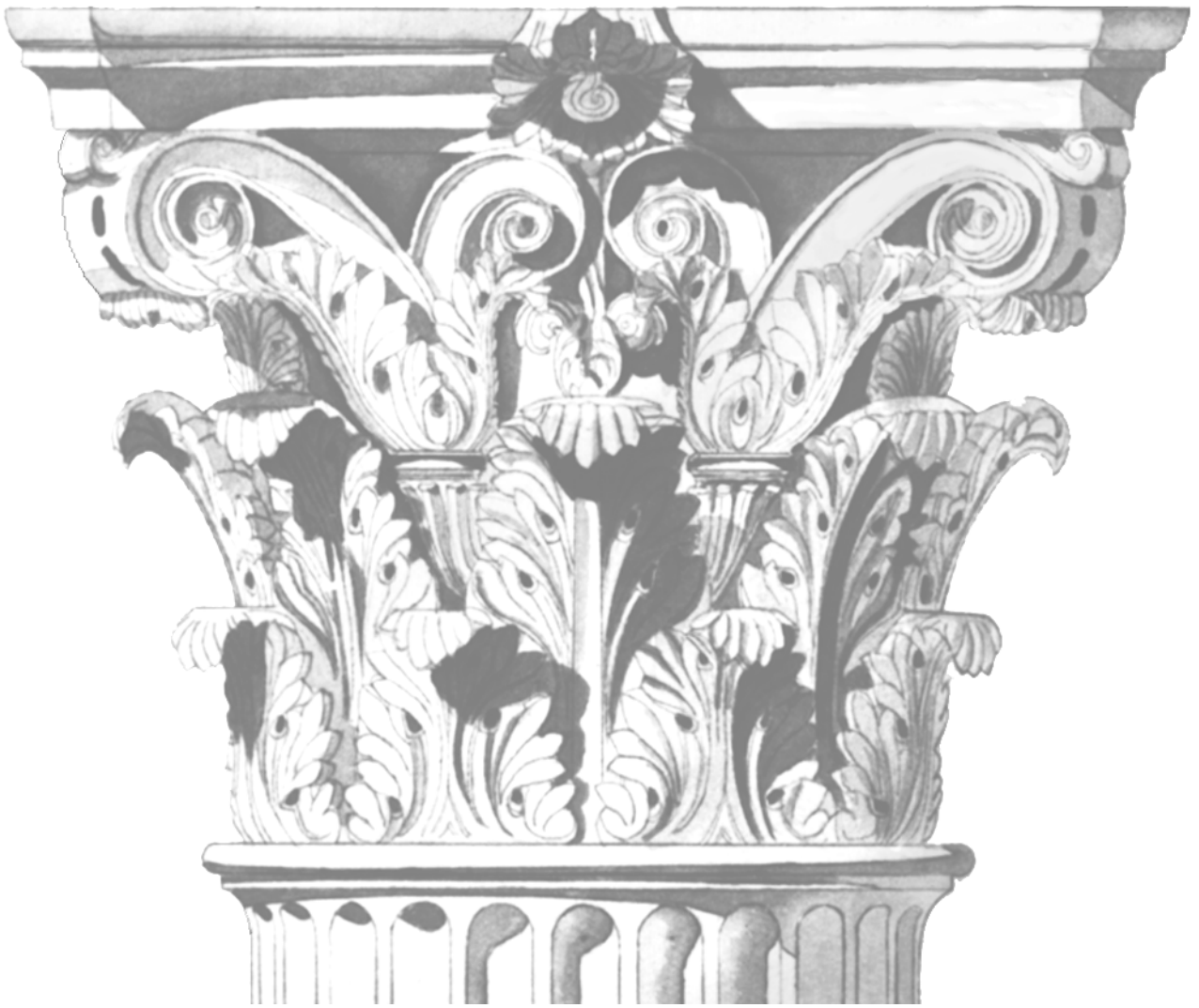

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Abstract

Epithelial cell-cell adhesions have a critical role in morphogenesis, establishment and maintenance of tissue architecture, cell-cell communication, normal cell growth and differentiation. These adhesions are disrupted during malignant transformation and tumour cell invasion. Several kinases, phosphatases and small GTPases regulate cell-cell contacts. In the present work we investigated the dynamics of cell-cell adhesion structures after microinjection of fluorophore tagged vinculin, during transformation caused by an active Src tyrosine kinase and during Helicobacter pylori infection. The regulatory role of Rac GTPase as well as the behaviour of actin and cadherin were analysed in all these conditions.

Microinjection of vinculin into bovine kidney epithelial MDBK cells induced release of actin, cadherin and plakoglobin to cytoplasm of the cells, caused disruption of protein complexes at adherens and tight junctions that finally led to formation of polykaryons. Activated Rac GTPase, in turn, enhanced accumulation of cadherin to membranes and thereby diminished the formation of polykaryons, whereas inactive Rac removed cadherin from membranes. Incorporation of vinculin to lateral membranes took place also in acidifying and depolarising conditions where cell fusions were prevented. Thus, the membrane potential seemed to control fusion ability. In src-MDCK cells, activation of Src kinase led to disintegration of adherens junctions. Clusters of junctional components and bundles of actin were seen at the basal surface already within 30 min after Src activation. p120ctn was the only component of adherens junction whose relocation correlated to its phosphorylation. Inhibition of Src by a specific inhibitor PP2 restored the cubic morphology of the cells and accumulated cadherin back to lateral walls. Still p120ctn remained in cytoplasm and thereby was not responsible for the epithelial phenotype. Activation of Rac GTPase by Tiam1 also increased the amount of cadherin at lateral membranes and maintained the morphology of src-MDCK cells practically normal after activation of Src kinase. In the same way, actin cytoskeleton was reorganised in gastric carcinoma cells in response to infection with H. pylori via activation of Rac signalling pathway. Hence, Rac and cadherin seem to be the major players in the maintenance of epithelial cell morphology.

Keywords: cadherins, cell adhesion, cytoskeleton, epithelial cells, Rac GTPase, Src-family kinases, vinculin

To Markus and Samuel

Tuo jylhä Puu.
Imee maasta voimaa,
kurkottaa päätänsä kohti valoa,
kylvää siemenensä tuulien mukaan.

Avaa suojan sinullekin,
ravitsee monta tarvitsevaa.

Jylhä Puu, tuulessa huojuen,
on siskosi ja veljesi,
elämämme.

Markku veli

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Oulu, December, 2002 Riitta Palovuori

Abbreviations

ADP	Adenosine diphosphate
AGS	Gastric adenocarcinoma
ASIP	atypical protein kinase C-specific interacting protein
ATP	Adenosine triphosphate
BPAG	Bullous pemphigoid antigen
BSA	Bovine serum albumin
C3	Clostridium botulinum exoenzyme
cagA	Cytotoxin-associated gene A
C. elegans	Caenorhabditis elegans
c-Src	Cellular Src
DAG	Diacylglycerol
D-MEM	Dulbecco's modified Eagle's medium
ECM	Extracellular matrix
EDTA	Ethylenediaminetetraacetic acid
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
EGTA	Ethyleneglycol-bis-(β-aminoethylether)-N,N,N',N'-tetraacetic acid
ERM	Ezrin, radixin, moesin
F-actin	Filamentous actin
FAK	Focal adhesion kinase
FCS	Foetal calf serum
FITC	Fluorescein 5-isothiocyanate
G-actin	Globular actin
GAP	GTPase activating protein
GDI	GDP dissociation inhibitor
GDP	Guanosine diphosphate
GEF	Guanosine nucleotide exchange factor
GFR	Growth factor receptor
GTP	Guanosine triphosphate
GTPase	Guanosine triphosphatase
HGF	Hepatocyte growth factor

H. pylori	Helicobacter pylori
IF	Intermediate filament
IL	Interleukin
JAM	Junctional adhesion molecule
JNK	Jun amino-terminal kinase
kD	Kilodalton
Le^b	Lewis b antigen
mAb	Monoclonal antibody
MAGUK	Membrane-associated guanylate kinase
MAPK	Mitogen-activated protein kinase
MARCKS	Myristoylated alanine-rich C kinase substrate
MDBK	Madin-Darby bovine kidney
MDCK	Madin-Darby canine kidney
MEKK	Mitogen-activated kinase kinase
MLC	Myosin light-chain
NF-κB	Nuclear transcription factor-κB
pAb	Polyclonal antibody
PAR	Partitioning-defective protein
p120ctn	p120-catenin
PDGF	Platelet-derived growth factor
$PI(4,5)P_2$, PIP_2	Phosphatidylinositol 4,5-bisphosphate
PIP5 kinase	Phosphatidylinositol 4-phosphate-5 kinase
PI3-kinase	Phosphatidylinositol 3-kinase, phosphoinositide 3-kinase
PH	Pleckstrin homology
PKB	Protein kinase B
PKC	Protein kinase C
PMA	Phorbol-12-myristate-13-acetate
PMSF	Phenylmethylsulfonyl fluoride
RSV	Rous sarcoma virus
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SFA	Stress fibre associated
SH3	Src homology 3
SOS	Son-of-sevenless
5-TAMRA, SE	5-carboxytetramethylrhodamine succinimidyl ester
Tiam1	T-lymphoma invasion and metastasis gene 1
TRITC	Tetramethylrhodamine
ts-Src	Temperature-sensitive Src
vacA	Vacuolating cytotoxin A
VASP	Vasodilator-stimulated phosphoprotein
v-Src	Viral Src
WASP	Wiskott-aldrich syndrome protein

List of original publications

The present thesis is based on the following original articles, which are referred to in the text by their Roman numerals:

I Palovuori R & Eskelinen S (2000) Role of vinculin in the maintenance of cell-cell contacts in kidney epithelial MDBK cells. Eur J Cell Biol 79:961-974.

II Palovuori R, Myrsky E & Eskelinen S (2002) Membrane potential and pH control disintegration of cell-cell adhesion and cell fusion in vinculin-injected MDBK cells. Submitted for publication.

III Palovuori R, Sormunen R & Eskelinen S (2002) Tiam1 antagonizes Src-induced disintegration of adherens junctions in MDCK cells and accumulates cadherin to lateral membranes. Submitted for publication.

IV Palovuori R, Perttu A, Yan Y, Karttunen R, Eskelinen S & Karttunen TJ (2000) Helicobacter pylori induces formation of stress fibres and membrane ruffles in AGS cells by Rac activation. Biochem Biophys Res Commun 269:247-253.

Contents

1 Introduction

Cell polarity is fundamental for the function of epithelial cells, but it is also important for the development and maintenance of tissue architecture. At molecular level, epithelial cell polarity is achieved by maintaining a different membrane composition at the basal side facing the extracellular matrix and at the apical side facing the lumen. This is achieved partly by polarised secretion and organisation of cytoskeleton, partly by preventing the migration of membrane components between the apical and basolateral membrane domains. The membrane domains are separated by tight junctions and adherens junctions. Polarised epithelial cells normally adopt a stationary and non-migratory phenotype, since the cells are tightly attached to each other and to the underlying basement membrane. During embryonic development, wound healing, or metastasis of tumours the cells may become motile and their intercellular adhesions are weakened. This process changes the expression and interactions of adhesion molecules and actin cytoskeleton, regulated by protein and lipid kinases and small GTPases. Particularly GTPases of the Rho family regulate actin cytoskeleton. Protein tyrosine kinases and different isoforms of protein kinase C (PKC) directly or indirectly regulate protein interactions of epithelial cell junctions.

In epithelial cells cell-cell adhesion complex is a rigid, but very dynamic structure. The components of the cell - cell adhesion sites are largely known and the steps in the formation of epithelium have been described in detail. In adherens junctions the intercellular adhesion is formed through the extracellular domains of cadherins, whereas their intracellular domains are associated to cytoskeletal actin filaments via catenins and vinculin. In tight junctions the paired strands consisting of claudin and occludin form a close lateral contact between the neighbouring cells. In this assembly ZO-1, as a peripheral membrane protein of tight junctions, binds to actin filaments and to α-catenin of adherens junctions. The desmosomes form tight adhesion between the cells by desmogleins, desmocollins and desmoplakins, whereas within the cells they are anchored to intermediate filaments. Gap junctions are responsible for the intercellular exchange of cytosolic molecules through connexin channels. The mutual role of all these adhesion proteins in the maintenance and disintegration of cell - cell contacts is still partly unclear. In general, small GTPases of Rho family promote actin polymerisation, whereas Ras family GTPases and Src tyrosine kinase

distort cell adhesion. The targets of small GTPases and Src kinase are partly unknown and the networking of the different signalling pathways is under intensive study.

This work was carried out to clarify the mechanisms that regulate the maintenance and disintegration of cell-cell adhesion in untransformed and transformed epithelial cells. Special interest was focused on the mechanism of disintegration. The levels of adhesion proteins were disturbed with microinjection of excess vinculin into bovine kidney (MDBK) cells and their phosphorylation was induced by activation of Src kinase in canine kidney (MDCK) cells. The effects of Rho GTPases, especially Rac GTPase, on adhesion were analysed by using microinjection or expression of its activator, a Rac GEF, Tiam1. The involvement of Rac and Cdc42 GTPases in reorganisation of actin cytoskeleton was also studied in gastric carcinoma (AGS) cells infected with Helicobacter pylori, a pathogen that causes gastric ulcer and gastric tumours.

2 Review of the literature

2.1 Polarity of epithelial cells

Epithelial cells line the cavities and the free organ surfaces of the body, or make up tubular and glandular structures in several organs. In complex organisms the cells form the interface between compartments and by their secretory and absorptive functions they alter the segregated compartments. For these functions the epithelial cells must be polarised to demark which side is the organism and which is the "outside" (reviewed by Rodriguez-Boulan & Nelson 1989, Matlin & Caplan 1992, reviewed by Rodriguez-Boulan & Powell 1992). The distinct feature of the polarised epithelial cells is the asymmetric distribution of macromolecules and organelles both in cytoplasm and in three plasma membrane domains. The polarised phenotype of the cell is mainly dependent on actin cytoskeleton, spectrin-based membrane skeleton and microtubules. Tight junctions also have a fundamental role during development of cell surface polarity, since they form an intramembrane diffusion fence that restricts diffusion of lipids in the plasma membrane. Besides tight junctions, other junctional complexes such as adherens junctions and desmosomes are responsible for the maintenance of polarity and vectorial transport functions of the cells. (Reviewed by Rodriguez-Boulan & Nelson 1989, reviewed by Mays *et al.* 1994, reviewed by Brown & Stow 1996.) Cell polarity is especially critical to epithelial cells lining the renal tubules that perform the crucial functions of cell volume regulation and vectorial transport of ions and nutrients.

2.1.1 Plasma membrane domains

The plasma membrane of the polarised epithelial cells is divided into apical, lateral and basal membrane domains with distinct protein and lipid compositions (Fig. 1, reviewed by Rodriguez-Boulan & Nelson 1989, reviewed by Brown & Stow 1996). The apical membrane domain, facing the external milieu (lumen), is often characterised by specialised structures such as microvilli and cilia. The lateral and basal membranes differ

from each other but often they are considered to be a single, basolateral domain. The lateral surface, responsible for the interactions with neighbouring cells, is distinguished from the other domains by the presence of tight and adherens junctional structures at the apical parts of the lateral walls. The basal surface closest to substratum communicates with the proteins of basal lamina, such as laminins, collagen IV and heparan sulfate proteoglycans. (Reviewed by Rodriguez-Boulan & Nelson 1989, Matlin & Caplan 1992, reviewed by Brown & Stow 1996.) The Madin-Darby canine kidney (MDCK) cell line is a widely used cell model for studies of polarity. Interaction of the cells with the substratum is adequate for the establishment of at least a rudimentary apical domain, but formation of basolateral polarity demands the establishment of cell-cell contacts (reviewed by Rodriguez-Boulan & Nelson 1989). This is shown in classical experiments of Wang *et al.* (1990). They cultivated MDCK cells in suspension where the aggregated MDCK cells, without contact to substratum, had the ability to organise into a cyst (a closed monolayer of the polarised cells) that surrounded the central lumen with the basal-lateral membrane facing the central lumen and the apical membrane the environment. The cells grown in collagen gel, in turn, have an opposite polarity in cysts with the apical side towards the lumen and the basal side towards the extracellular matrix, ECM (Wang *et al.* 1990). Thus, ECM creates the apico-basal axis and determines the apical surface of epithelium.

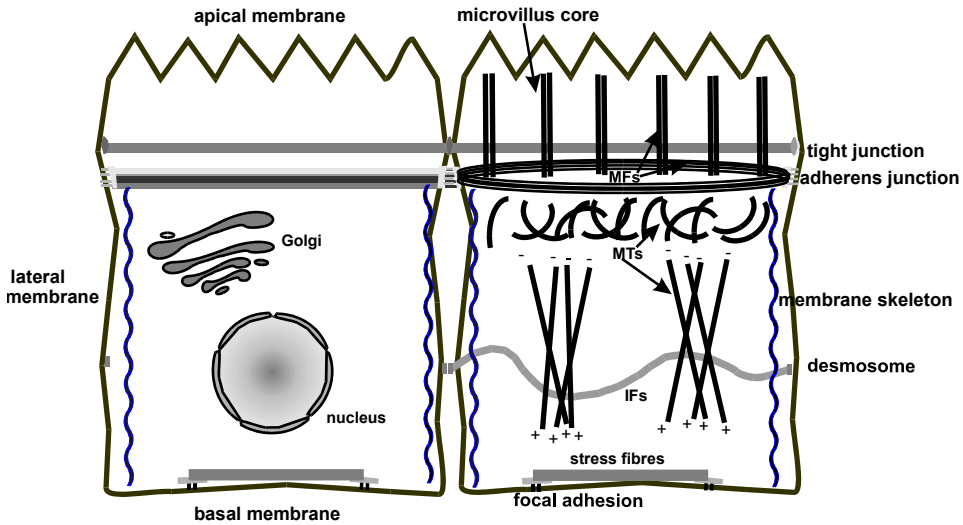

Fig. 1. Some features of the polarised epithelial cell. Microfilaments (MFs), microtubules (MTs), intermediate filaments (IFs).

The polarised epithelial cells are responsible for the movement of ions, water and macromolecules between biological compartments. The existence of distinct sets of membrane proteins on the apical and basolateral surfaces enables the different functions of these membrane domains. The apical domain, whose primarily function is in absorption and secretion, contains for example hydrolases and sodium-dependent transporters that are not present in the plasma membrane of nonepithelial cells. The basolateral domain, in turn,

specialises in the maintenance of the normal physiological state and the recognition and transduction of signals. Thus, ion channels, transporter proteins, enzymes of the cell surface, cellular adhesion molecules and junctional complexes, receptors of growth factors and signalling molecules are present either on lateral and basal membrane domains or only one of them. (Reviewed by Rodriguez-Boulan & Nelson 1989.) The differences between the apical and basolateral membrane domains surface are evident also in the levels of expressed lipids: cholesterol and sphingomyelin are dominant lipids on the apical membrane, whereas phosphatidylcholine and phosphatidylinositol dominate on the basolateral membrane. The variable ratios of lipids on these membrane domains affect the fluidity of membrane, the velocity of diffusion across the membrane and the function of intrinsic membrane proteins (reviewed by Leiser & Molitoris 1993).

During development of epithelial polarity the localisation, organisation and function of cellular organelles and cytoskeletal structures are strictly determined (reviewed by Rodriguez-Boulan & Nelson 1989, reviewed by Gumbiner 1992, reviewed by Mays *et al.* 1994). The Golgi complex, usually positioned apically above the nucleus (Fig. 1, Bacallao *et al.* 1989), plays an important role in the sorting of apico-basolateral components into carrier vesicles (Griffiths & Simons 1986, Bennett *et al.* 1988). The spectrin-based membrane cytoskeleton that forms the linkage between transmembrane proteins and actin filaments has a central role in targeting and positioning of different membrane proteins in polarised cells by forming complexes with transmembrane proteins. For example, in MDCK cells Na^+-K^+-ATPase is bound to ankyrin and thus, to membrane skeleton on the lateral membrane domains (Nelson *et al.* 1990), whereas in retinal pigment epithelial cells Na^+-K^+-ATPase-cytoskeleton complexes are localised to apical membrane domains (Huotari *et al.* 1995). The polarised cells might even have two spectrin/ankyrin-based membrane skeletons that are distributed to apical and basolateral membranes (Huotari *et al.* 1995). Actin microfilaments, intermediate filaments, microtubules and their associated proteins are also involved in the establishment of cell polarity (Fig. 1, reviewed by Carraway & Carraway 1989, reviewed by Mays *et al.* 1994, reviewed by Yeaman *et al.* 1999).

2.1.2 Cytoskeleton

Cytoskeleton is a combination of dynamic cytoplasmic filamentous structures including actin filaments, intermediate filaments and microtubules. The cytoskeleton of the cells is not only a structural element, but it also contributes to many cellular processes such as maintenance of cell integrity, transport and position of organelles, mitosis, cytokinesis, secretion and formation of cell extensions.

2.1.2.1 Actin cytoskeleton

Actin as a component of cytoskeleton has an evident contribution to the structure and motility of the cells (Pollard 1976, Stossel 1984). In cultured fibroblasts, well-known

assemblies of actin are stress fibres, lamellipodia and filopodia. The stress fibres are large bundles of actin filaments in the cytoplasm of the cells. In these fibres, actin and myosin II are in bipolar arrangement and this feature contributes to the contraction and tension of fibres. In cultured cells the stress fibres terminate in focal adhesions where integrins mediate cell attachment to extracellular matrix (ECM) proteins including collagen, laminin and fibronectin (reviewed by Jockusch *et al.* 1995, Schwartz *et al.* 1995). The lamellipodium or leading edge is a characteristic, extensional structure for the spreading cells at the cell periphery, whereas filopodia or microspikes are an actin-rich element seen either as an extension from lamellipodia or found within this assembly (reviewed by Small 1988, reviewed by Small *et al.* 1999a). Lamellipodia consist of planar meshworks in which unipolar actin filaments grow outwards. Lamellipodia and filopodia are required for the spreading and motility of cells. For movement the cells use precursor contacts in ruffling lamellipodia or beneath filopodia. Typically a lamellipodium is made towards the direction of a chemoattractant stimulus that induces cell migration, e.g. in orientation of metastatic tumour cells towards blood vessels. Other examples are wound healing and chemotaxis of macrophages.

The ability of cells to move is largely based on the formation of actin filaments from the actin monomers near the plasma membrane and on myosin motors that contract the filaments. In the physiological ATP-Mg-rich cell medium, actin filaments (F-actin) and monomeric actin (ATP-G-actin) are maintained in a dynamic steady state. Actin filament polymerisation is a controlled process at the leading edge of the cell. As a result of polymerisation reaction, the flux of ATP-G-actin association onto available barbed, fast growing ends is balanced by the dissociation flux of actin subunits from the pointed (slower growing) ends. The process is known as treadmilling cycle of F-actin. Several proteins regulate this process by binding either to barbed or pointed ends of the filaments and thereby inhibiting the addition or loss of subunits at the ends. (Reviewed by Machesky & Way 1998.) These proteins can be classified into six groups: monomer-binding, sequestering, severing, capping, cross-linking and nucleating proteins. Monomer-binding proteins, such as profilin, control the dynamics of actin polymerisation, and affect the formation of specific F-actin structures through monomer sequestration, catalytic enhancement of ADP/ATP exchange and enhancement of ATP-dependent monomer addition to the barbed ends of actin filaments. Sequestering proteins, such as thymosin-β_4, bind G-actin and change its conformation. This in turn leads to inhibition of actin polymerisation and nucleotide exchange (De La Cruz *et al.* 2000). The severing proteins, such as gelsolin and cofilin families, regulate the number and distribution of actin filaments. The capping proteins, such as gelsolin, cause lamellipodial protrusion (or Listeria movement) by binding tightly to barbed ends, but they also sever filaments. During crosslinkage of actin the proteins, such as fimbrin, α-actinin, dystrophin and spectrin, organise the individual actin filaments either to tight or loose well-organised assemblies (reviewed by Carlier 1998, reviewed by Puius *et al.* 1998). The formation of new actin filaments from actin monomers is called actin nucleation, and it is regulated by multiple actin-nucleating factors and Arp2/3 complex (reviewed by Welch & Mullins 2002).

Actin filaments are widely distributed in polarised epithelial cells. The core of each apical microvillus is composed of a bundle of actin filaments (Fig. 1), and a meshwork of actin filaments, spectrin and other cytoskeletal proteins form the terminal web underneath the apical membrane into which microvillar actin bundles insert in intestinal epithelium

(reviewed by Mooseker 1985). Prominent bundles of actin filaments encircle the apex of the lateral membrane associated to tight and adherens junctions (Fig. 1, Kartenbeck *et al.* 1991, reviewed by Mays *et al.* 1994). The stress fibres of cultured epithelial cells are short and few.

2.1.2.2 Membrane skeleton

Actin-based cytoskeleton is connected to the plasma membrane via a lattice-like network of actin-binding proteins that form the membrane skeleton or membrane-associated cytoskeleton (reviewed by Bennett & Gilligan 1993). Originally this detergent-insoluble structure was identified from the preparations of human erythrocyte membranes (Yu *et al.* 1973) and later on it was also found in non-erythroid cells. In erythrocytes, membrane skeleton extends over the entire cytoplasmic surface of plasma membrane, providing a structural support for the lipid bilayer and affecting the survival of the cells in circulation (Liu *et al.* 1987, reviewed by Bennett 1990, Hartwig & DeSisto 1991). In polarised kidney epithelial cells the membrane skeleton is localised to the basolateral plasma membrane domain (Fig. 1, reviewed by Nelson 1991, reviewed by Mays *et al.* 1994, reviewed by Brown & Stow 1996). The membrane skeleton of the polarised cells forms a functional and structural linkage between transmembrane proteins and actin cytoskeleton, but it also determines the destination of other membrane proteins (reviewed by Brown & Stow 1996).

The major structural component of the membrane skeleton is spectrin (also referred to as fodrin in non-erythroid cells), a flexible rod-shaped molecule composed of homologous, but non-identical α- and β-subunits. Each subunit is composed of multiple 106 amino acid residue repeats and non-homologous sequences at the N- and C-terminus of the molecule (reviewed by Bennett & Gilligan 1993, reviewed by Winkelman & Forget 1993, reviewed by Hartwig 1994). α -and β-subunits form antiparallelly aligned heterodimers. Two heterodimers assemble into tetramers by lateral head to head association, and tetramers bound together via actin oligomers form higher order oligomers (reviewed by Bennett & Gilligan 1993). Spectrin binds to biological membrane either directly via association of membrane lipids with at least three regions of β-spectrin (Wang & Shaw 1995) or indirectly via membrane-associated proteins (Davis & Bennett 1994, Lombardo *et al.* 1994). The linkages are formed e.g. via adducin and members of the protein 4.1 family (reviewed by De Matteis & Morrow 2000, Parra *et al.* 2000). Spectrin also binds ankyrin and is thus associated to transmembrane proteins including ion channels or pumps and cell adhesion molecules of the immunoglobulin (Ig) superfamily. In some polarised cells ankyrin forms a complex with Na^+-K^+-ATPase on the lateral membrane (Nelson & Veshnock 1987), whereas in retinal pigment epithelial cells there are likely two spectrin/ankyrin skeletons that are apically and basolaterally orientated (Huotari *et al.* 1995). In epithelial cells the association of spectrin to adherens and tight junctions is mediated by α-catenin (Pradhan *et al.* 2001) and ZO-1 (Itoh *et al.* 1991), respectively. Interaction of spectrin with ZO-1 suggests a role for spectrin in the maintenance of tight junctional structure (Itoh *et al.* 1991). Spectrin-based membrane skeleton also exists in the Golgi complex (Beck *et al.* 1994).

2.1.2.3 Other actin and membrane associated proteins

Several proteins have both structural and signalling function. In epithelial cells the ezrin-radixin-moesin (ERM) family regulates organisation of actin cytoskeleton both during development and in adult tissues (reviewed by Bretscher *et al.* 1997, reviewed by Tsukita *et al.* 1997a). The proteins are also involved in the formation of cadherin- and integrin-based adherens junctions (Takeuchi *et al.* 1994) and tightly linked to a signalling pathway of small GTPases of the Rho family (reviewed by Tsukita *et al.* 1997b, Mackay *et al.* 1997, Matsui *et al.* 1998). ERM proteins form a group of closely related cross-linkers between plasma membranes and actin filaments. They are mainly localised at cleavage furrows during cytokinesis (Sato *et al.* 1991), microvilli, ruffling membranes and cell-cell/cell-matrix adhesion sites (Sato *et al.* 1992, Berryman *et al.* 1993). The molecular structure of ERM molecules consists of a N-terminal globular domain, an extended α-helical domain and a charged C-terminal domain (reviewed by Arpin *et al.* 1994). Similarly to vinculin, ERM proteins exist in a closed conformation in which the amino- and carboxy-terminal halves link to each other in a head-to-tail manner, preventing the association of proteins to membrane and actin (Magendantz *et al.* 1995). In opened and activated state the proteins bind to actin filaments and to membrane via integral membrane proteins such as CD44, H^+/K^+-ATPase pump, CD43 or ICAM-1 proteins (reviewed by Tsukita *et al.* 1997a, b). Due to a common highly conserved globular domain ERM proteins belong to a larger superfamily of proteins including protein 4.1 (band 4.1 in erythrocytes) and its homologous proteins, such as talin. Since the merlin/schwannomin, NF2 protein highly resembles ERM proteins, the collective name MERM proteins is also used (reviewed by Vaheri *et al.* 1997, reviewed by Mangeat *et al.* 1999).

Vinculin is an important regulatory and structural protein in the architecture of adherens junctions and focal adhesions (Figs. 2, 3), functionally related to α-catenin. Besides α-catenin, it has an essential role in the regulation and maintenance of adherens junction assembly (reviewed by Rüdiger 1998), but also in embryogenesis (Barstead & Waterston 1991, Xu *et al.* 1998) and in the spreading and motility of the cells (Rodriguez Fernandez *et al.* 1992, 1993, Volberg *et al.* 1995, Xu *et al.* 1998). As a multiligand protein vinculin binds to actin filaments (Johnson & Craig 1995a), to microfilament-associated proteins like talin (Otto 1983) and α-actinin (Belkin & Koteliansky 1987), to α-catenin (Weiss *et al.* 1998), to acidic phospholipids (Johnson & Craig 1995b, Johnson *et al.* 1998), to vasodilator-stimulated phosphoprotein, VASP (Brindle *et al.* 1996, Hüttelmaier *et al.* 1998), to paxillin (Turner *et al.* 1990), to vinexin (Kioka *et al.* 1999) and to ponsin (Mandai *et al.* 1999). Recently raver 1 protein has been characterised, which is transported between the nucleus and the cytoplasm, but it also forms complexes with vinculin/metavinculin and α-actinin (Hüttelmaier *et al.* 2001). Vinculin (117 kD) is composed of a 95 kD, globular head domain at N-terminus that is connected to C-terminal, 30 kD tail domain. Vinculin head binds and stabilises the helical bundle conformation of the vinculin tail in close conformation and thereby prevents the binding of vinculin to talin, α-actinin, VASP and F-actin. Binding of the vinculin tail to acidic phospholipids including phosphatidylinositol 4,5-bisphosphate (PI4,5P_2, PIP_2) opens the conformation of molecule and allows protein associations (Gilmore & Burridge 1996, Weekes *et al.* 1996, Bakolitsa *et al.* 1999). Vinculin binds to focal adhesions through its C-terminal and N-terminal domains (Bendori

et al. 1989), whereas the head domain of vinculin recruits the protein to adherens junctions via binding to tail domain of α-catenin (Weiss *et al.* 1998). The proline-rich region of vinculin between head and tail is also available for ligand binding, such as Src homology 3 (SH3) domains of ponsin (Mandai *et al.* 1999). Vinculin is also a substrate for Src kinase (Sefton *et al.* 1981) and a target of protein kinase C (Werth *et al.* 1983, Perez-Moreno *et al.* 1998).

α-actinin is a rod-shaped molecule composed of two 100 kD anti-parallel monomers. Each domain has an amino-terminal actin-binding domain, the central region contains four spectrin-like repeats and calmodulin-like domain is at C-terminus (Castresana & Saraste 1995, Davison & Critchley 1988, Trave *et al.* 1995). α-actinin is a widely expressed actin cross-linking protein (reviewed by Blanchard *et al.* 1989). Nonmuscle α-actinin binds to F-actin in a calcium-sensitive manner, whereas actin binding of muscle α-actinin is independent of calcium. In Z-disks of skeletal and cardiac muscle α-actinin cross-links anti-parallel actin filaments of adjacent sarcomeres and the binding of protein to titin controls the assembly (Ohtsuka *et al.* 1997, Young *et al.* 1998). In non-muscle cells α-actinin is involved in the organisation of the cortical cytoskeleton close to adherens and tight junctions. At cell-cell contacts its association to α-catenin forms a linkage between actin cytoskeleton and cadherin/catenin complexes (Knudsen *et al.* 1995). In focal contacts of cultured fibroblasts α-actinin cross-links actin filaments and forms linkages to numerous cytoskeletal and membrane-associated proteins (reviewed by Blanchard *et al.* 1989). As vinculin, α-actinin is also a PIP_2-binding protein (Fukami *et al.* 1994).

2.1.2.4 Intermediate filaments

Originally intermediate filaments (IFs) were characterised as a separate cytoplasmic structure (Ishikawa *et al.* 1968) that was recognised by animal and human autoantibodies (Kurki *et al.* 1977). In most cells they form a fine fibrillar network in cytoplasm of the cells linking the nucleus to cell surface (Lehto *et al.* 1978, reviewed by Fuchs & Cleveland 1998). In stratified epithelia, IFs are linked to cell-cell adhesion via desmoplakin of desmosomes (reviewed by Garrod 1993) and are also anchored to integrin-mediated junctions, hemidesmosomes of the epidermal and muscle cells (Guo *et al.* 1995). On the basis of amino acid and cDNA (complementary DNA) sequence similarities, IF proteins are classified into at least five types expressed in different cell types: type I and II keratins are mostly expressed in epithelial cells, type III IFs such as vimentin exist in mesenchymal cells, type IV IFs are so-called neurofilaments and type V IFs are nuclear lamins (reviewed by Steinert & Liem 1990, reviewed by Fuchs 1996). Vimentins and lamins have dynamic roles during mitosis of the cells, since in the beginning of the event they are disassembled into subfilamentous particles and are phosphorylated (Dessev *et al.* 1988, Chou *et al.* 1989). After mitosis they are dephosphorylated and form reformed structures around the chromatin and centrosomes.

2.1.2.5 Microtubules

Microtubules are highly dynamic tubular structures found in all dividing and differentiated eukaryotic cells. Microtubules are composed of α- and β-tubulin dimers that assemble to polar microtubules having a fast growing plus (+) end and another minus (-) end. In polarised epithelial cells, the microtubuli traverse the cells in an apico-basal direction in such a way that the minus ends are directed to apical pole of the cells and the plus ends toward the basal pole (Fig.1, reviewed by Mogensen 1999).

Besides actin cytoskeleton, microtubules have an important role in the generation and maintenance of polarity in epithelial cells. In transporting polarised epithelial cells the microtubules regulate the precise distribution of proteins and lipids by directing the proteins from the Golgi complex to the apical plasma membrane domain of the cells and by targeting the transport vesicles between the basolateral and apical membrane domains (transcytosis, Brändli et al. 1990). In intracellular trafficking vertically and laterally orientated microtubules have distinct functions. For instance, in kidney cells the highly orientated microtubules beneath the apical membrane are responsible for recycling of proteins and vesicles, whereas the others are involved in their movement from trans-Golgi towards plasma membrane (Rindler et al. 1987, Breitfeld et al. 1990, Boll et al. 1991). Besides the vesicles, the microtubules move other intracellular organelles such as mitochondria, lysosomes and the rough endoplasmic reticulum (reviewed by Brown & Stow 1996). The disruption of the microtubules with nocodazole or colchicine (Rindler et al. 1987, Matter et al. 1990, Breitfeld et al. 1990) interferes with the secretion of the cells and causes a dramatic redistribution of organelles and membrane proteins (reviewed by Brown & Stow 1996). Microtubules and mitotic motors also play a central role during mitosis since they are major components of the spindle fibres that coordinate chromosome movements in dividing cell (reviewed by Scholey et al. 2001).

2.2 Cell adhesion structures

Cell adhesion is a prerequisite for the assembly of individual cells into the three-dimensional organisation of tissues in animals. The connection of the adjacent cells together and/or to extracellular matrix and the linkage of the adhesions systems to the intracellular cytoskeleton occur via different cell adhesion mechanisms. The proteins responsible for the cell adhesion are typically divided into three classes; 1) cell adhesion molecules/adhesion receptors usually consist of transmembrane glycoproteins, such as cadherin at cell-cell contact and integrin at matrix contact, 2) the extracellular matrix (ECM) proteins, large fibrillar glycoproteins like collagens linked tightly to adhesion receptors, 3) the cytoplasmic plaque/peripheral membrane proteins, such as catenins of the cell-cell contact sites that are associated to adhesion receptors at the intracellular surface of the plasma membrane and form a linkage between the adhesion systems and actin cytoskeleton. The latter proteins regulate the cell adhesions and have a key role in the transduction of the signals from the cell surface. (Reviewed by Gumbiner 1996.)

2.2.1 Cell-cell contacts

The adhesive elements between the individual epithelial cells can be divided into three groups; 1) communicating junctions, such as gap junctions (reviewed by Bennett *et al.* 1991), 2) anchoring junctions, such as desmosomes and adherens junctions (reviewed by Steinert & Roop 1988, reviewed by Tsukita *et al.* 1992) and 3) sealing junctions, such as zonula occludens or tight junctions (reviewed by Schneeberger & Lynch 1992, reviewed by Anderson *et al.* 1993). The physical adhesion of the cells to their surrounding extracellular matrix (hence the name adherence or adherens junctions) plays an important role in the maintenance of tissue organisation. Morphologically the best-known adherens junctions are focal adhesions, desmosomes, hemidesmosomes, and the junctions of epithelial cells named zonula adherens, ZA (reviewed by Borrmann *et al.* 2000).

2.2.1.1 Cadherin-based adherens junctions

In epithelial cells cadherin-based cell-cell contact is a specialised region of the plasma membrane, where cadherin molecules of the adjacent cells interact in a calcium-dependent manner. Actin filaments are associated to this structure through catenins located at the undercoat of the adherens junction (Ozawa *et al.* 1990, reviewed by Geiger & Ginsberg 1991, Rimm *et al.* 1995, reviewed by Takeichi 1995). Cadherins are subdivided into classic cadherins (E; epithelial, P; placental, N; neuronal cadherins) found in adherens junctions, and desmosomal cadherins, found in desmosomes (reviewed by Yap *et al.* 1997). Classical cadherins have an extracellular part consisting of five distinct domains (EC1-5) and a conserved cytoplasmic domain. The extracellular part interacts homotypically with cadherins of the neighbouring cells, and the conserved cytoplasmic tail associates with intracellular proteins involved in the formation of the junctional structure. E-cadherin mediates the assembly of adherens junctions, but it also affects the formation of desmosomes and tight junctions (Gumbiner *et al.* 1988, Wheelock & Jensen 1992).

The carboxy-terminal part of E-cadherin cytoplasmic domain binds to β-catenin or to γ-catenin (plakoglobin) which, in turn, form the linkage to α-catenin (Fig. 2). Furthermore, through a site near its transmembrane domain, E-cadherin binds directly to a special catenin, called p120ctn (p120cas, Reynolds *et al.* 1994, Yap *et al.* 1998, Thoreson *et al.* 2000) that was originally characterised as a substrate of v-Src kinase (Reynolds *et al.* 1992). In the complex of E-cadherin, α-catenin is the only catenin that forms either direct or indirect linkage to actin cytoskeleton through α-actinin, vinculin, ZO-1, spectrin and a number of other molecules associated to cadherin complex (reviewed by Yamada & Geiger 1997).

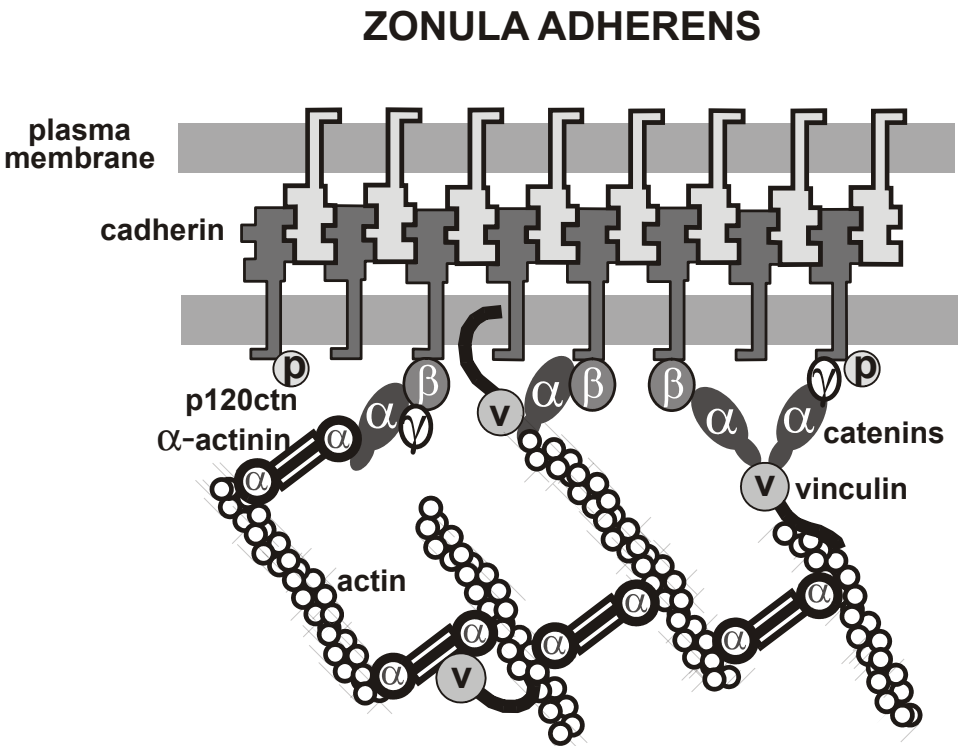

Fig. 2. A schematic drawing of principal interactions of structural proteins at cadherin-based adherens junction. Actin filaments are linked to α-actinin and to membrane through vinculin. The head domain of vinculin associates to E-cadherin via α-, β- and γ-catenins. The tail domain of vinculin binds to membrane lipids and to actin filaments.

The function of cadherins is not only limited to formation of protein complexes inside the cells and linkage of the cells together, but they also regulate the signalling events during differentiation, proliferation and migration (reviewed by Knudsen *et al.* 1998). Cadherins contribute either to the organisation of signalling components or, by formation of close cell-cell contact, they affect the signalling mechanisms indirectly (reviewed by Fagotto & Gumbiner 1996). Cell-cell contacts are also involved in the long-term signalling events that regulate cell growth and gene expression. During differentiation the cells express the characteristic, unique cadherins and in several cases cadherins determine the differentiated phenotype of the cells. The presence of many signalling molecules, such as Src family tyrosine kinases, receptor kinases and phosphatases in the adherens junctions of the epithelial cells (Tsukita *et al.* 1991, reviewed by Woods & Bryant 1993, Brady-Kalnay *et al.* 1995) may be important for the regulation of the function of cadherin as well as for transduction of the signals from cadherin or adherens junction into the cell. In tissues, the establishment of strong adhesion or contractility is dependent on the assembly of adherens junction. On the other hand, in epithelial cells, the apico-lateral belt of adherens junction strengthens the adhesion by linking the actin cytoskeleton to sites of strong adhesion. The absence of E-cadherin weakens the intercellular adhesion by affecting other junctional

proteins, and loss of its expression or function is associated with tumour cell invasion of epithelial cancers (reviewed by Birchmeier & Behrens 1994).

β-catenin is a key component of cell-cell adhesion linking cadherin receptors to the cytoskeleton (Fig. 2). Moreover, it is also part of the Wnt/Wingless signalling pathway that controls numerous events in development, including differentiation, proliferation and morphogenesis (reviewed by Wodarz & Nusse 1998). β-catenin uses this pathway for transmission of signals from cell-adhesion components or Wnt protein to the nucleus. In the presence of Wnt signals unphosphorylated β-catenin regulates gene expression through its association with transcription factors, LEF-1 (lymphocyte-enhancer factor-1) and TCFs (reviewed by Behrens *et al.* 1996, reviewed by Seidensticker & Behrens 2000). In the absence of Wnts β-catenin is phosphorylated and degraded in proteasomes. In tumours degradation of β-catenin is blocked due to mutation of β-catenin or tumour suppressor gene APC (adenomatous polyposis coli). This leads to formation of TCF/β-catenin complexes and activation of oncogenes (reviewed by Seidensticker & Behrens 2000).

2.2.1.2 Tight junctions

In epithelial and endothelial cells, tight junctions are the most apical intercellular junctions that function as selective (semipermeable) diffusion barriers between individual cells. They maintain the different composition of proteins and lipids between the apical and basolateral plasma membrane domains ("fence" function). Furthermore, the tight junctions regulate the growth and differentiation of the cells. (Reviewed by Balda & Matter 1998, reviewed by Tsukita *et al.* 1999.)

The tight junction is identified as a belt-like region in which two lipid-apposing membranes lie close together (tight junction strands). Tight junction strands of the adjacent cells form tightly connected pairs. The proteins involved in the formation of tight junctions are divided into two categories: 1) integral membrane proteins, such as occludin, claudin and junctional adhesion molecule, JAM and 2) peripheral membrane proteins (cytoplasmic plaque proteins), MAGUK (membrane-associated guanylate kinase) homologue proteins, such as ZO-1, 2, 3, cingulin, symplekin, 19B1, and AF-6. Moreover, various signalling proteins (protein kinases, heterotrimeric G-proteins and small GTP-binding proteins) are either localised at the cytoplasmic plaque domain of tight junction, or they have a central role in the assembly or function of junction (reviewed by Tsukita *et al.* 2001). ZO-1 was the first tight junctional protein identified (Stevenson *et al.* 1986). In epithelial cells ZO-1 is a critical protein in the initial steps during the formation of cell-cell junctions, because it accumulates together with cadherin to tips of cellular protrusions of the adjacent cells forming spot-like junctions (Yonemura *et al.* 1995). As polarisation of the cells proceeds, occludin is gradually carried to spot-like junctions of ZO-1 to form a belt-like tight junction, whereas E-cadherin is released from ZO-1 junctions in order to form adherens junction (Ando-Akatsuka *et al.* 1999). In non-epithelial cells ZO-1 has also been found in other subcellular sites, e.g. in the nucleus of growing cells (Gottardi *et al.* 1996) and in adherens junctions of cells which lack tight junctions, such as fibroblasts (Howarth *et al.* 1992, Itoh *et al.* 1993). These variations in the distribution of ZO-1 might be a consequence

of its ability to bind both occludin (Furuse *et al.* 1994) and α-catenin (Itoh *et al.* 1997), or the presence of forms of the protein of varying degrees of solubility.

In tight junctions of the epithelial cells, ZO-1 interacts with ZO-2, ZO-3, occludin and claudin (reviewed by Tsukita *et al.* 1999). Other molecules also associate directly with ZO-1, such as AF-6 (afadin), a putative target for Ras (Yamamoto *et al.* 1997), gap junctional protein connexin-43 (Toyofuku *et al.* 1998, Giepmans & Moolenaar 1998) and spectrin (Itoh *et al.* 1991). Occludin and claudin are linked to actin cytoskeleton through the C-terminal half of ZO-1 (Itoh *et al.* 1997). This part of ZO-1 contains various alternatively spliced domains, of which the α-domain corresponds to the plasticity of tight junctions (Balda & Anderson 1993, Sheth *et al.* 1997). Occludin and claudin have four transmembrane domains with N- and C-termini in the cytoplasm and two extracellular loops (reviewed by Tsukita *et al.* 1999). The extracellular domains and at least one of the transmembrane domains of occludin are responsible for regulation of paracellular permeability of tight junctions (McCarthy *et al.* 1996). Occludin is not only the structural and functional component of tight junction, but it also affects the phenotype of the epithelial cells during transformation (Li & Mrsny 2000). In Ras-transformed MDCK cells occludin is localised to cytoplasm together with claudin-1 and ZO-1, but the proteins are recruited to membrane if activation of mitogen-activated protein kinase (MAPK) is blocked (Chen *et al.* 2000). Junctional adhesion molecules (JAMs) are a family of immunoglobulin-like single-span transmembrane molecules that are expressed in endothelial cells, epithelial cells, leukocytes and myocardia. JAM has been suggested to contribute to the adhesive function of tight junctions and to regulate leukocyte transmigration (reviewed by Fanning *et al.* 1999). At tight junction JAM is linked to strands of claudin through ZO-1. JAM regulates the polarity of epithelial cells through its association with and recruitment of ASIP (atypical protein kinase C-specific interacting protein)/PAR3-atypical PKC complex to tight junction (Ebnet *et al.* 2001). In man, the claudin superfamily consists of at least 18 members, which are involved on paracellular transport as structural and functional components of tight junction. Claudins are directly associated with ZO-1, 2 and 3 and indirectly with AF-6 and cingulin (Itoh *et al.* 1999, reviewed by Tsukita *et al.* 1999). The tight junction of choroids plexus epithelium has a unique molecular composition of claudins composed of claudin-1, 2 and 11 that have a unique regulatory role in barrier function of blood-cerebrospinal fluid (Wolburg *et al.* 2001).

Nectin, afadin and ponsin are the components of a recently found cell-cell adhesion system at adherens junctions that are likely involved in the formation of cadherin– and claudin-based junctions (Mandai *et al.* 1997, Asakura *et al.* 1999, Ikeda *et al.* 1999). Nectin is a Ca^{2+}-independent immunoglobulin-like adhesion molecule (Takahashi *et al.* 1999). The nectin family consists of at least three members, nectin 1, 2 and 3. Most nectins interact with the PDZ domain of afadin through their conserved motif of C-terminus (Takahashi *et al.* 1999, Satoh-Horikawa *et al.* 2000). A family of afadins consists of at least two members, larger (l) –and smaller (s)-afadin, of which l-afadin is ubiquitously expressed in epithelial cells, whereas s-afadin is found in neural tissues. Both afadins have one PDZ domain, but s-afadin lacks the F-actin binding domain and there are also variations in the proline-rich region of the proteins (Mandai *et al.* 1997). Human s-afadin is identical to AF-6, the protein that forms a complex with ZO-1 at tight junction of epithelial cells (Yamamoto *et al.* 1997). L-Afadin is linked to cadherin-based adherens junction via ponsin, which in turn associates

through its SH3 domain to the proline-rich region of vinculin (Mandai *et al.* 1999). The nectin-afadin-ponsin complex is important in the formation of primordial spot-like junctions of epithelial cells, but it is not sensitive to disruption of existent cell-cell contacts induced by low Ca^{2+} concentration (Asakura *et al.* 1999). Thus, the nectin-afadin-ponsin complex serves as an independent adhesion system at epithelium.

2.2.1.3 Desmosomes and gap junctions

Desmosomes are specialised junctional structures that form a tight connection between all the epithelial cells and cardiac myocytes (reviewed by Schwartz *et al.* 1990). Within the cells, they are linked to cytokeratins, a class of intermediate filaments. The complex desmosomal structure consists of several transmembrane adhesive glycoproteins and cytoplasmic plaque proteins (reviewed by Garrod 1993). The glycoproteins, such as desmogleins and desmocollins, belong to the cadherin superfamily and the others, such as desmoplakins link the intermediate filaments to the membrane. Plakoglobin, a member of the cytoplasmic desmosomal plaque proteins, is directly bound to desmosomal cadherins; desmoglein-1 (Korman *et al.* 1989, Mathur *et al.* 1994, Troyanovsky *et al.* 1994) and desmocollin-1 (Troyanovsky *et al.* 1994). It is also found at adherens junction of the epithelial cells associated to classical cadherin (Cowin *et al.* 1986, Peifer *et al.* 1992) and α-catenin (Huber *et al.* 1997).

Gap junctions are intercellular structures that make possible the passive diffusion of the ions and small molecules in aqueous intercellular channels (connexons) between the cytoplasms of the neighbouring cells (reviewed by Kumar & Gilula 1996). Most cells of the normal tissues, except skeletal muscle cells, erythrocytes and circulating lymphocytes, generally communicate via these junctions. Gap junctions are specialised regions of the cell membrane in which each gap junction pore is formed by a juxtaposition of two hemichannels in neighbouring cells. These interact to span the plasma membranes of two adjacent cells joining the cytoplasms of the cells. The hemichannels are composed of connexins, highly related transmembrane proteins consisting of at least 13 members (reviewed by Beyer *et al.* 1990, reviewed by Saez *et al.* 1993, reviewed by Goodenough *et al.* 1996). To date, cadherins and catenins are excluded from the gap junction plaques but cadherin-catenin cell adhesion system may be involved in the formation of gap junctions (Fujimoto *et al.* 1997). The gap junctions are not only communicating channels but they also promote the fusions of placental cytotrophoblasts (Cronier *et al.* 1997). In fibroblasts proteins such as connexin 43 affect cell growth independently of gap junction formation (Moorby & Patel 2001). Inhibition of gap junction either with heptan-1-ol treatment or culturing cells at low density has no effect on connexin 43 to control cell growth.

2.2.2 Cell-substratum contacts

2.2.2.1 Focal adhesions

Most cultured and stationary cells adhere tightly to the underlying growth substratum through distinct regions of their plasma membrane called cell-matrix junctions, known as focal adhesion plaques, focal contacts, or focal adhesions, FAs (Abercrombie *et al.* 1971, reviewed by Burridge *et al.* 1988). At these sites, transmembrane receptors, or integrins, interact with extracellular matrix (ECM) proteins e.g. fibronectin, collagens, laminins and vitronectin. On the cytoplasmic side of focal adhesions, integrins together with cytoskeletal proteins link the large bundles of microfilaments, stress fibres, to these structures. Thus, the focal adhesions are a structural connection between the ECM and actin cytoskeleton (Fig. 3). The focal adhesions of the cultured cells are typically 2-10 μm long and 0.25-0.5 μm wide.

Integrin ligation is the first step in the formation of the focal adhesions. Thereafter, integrin makes the linkage with actin cytoskeleton via vinculin, talin, α-actinin, paxillin and p125 focal-adhesion kinase, FAK (reviewed by Yamada & Miyamoto 1995) and other components. Various regulatory proteins, such as calcium-dependent protease; calpain II, protein kinase C, FAK and Src family tyrosine kinases control the assembly of focal adhesion (Beckerle *et al.* 1987, Jaken *et al.* 1989, reviewed by Burridge & Chrzanowska-Wodnicka 1996, Kaplan *et al.* 1994, reviewed by Zamir & Geiger 2001). In many cultured cells, a number of proteins in focal adhesions are highly tyrosine phosphorylated (Burridge *et al.* 1992), and the formation of focal adhesions can be prevented by inhibitors of tyrosine kinases, such as herbimycin (Burridge *et al.* 1992) or by serum starvation, or it can be stimulated by inhibition of tyrosine phosphatases with vanadyl hydroperoxide (Barry & Critchley 1994, Chrzanowska-Wodnicka & Burridge 1994, Retta *et al.* 1996). In living, migrating fibroblasts microtubules and small GTPases of Rho family control the turnover of contact sites (Ridley & Hall 1992, reviewed by Small *et al.* 1999b).

2.2.2.2 Hemidesmosomes

Hemidesmosomes are multimeric protein complexes that attach epithelial cells to their underlying matrix and serve as cell surface anchorage sites for the keratin cytoskeleton. They are morphologically similar to desmosomes and localised to the basal surface of some epithelial cells (reviewed by Schwartz *et al.* 1990, reviewed by Garrod 1993). This kind of structures are typically described in epidermis of the skin. In spite of the morphological similarities (cytoplasmic plaques and connection to cytokeratin filaments) with desmosomes, the protein composition is distinct. Hemidesmosomes contain α6β4 integrin that is important for hemidesmosomal attachment. The plaque protein BP230 (BPAG1, bullous pemphigoid antigen 1) is sequencially related to desmoplakin (Green *et al.* 1999), and antigen BP180 (BPAG2) is a collagen-like transmembrane protein. Keratin

bundles associate to the hemidesmosome plaque via complex of BP230, BP180 and α6β4 integrin (Hopkinson & Jones 2000).

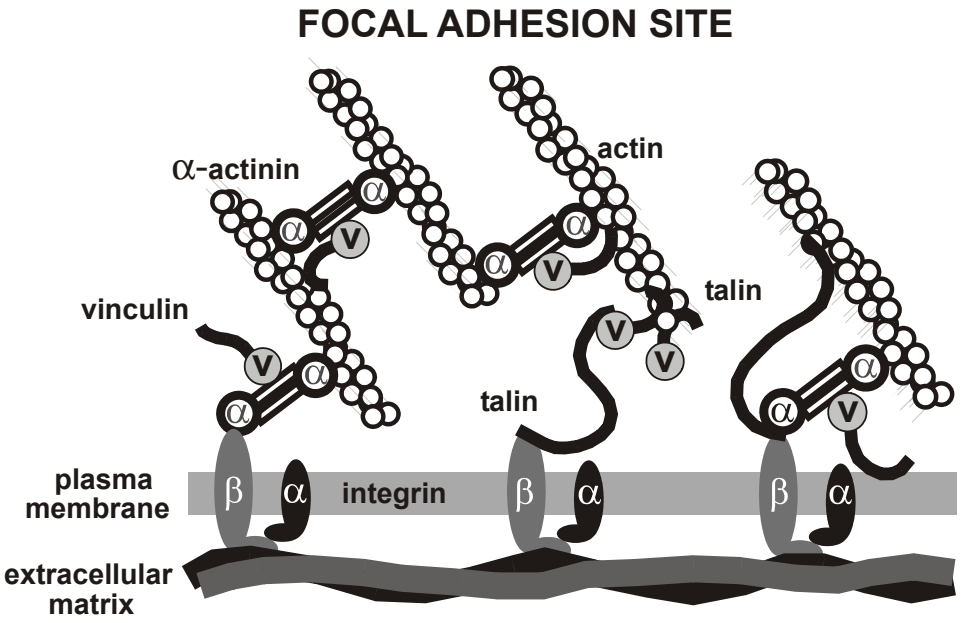

Fig. 3. A schematic drawing of protein interactions at focal adhesions based on *in vitro* studies. At focal adhesions α-actinin forms a bridge between the actin filaments as well as links them with integrins. The head domain of vinculin binds talin and α-actinin, whereas the vinculin tail is associated to lipids of plasma membrane and to actin filaments.

2.3 Small GTPases of Rho family

The Rho family of guanine nucleotide (GTP)-binding proteins consists of Rho, Rac and Cdc42 subfamilies. They were originally found during search for proteins homologous to Ras proto-oncoprotein (reviewed by Satoh *et al.* 1992). Rho GTPases belong to the Ras superfamily which is composed of more than 50 members and has been divided into six families: Ras, Rho, Arf, Sar, Ran and Rab (reviewed by Takai *et al.* 2001). The proteins exist in two interconvertible forms: the GDP-bound inactive and GTP-bound active forms. Active GTPases interact with their specific downstream targets and perform their cellular functions, whereas GTP-hydrolysis and liberation of phosphate inactivate the GTPases (Fig. 4). The GTP-GDP exchange reactions are regulated by guanine nucleotide exchange factors (GEFs), guanine nucleotide dissociation inhibitors (GDIs) and GTPase-activating proteins (GAPs, reviewed by Narumiya 1996, reviewed by Lamarche & Hall 1994). Small GTPases of the Rho family control cell growth, morphogenesis, cell motility, cytokinesis, trafficking and organisation of cell cytoskeleton, but they are also involved in transformation and metastasis (reviewed by Jaffe & Hall 2002).

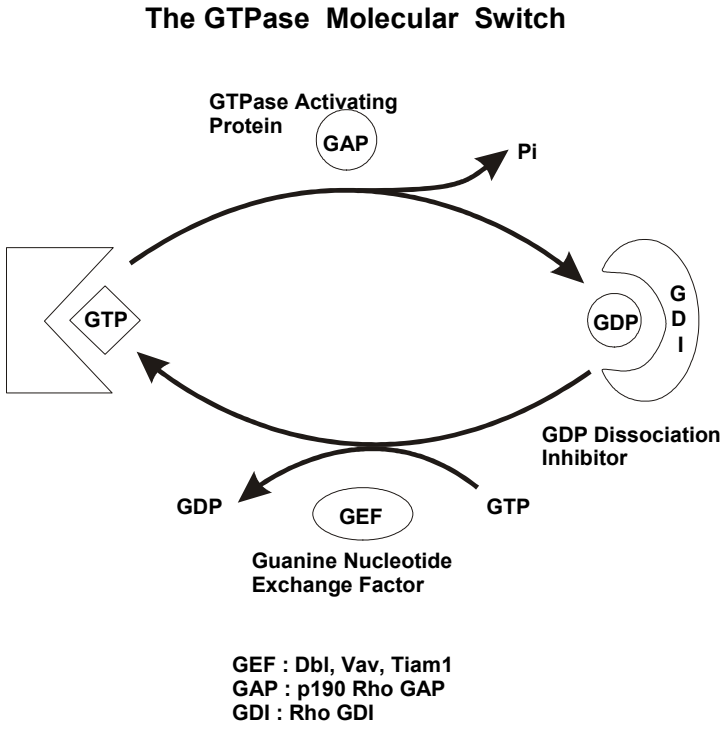

Fig. 4. The GTPase molecular switch. Guanine nucleotide exchange factors (GEFs) release guanosine diphosphate (GDP) from Rho GTPases promoting the binding of guanosine triphosphate (GTP) and activation of Rho GTPases. GDP dissociation inhibitor (GDI) inhibits the dissociation of GDP from Rho GTPases and thus prevents association of GDP-GTPase to cell membrane. GTPase activating proteins (GAPs) stimulate the intrinsic GTPase activity of Rho GTPases and convert GTP-bound form of Rho GTPases to inactive GDP-GTPases. In resting cells Rho GTPases exist mostly in GDP-bound form and in complexes with Rho GDI in the cytosol. The GTP-bound form of Rho GTPases is associated with cell membranes.

2.3.1 Regulation of actin cytoskeleton in fibroblasts

During recent years the regulation of the assembly and organisation of actin-based structures has obtained a great deal of interest in cell biology. Rho GTPases are important regulators of the actin cytoskeleton and consequently influence the shape and movement of the cells. Several lines of evidence have implicated that small GTPases of the Rho family are the major regulators of signalling pathways that link the extracellular growth factors or their receptors to the assembly of focal adhesions and associated structures

(Ridley & Hall 1992, Ridley *et al.* 1992, Nobes *et al.* 1995, Kozma *et al.* 1995). The first remarkable observation was the capability of serum or lysophosphatidic acid (LPA) to trigger activation of Rho that induces formation of focal adhesions and actin stress fibres in serum-starved Swiss 3T3 fibroblasts (Ridley & Hall 1992). On the other hand, growth factors and hormones such as PDGF, insulin and bombesin are able to activate Rac, whereas bradykinin activates Cdc42 (Ridley *et al.* 1992, Nobes *et al.* 1995, Kozma *et al.* 1995). Cdc42 triggers formation of filopodia, whereas Rac1 is responsible for the formation of lamellipodia (pleat-shaped protrusions at the cell periphery) and membrane ruffles (Kozma *et al.* 1995, Nobes & Hall 1995, Ridley *et al.* 1992). In Swiss 3T3 fibroblasts these GTPases activate each other in a hierarchical cascade in which Cdc42 activates Rac, which in turn activates Rho (Ridley *et al.* 1992, Nobes & Hall 1995). Active Rho and Rac GTPases together with extracellular matrix components are needed for the assembly of focal complexes (Hotchin & Hall 1995).

Numerous proteins bind Rho, Rac and Cdc42 in a GTP-dependent manner and mediate their effects on actin cytoskeleton. Serine/threonine kinases, Rho-kinase (Matsui *et al.* 1996), ROKα (RhoA-binding kinase α) and its close relative p160ROCK (ROKβ) have obtained particular interest (reviewed by Van Aelst & D'Souza-Schorey 1997). Rho GTPase binds to these kinases and elevates their activity (Amano *et al.* 1997, Leung *et al.* 1995). Activated Rho-kinase can phosphorylate myosin binding subunit of myosin light-chain (MLC) through inactivation of MLC phosphatase and also probably through direct phosphorylation of MLC (Kimura *et al.* 1996, reviewed by Ridley 2001). This, in turn, enhances the binding of myosin to actin filaments and subsequently the formation of stress fibres (Leung *et al.* 1995, Chrzanowska-Wodnicka & Burridge 1996, Amano *et al.* 1997). Other identified targets of Rho are phosphatidylinositol 4-phosphate 5-kinase (PIP5-kinase) and p140mDia that regulate actin polymerisation (Tolias *et al.* 1995, Ren *et al.* 1996, Watanabe *et al.* 1997). In MDCK cells mDia and Rho-kinase are responsible for the formation of thin and thick actin filaments, respectively (Nakano *et al.* 1999, Amano *et al.* 1997).

GTP-bound forms of Rac1 and Cdc42 have several common downstream targets that regulate actin cytoskeleton. Serine threonine kinase Pak1 serves as an effector for Cdc42 and Rac promoting the motility of fibroblasts (Sells *et al.* 1997). However, Pak1 is not required for Rac dependent membrane ruffling and lamellipodia formation nor for Cdc42 induced filopodia formation (Joneson *et al.* 1996, Lamarche *et al.* 1996). This refers to its possibility to function independently of Rho GTPases. POR1, in turn, as a target of Rac plays a role in Rac-mediated membrane ruffling in fibroblasts (Van Aelst *et al.* 1996). It binds directly to GTP-bound form of Rac1. Membrane ruffling and interaction are prevented if the effector-binding loop of Rac is mutated. Wiskott-aldrich syndrome protein (WASP), in turn, forms the linkage of Cdc42 and Rac to the Arp2/3 complex that is found in the zone of lamellipodia (reviewed by Bi & Zigmond 1999). WASP and its related N-WASP are activated through its binding to Cdc42 and phosphatidylinositol 4,5-bisphosphate (PIP_2). Activation of N-WASP by Cdc42 recruits the Arp2/3 complex to leading edge and thereby promotes formation of filopodia (reviewed by Carlier *et al.* 1999). POSH as a target of Rac activates the jun amino-terminal kinase/stress-activated protein kinase (JNK/SAPK) pathway and thereby regulates gene transcription. POSH may also play a role in the activation of transcription factor NF-κB and induce its nuclear

tranlocation. Overexpression of the protein leads to apoptosis of fibroblasts independently of JNK activation. (Tapon *et al.* 1998, reviewed by Aspenström 1999.)

2.3.2 Regulation of cell-cell adhesion

Small GTPases of the Rho family regulate the cadherin mediated cell-cell adhesions in many ways (Braga *et al.* 1997, 1999, Hordijk *et al.* 1997, Kuroda *et al.* 1997, Takaishi *et al.* 1997, Zhong *et al.* 1997). Transfection studies of MDCK cells with RhoA, Rac1 and Cdc42 constructs have indicated that Rac strengthens the cadherin-based cell-cell adhesion by increasing the amounts of actin filaments, E-cadherin and β-catenin to the cell-cell adhesion sites (Takaishi *et al.* 1997). In contrast, Rac disrupts cell-cell adhesion in small preconfluent colonies of keratinocytes (Braga *et al.* 2000). The molecular mechanism for the regulation of cell-cell adhesion is largely unknown, and there are several candidates for downstream effectors. One effector is IQGAP1 that binds to activated Cdc42 and Rac (Kuroda *et al.* 1996). This interaction prevents association of IQGAP1 with β-catenin and results in strong cell-cell adhesion. Inactive Cdc42 and Rac, in turn, are unable to interact with IQGAP1, which then promotes its binding to β-catenin and dissociation of α-catenin from the cadherin-catenin complex, leading to weak adhesion in mouse L fibroblasts expressing E-cadherin (Kuroda *et al.* 1998). The significance of this interaction *in vivo* is questioned by the observation that complete lack of IQGAP1 had hardly any effect on the cadherin-based adhesion of gastric cells in mice during embryogenesis, although it increased susceptibility to gastric hyperplasia (Li *et al.* 2000). Another regulator is p120-catenin (p120ctn) that binds cadherin and promotes its clustering together with RhoA resulting in strong adhesion. At the same time it inactivates or activates Rho signalling. RhoA inhibition by p120ctn and the interaction of p120ctn with cadherins are mutually exclusive, suggesting a mechanism for regulating the recruitment and exchange of RhoA at nascent cell-cell contacts (Anastasiadis *et al.* 2000, reviewed by Anastasiadis & Reynolds 2001). Increased association of p120ctn with cadherins likely releases RhoA at the junction and promotes its activation by Rho GEFs (reviewed by Anastasiadis & Reynolds 2001). On the other hand, overexpression of p120ctn in cytoplasm inhibits RhoA and increases cell motility through its action on other RhoGTPases and thereby on cell-ECM contact (reviewed by Anastasiadis & Reynolds 2001). By affecting RhoA activation, p120ctn could modulate cadherin functions, including suppression of invasion, neurite extension and junction formation (Anastasiadis *et al.* 2000, Noren *et al.* 2000). Rho has multiple functions in epithelial cells, since ROCK, a downstream effector of Rho GTPase disrupts the adhesion of MDCK cells, whereas another Rho downstream regulator, mDia, stabilises it (Sahai & Marshall 2002).

Tiam1 was originally identified as the murine invasion- and metastasis-inducing gene (Habets *et al.* 1994). Tiam1 protein shares a Dbl homology (DH) domain with an increasing number of oncoproteins, some of which have been shown to function as GDP dissociation stimulators (GDSs) or GDP exchange factors (GEFs) for small GTPases of the Rho family. Tiam1 is GEF for Rac1 (Hordijk *et al.* 1997). It induces metastasis of lymphomas (Habets *et al.* 1994), but prevents HGF-induced cell scattering of MDCK cells, probably by increasing accumulation of E-cadherin accompanied by actin polymerisation at cell-cell

contacts (Hordijk *et al.* 1997). In Ras-transformed MDCK cells, expression of Tiam1 or Rac restored E-cadherin-mediated adhesion, resulting in phenotypic reversion of the fibroblast phenotype towards the epithelial phenotype and loss of invasiveness (Hordijk *et al.* 1997). Hence, Tiam1 and Rac function as invasion-suppressors in epithelial cells. Moreover, activation of Rac by Tiam1 requires PI3-kinase activity. PI3-kinase also regulates Tiam1-incuded migration as well as E-cadherin-mediated cell-cell adhesion, indicating that PI3-kinase acts upstream of Tiam1 and Rac (Sander *et al.* 1998). In NIH3T3 fibroblasts, Tiam1 increases the cell-substrate interactions and induces the epithelial-like phenotype by strengthening the cell-cell adhesion with N and P-cadherin through Rac– and Cdc42-induced down-regulation of Rho activity and inhibition of cell migration (Sander *et al.* 1999).

Rho and Rac GTPases also regulate the tight junctions. Active GTPases perturb the gate function of junction as well as change the morphology of junction strands and the localisation of tight junctional components, occludin and ZO-1. Inactive GTPases only perturb the gate function (Jou *et al.* 1998). Active Rho also protects the tight junctions from damage during ATP depletion (Gopalakrishnan *et al.* 1998). RhoA-ROCK dependent and independent mechanisms, in turn, control the permeability of tight junction and phosphorylation of occludin (Hirase *et al.* 2001).

2.3.3 Rho GTPases in inflammation

One of the major functions of the Rho GTPases is the reorganisation of actin cytoskeleton in response to various extracellular stimuli. Thus, they especially regulate the cellular processes where filamentous actin plays a central role. Furthermore, they are involved in diverse vital cellular processes such as regulation of transcription via the c-Jun N-terminal kinase (JNK) signalling pathway, induction of apoptosis, cell growth control, inflammation and malignant transformation (reviewed by Mackay & Hall 1998, Chuang *et al.* 1997, reviewed by Van Aelst & D'Souza-Schorey 1997).

Some bacterial toxins inactivate or activate Rho GTPases (reviewed by Aktories 1997) and in this way affect the actin cytoskeleton of the host. Cytotoxic necrotising factor 1 (CNF1) of Escherichia coli activates RhoA, Cdc42 and Rac (Schmidt *et al.* 1997, Flatau *et al.* 1997, Lerm *et al.* 1999). In cultured cells, the toxin increases the amount of actin stress fibres and focal contacts. Clostridium botulinum exoenzyme (C3) inhibits Rho by ADP-ribosylation (Saito 1997) and Clostridium toxins A and B promote glucosylation of Rho GTPases. Clostridium sordellii lethal toxin inhibits Rac, Ras and Rap GTPases with the same mechanism (Popoff *et al.* 1996, Just *et al.* 1996, 2001).

Cytokines are soluble proteins produced by nucleated cells throughout the body and secreted into circulation. They affect cell growth and differentiation but also mediate immune responses and have a role in tissue repair. Recent studies indicate that cytokines produced by cells of the innate defense system play an essential role in influencing the immune response towards protective anti-tumour immunity. These cytokines might act as first 'danger signals' in alerting the immune system. (Dunham 1999.) Interleukin-1β (IL-1β) is one of the inflammatory cytokines that exerts its effect through Rho GTPases (Bagrodia *et al.* 1995, Zhang *et al.* 1995, Whitmarsh *et al.* 1997). For induction of infection bacterium

binds to cell surface and/or enters the cell interior. Helicobacter pylori is a human-specific pathogen that causes chronic active gastritis and peptic ulcer disease (reviewed by Dubois 1995). Its adherence to surface of the gastric cells promotes activation of Rho GTPases and sequential activation of jun and p38 kinases, responsible for stimulation of transcription. As a result transcription factors, such as NF-κB are activated, followed by secretion of interleukins 1, 6, 8 and tumour necrosis factor-α, TNF-α (Perona *et al.* 1997, Sharma *et al.* 1998, Wessler *et al.* 2000). The molecular linkages (possible other kinases, like SEK1 and PAKs) between the small GTPases and jun/p38-kinases are largely unknown, but they are also dependent on cell type and extracellular stimulus. Another well-known bacterium Listeria monocytogenes enters target cells and induces massive actin polymerisation. The surface protein of this pathogen, ActA, induces actin-driven movement of bacteria in the cytoplasm of infected host cells and initiates actin polymerisation by binding and activating the host Arp2/3 complex (reviewed by Cossart 2000, reviewed by Higgs & Pollard 2001).

2.3.4 Rho GTPases in cancer

Ras is a well-known oncogene that has been identified in an activated state in various human cancers including epithelial carcinomas of the lung, colon, and pancreas (reviewed by Barbacid 1987, Hamad *et al.* 2002). The Ras proteins were originally identified in retroviruses that trigger sarcoma-type tumours in rats (Rasheed *et al.* 1983). They are crucial switchers of intracellular signalling networks that regulate cell growth and differentiation. Activation of Ras proteins proceeds through their activated kinase receptors (most notably receptor tyrosine kinases, RTKs) or G protein-coupled receptors, and when activated they control cellular signalling via multiple downstream effectors (reviewed by Vojtek & Der 1998). A major intracellular signalling pathway mediated by Ras is initiated by the epidermal growth factor (EGF) receptor (EGFR) leading to cell proliferation. Depending on cell type and environment EGFR signalling can induce mitosis or apoptosis, proliferation, oncogenic transformation, enhanced motility, protein secretion, and differentiation or dedifferentiation (reviewed by Seger & Krebs 1995, reviewed by Wells 1999). The best understood effector pathway is activation of Ras by the recruitment of son-of-sevenless (SOS) to the cell membrane via the adapter proteins Shc and/or Grb2. This promotes interaction of GTP-bound Ras with downstream effectors such as the kinase Raf, which ultimately leads to the activation of mitogen-activated protein kinase (MAPK, reviewed by Force & Bonventre 1998).

Rho GTPases and especially their activating GEFs are also involved in the evolution of cancer. Ras-transformed mammary epithelial cells or MDCK cells resemble fibroblasts with decreased junctions but increased focal adhesions and stress fibres (Zhong *et al.* 1997, Zondag *et al.* 2000). Activated Rho is responsible for some characteristics of Ras-transformed mammary cells. Its inhibition by C3 exoenzyme or the presence of inactive Rho restores partially the normal epithelial phenotype of the cells with poor contacts but leads to loss of stress fibers and focal adhesions. Thus, high level of activated Rho is responsible for the cytoskeletal alterations in these cells (Zhong *et al.* 1997). On the other hand, activation of Rac promotes epithelial phenotype in MDCK cells, showing that active Rac restores E-cadherin-mediated adhesion resulting in phenotypic reversion.

However, Ras is able to decrease Rac activity (down-regulation) and increase Rho activity leading to mesenchymal MDCK cells (Zondag *et al.* 2000).

The guanine-nucleotide exchange factors (GEFs) for Rho GTPases are proteins that have been identified as oncogenes in transfection assays. According to general assumption all of them share two common structural motifs: a Dbl homology (DH) domain and a pleckstrin homology (PH) domain. In these proteins the DH domain is responsible for the GEF activity and also required for cellular transformation. The PH-domain in turn mediates cellular transformation by targeting the Dbl protein to specific cytoskeletal locations to activate Rho GTPases (Zheng *et al.* 1996, reviewed by Van Aelst & D'Souza-Schorey 1997). These domains are found in GEFs, e.g. Vav, Ost, Bcr (GEFs for Rho GTPases) and Sos (GEF for Ras). Several GEFs also have other domains that typically exist in signalling molecules, such as Src homology (SH3) domain.

Tiam1, a GEF for Rac GTPase is considered an invasion-inducing gene (Habets *et al.* 1994) that contains Dbl and PH domains. Overexpression of activated Tiam-1 or activated Rac induces invasion and metastasis of T-lymphoma cells (Michiels *et al.* 1995). In contrast, in MDCK cells Tiam1 promotes accumulation of cadherin to lateral walls (Hordijk *et al.* 1997). In Tiam1 deficient mice development of Ras-induced skin tumours depends on the initiator. The mice exposed to 7,12-dimethylbenzanthracene were resistant, whereas 12-O-tetradecanoylphorbol-13-acetate promoted tumour development. Moreover, the growth of skin tumours was slower than in Tiam1 expressing mice. On the other hand, those tumours that grew were more aggressive. (Malliri *et al.* 2002.) Thus, effects of Tiam1 seem to depend on cell type and environment.

2.4 Src protein tyrosine kinases

Tyrosine phosphorylation is a central event in the regulation of a variety of biological processes such as cell proliferation, migration, differentiation and survival. Several families of receptor and non-receptor tyrosine kinases control these events by catalysing the transfer of phosphate from ATP to a tyrosine residue of specific cell protein targets.

Transformation is the process that in animals leads to formation of tumours and in cell culture induces structural, biochemical and behavioural changes in the cells. The prototype member of the Src family protein tyrosine kinases was originally identified as the transforming protein (v-Src) of the oncogenic retrovirus, Rous sarcoma virus, RSV (Brugge & Erikson 1977, Purchio et al. 1978, Hamaguchi et al. 1995). Viral v-Src is a mutated and activated version of a normal cellular protein (c-Src) with intrinsic tyrosine kinase activity (Collett & Erikson 1978, Levinson et al. 1978). This kinase phosphorylates its protein substrates exclusively on tyrosyl residues (Hunter & Sefton 1980). In epithelial cells it is associated with adherens junctions and focal contacts suggesting a role in cell adhesion (Tsukita et al. 1991, reviewed by Frame et al. 2002).

Src protein tyrosine kinases are 52-62 kD proteins composed of six distinct functional domains: SH4 (src homology 4), a unique domain, SH3, SH2, SH1 and a C-terminal regulatory region (reviewed by Brown & Cooper 1996). SH4 domain contains the myristylation signals that guide the Src molecule to the cell membrane. This unique domain

of Src proteins is responsible for their specific interaction with particular receptors and protein targets (reviewed by Thomas & Brugge 1997). Moreover, it binds to atypical protein kinase C (Seibenhener *et al.* 1999). The modulating regions, SH3 and SH2, control intra- as well as intermolecular interactions with protein substrates which affect Src catalytic activity, localisation and association with protein targets (reviewed by Pawson 1995). The kinase domain, SH1, found in all proteins of the Src family, is responsible for the tyrosine kinase activity and has a central role in binding of substrates. The N-terminal half of Src kinase contains the site(s) for its tyrosine phosphorylation, and phosphorylation of tyrosine (Y) 416 regulates the catalytic activity of Src (reviewed by Thomas & Brugge 1997). v-Src differs from cellular Src (c-Src) on the basis of the structural differences in C-terminal region responsible for regulation of kinase activity. V-Src always exists in opened, active conformation, whereas c-Src is flexible and normally inactive (reviewed by Thomas & Brugge 1997). Activation of c-Src is critically involved in carcinoma cell migration and metastasis (Sakamoto *et al.* 2001).

2.4.1 Targets of Src kinases

Src substrates are proteins that become tyrosine phosphorylated as a result of src gene function and are direct or indirect targets of Src. The best-known Src substrates are found in transformed cells. Most of them, found at focal adhesions, are key components in the integrin-mediated signal transduction and bound to actin or integrin, e.g. vinculin, cortactin, talin, paxillin, FAK, tensin, ezrin and p130cas. Furthermore, junctional proteins, such as β- and γ-catenin, ZO-1, occludin, p120ctn, connexin 43, nectin-2 delta are identified as major sites of tyrosine phosphorylation by Src kinases (reviewed by Parsons & Parsons 1997, reviewed by Thomas & Brugge 1997, Tsukamoto & Nigam 1999, reviewed by Abram & Courtneidge 2000, Kikyo *et al.* 2000). The intercellular communication via gap junctions is prevented when two tyrosines of connexin 43 are phosphorylated by v-Src (Lin *et al.* 2001). The other known targets of Src are enzymes involved in phospholipid metabolism, such as PLC-γ, p85 subunit of PI3-kinase and the signalling molecules p190RhoGAP, p120rasGAP and EGF receptor substrate, Eps8 (reviewed by Thomas & Brugge 1997, reviewed by Abram & Courtneidge 2000, Gallo *et al.* 1997).

2.4.2 Regulation of cell adhesion by Src

The regulation of cellular adhesion by v-Src kinase is still poorly understood. It is known that Src has an essential role in bone formation, since its mutation induces defects in bone remodelling of mice including impaired osteoclast function and developed osteopetrosis (Soriano *et al.* 1991). Experiments with Src-/- fibroblasts have shown that Src is not essential for the formation of focal adhesions but it is required for the optimal adhesion and spreading on fibronectin matrix mediated by integrin receptors (Felsenfeld *et al.* 1999, Cary *et al.* 2002). Attachment to fibronectin stimulates phosphorylation of focal

adhesion kinase (FAK) induced by integrin engagement and activated Src (McLean *et al.* 2000). In MDCK cells low expression of v-Src predominantly disturbs the formation of adherens junctions but has no effect on the assembly of tight junctions and desmosomes (Warren & Nelson 1987). Moreover, in v-src transformed MDCK cells activation of v-Src weakens the cadherin-based adhesion (Volberg *et al.* 1992, Behrens *et al.* 1993, Takeda *et al.* 1995). The tyrosine phosphorylation of the cellular proteins (especially proteins of zonula adherens) such as β-catenin is increased in these cells, correlating with their dedifferentiation and promotion of the invasiveness (Takeda *et al.* 1995, reviewed by Noe *et al.* 1999). In src-MDCK cells activation of v-Src also releases spectrin from the cell membranes (Sormunen *et al.* 1999). To date it is unclear whether v-Src or c-Src interacts directly with the components of adherens junctions *in vivo*. Moreover, tyrosine phosphorylation of β-catenin in response to growth factors and cell transformation is not connected with the shift of adhesion from strong to weak (Takeda *et al.* 1995). Although p120ctn was first identified as a Src substrate and its constitutive tyrosine phosphorylation correlates with cell transformation, it is not clear what is the significance of tyrosine phosphorylation of p120ctn and how it affects on Rho or cadherin function (Anastasiadis & Reynolds 2000). However, in Ras-transformed breast epithelial MCF10-A cells p120ctn is heavily phosphorylated, adherens junctions are loosened and p120ctn is efficiently bound to cadherin (Kinch *et al.* 1995). Expression of cadherin also controls recruitment of p120ctn to cell-cell contacts and association of p120ctn to E-cadherin is a prerequisite for tight adhesion (Thoreson *et al.* 2000).

2.5 Inositol –and serine/threonine kinases involved in cell signalling

2.5.1 PI-kinases

Phosphatidylinositol is a cellular phospholipid that is an important precursor of several second-messenger molecules in cellular signalling. It contains five free hydroxyls that are available for phosphorylation by phosphoinositide kinases (PI-kinases). The kinases are categorised into three families: phosphoinositide 3-kinases (PI3-Ks), phosphoinositide 4-kinases (PtdIns4Ks), and phosphoinositide-P (PIP) kinases (PIPKs). PI3-kinase is an enzyme-complex that phosphorylates the 3’ hydroxyl position of the inositol ring of phosphatidylinositol, phosphatidylinositol 4-phosphate or phosphatidylinositol 4,5-bisphosphate, PIP_2. The enzyme contains a 85 kD regulatory subunit and a 110 kD catalytic subunit. PI3-kinase regulates many biological activities, such as DNA synthesis, cell survival, differentiation, lamellipodia formation, chemotaxis and migration, adhesion, and neurite outgrowth. (Reviewed by Vanhaesebroeck *et al.* 1996, reviewed by Fruman *et al.* 1998.)

The phosphoinositide 3-kinase family of enzymes play a central role in growth factor receptor signal relay. Stimulation of the cells with growth factors causes rapid accumulation of the PI3-kinase products, 3' phosphoinositide lipids (Laffargue *et al.* 1999, Crljen *et al.* 2002) and further association of PI3-kinase with tyrosine-phosphorylated

growth factor receptors or their tyrosine-phosphorylated products. The lipid products of PI3-kinase act as second messengers by binding to and activating diverse cellular target proteins. These events constitute the start of a complex signalling cascade, which ultimately results in the mediation of cellular activities such as proliferation, differentiation, chemotaxis, survival, trafficking, and glucose homeostasis (reviewed by Katso *et al.* 2001).

The downstream effectors of Src, GAPs and GEFs promote the cytoskeletal changes via Rho family GTPases (reviewed by Abram & Courtneidge 2000). PI3-kinase is known to coordinate some of the responses, such as growth factor-induced membrane ruffling which Rac GTPase also regulates. This is based on the fact that active Rac and also Cdc42 are able to form the complex with PI3-kinase and the former linkage is stimulated by PDGF (Carpenter *et al.* 1997). Moreover, it is known that Rac acts as a downstream regulator of the scatter factor/hepatocyte growth factor (SF/HGF) receptor p190Met and induces membrane ruffling through PI3-kinase (Ridley *et al.* 1995). The PI3-kinase pathway is also important for efficient transformation of mammalian cells by Ras oncogenes and in Ras-induced rearrangement of cortical actin (Rodriguez-Viciana *et al.* 1997).

2.5.2 Protein kinase C

The protein kinase C (PKC) family is composed of at least 10 closely related multifunctional members of serine/threonine kinases that can be divided into three subgroups: conventional PKCs (α, βI, βII, Cγ), novel PKCs (δ, ε, η, θ), and atypical PKCs (ξ, λ/ι, reviewed by Nishizuka 1995). All of them are important in the regulation of cell growth, differentiation, proliferation and neurotransmission. The enzymes are activated by lipid-derived metabolites, like diacylglyserols (DAG), ceramide and phosphatidylinositol 3,4,5-triphosphate and in this way transmit the extracellular stimuli to the cellular signalling networks (Nishizuka & Nakamura 1995, Lozano *et al.* 1994, Müller *et al.* 1995, Nakanishi *et al.* 1993). Conventional PKCs also need calcium for their maximal activation, whereas novel PKCs are activated in the absence of calcium (reviewed by Nishizuka 1995).

Besides DAG some tumour promoting phorbol esters, such as phorbol myristoyl acetate (PMA) induce a prolonged activation of PKC. This discovery created for the first time a connection between the processes of signal transduction and tumour promotion (reviewed by Anderson *et al.* 1985). PMA is able to substitute for DAG in the activation of PKC (reviewed by Nishizuka 1989). In addition to DAG, PKC is regulated by two mechanism: phosphorylations of newly synthesised enzyme affect the alignment of its residues for catalysis and guide PKC to cytosol, whereas binding of ligands or substrate promotes activation of enzyme by removing the pseudosubstrate from the substrate-binding site. PKC is targeted to membrane by its interactions with DAG and phosphatidylserine. Conventional PKCs also need Ca^{2+} (reviewed by Newton 1993). PKC controls opening and closing dynamics of tight junction in the frog urinary bladder (Lacaz-Vieira 2000). In epithelial cells, such as MDCK cells it regulates formation and paracellular permeability of tight junction (Ellis *et al.* 1992, Dodane & Kachar 1996).

Activation of PKC leads to phosphorylation of its target proteins. One of the major cellular substrates is the myristoylated alanine-rich C kinase substrate (MARCKS) involved in a variety of cellular processes in which the regulated rearrangement of actin cytoskeleton is needed. The other important substrates of PKC are macMARCKS and RACK (receptor for activated C-kinase, reviewed by Jaken 1996). MARCKS binds calmodulin in a calcium-dependent fashion (Graff *et al.* 1989) and cytoskeletal actin in a calmodulin-dependent manner (Hartwig *et al.* 1992). It associates to phospholipid membranes by the N-terminal myristoyl moiety and the basic effector domain (Taniguchi & Manenti 1993). In epithelial cells, the role of MARCKS is poorly known. However, in MDCK cells phosphorylated MARCKS likely reduces its actin-crosslinking capacity, promotes the disintegration of membrane skeleton and depolymerisation of stress fibres (Vääräniemi *et al.* 1999). Adducin, another substrate for PKC, is a membrane-skeletal protein localised at spectrin-actin junctions and originally found in human erythrocytes (Derick *et al.* 1992). In epithelial cells it associates to cell-cell contact sites in a calcium-dependent manner together with spectrin (Kaiser *et al.* 1989, Hu *et al.* 1995). Adducin promotes association of spectrin with actin (Gardner & Bennet 1987) via its MARCKS-related domain (Li *et al.* 1998). The same domain is also phosphorylated by PKC and binds to calmodulin (Matsuoka *et al.* 1996). Rho-kinase phosphorylates adducin and thus enhances its F-actin-binding activity (Kimura *et al.* 1998, reviewed by Fukata *et al.* 1999). Besides these regulations, PMA-activated PKC has a deleterious effect on actin-based structures in fully confluent tightly associated epithelial cells, whereas in cells without contacts or loose contacts, activation of PKC improves actin-based cytoskeletal structures (Vääräniemi *et al.* 1999). In tight adhesion, membrane skeleton is disintegrated and stress fibers are depolymerised, whereas in cells with loose contacts activation of PKC assists formation of these structures (Vääräniemi *et al.* 1994, 1997, 1999, Huotari *et al.* 1996).

Atypical PKCs have been found in the cell junctional complex of renal epithelial MDCK cells (Dodane & Kachar 1996, Izumi *et al.* 1998). Isotypes of ξ and λ of atypical PKC take part in signalling through lipid metabolites, such as phosphatidylinositol 3-phosphates. On the other hand, their association with atypical PKC-specific interacting protein (ASIP) and PAR proteins allows them to regulate the establishment and/or maintenance of cell polarity and development of junctional structures in epithelial cells (Izumi *et al.* 1998, Suzuki *et al.* 2001).

2.5.3 Protein kinase B and PAR

Protein kinase B (PKB or Akt) is a serine/threonine kinase and an integral component of survival pathways that is regulated by growth factors and extracellular stimuli (reviewed by Downward 1998). In epithelial cells, both matrix adhesion and cell-cell interaction promote the survival of the cells via activation of PI3-kinase and protein kinase B (Khwaja *et al.* 1997, King *et al.* 1997, Pece *et al.* 1999, Watton & Downward 1999). PI3-kinase is a key enzyme in PKB activation, but activation of PKB may also proceed through PI3-kinase independent pathway including PKCα/β (Kroner *et al.* 2000). Moreover, PKCζ has a clear inhibitory effect on the activity of PKB (Doornbos *et al.*

1999). Activation of PKB by receptor tyrosine kinases via PI3-kinase promotes formation of basement membrane by inducing synthesis of matrix components, laminin β1 and collagen IV (Li *et al.* 2001). PKB interacts with several targets of which trh (trachealess) is important for development of some tubular organs in Drosophila (Jin *et al.* 2001). Besides PKC, PKB has a central role in apoptosis (reviewed by Cross *et al.* 2000) and thereby it binds to apoptotic targets such as Bad, Forkhead transcription factors and the cyclic AMP response element-binding protein, CREB (Yamaguchi & Wang 2001). PKB is also able to prevent apoptosis of the cells by inhibiting conformational change of the pro-apoptotic Bax protein (Yamaguchi & Wang 2001).

The discovery of PAR proteins gave a new insight into molecular mechanism of cell polarity. PAR-3, PAR-6 and atypical PKC are needed for cellular polarity of C. elegans (reviewed by Doe & Bowerman 2001). In mammalian cells these proteins exist as a multiprotein complex localised to the apical site of the epithelial cells. They control the polarity and growth of the cells by confirming the asymmetric cell division (Kim 2000). Moreover, human PAR-6 also interacts with active Cdc42 and Rac, referring to its capability to function as an effector of these GTPases (Johansson *et al.* 2000, Joberty *et al.* 2000, Lin *et al.* 2000, Qiu *et al.* 2000). At tight junction of mammalian epithelial cells, PAR-3, also called ASIP, interacts with atypical PKC and PAR-6 (Izumi *et al.* 1998, reviewed by Ohno 2001). The localisation of PAR-3, PAR-6, ZO-1, occludin and claudin at tight juction is regulated by atypical PKC, which thereby affects the development of functional tight junctions (Gao *et al.* 2002) and the establishment of cell polarity (reviewed by Ohno 2001).

2.6 Carcinomas and cytoskeleton

In normal epithelium the interacting cells form highly organised sheets and the cells are often poorly motile. In carcinoma cells the morphology and migratory behaviour have changed to resemble the properties of fibroblasts or mesenchymal cells (reviewed by Birchmeier & Birchmeier 1993). On malignant progression actin structures are disorganised and actin bundles are hardly visible. Thus, in malignant transformation the mechanisms that regulate polymerisation and binding of actin seem to change. This leads to disturbances of cellular function. Another difference in transformed cells is the appearance of tyrosine-phosphorylated proteins in the cell cytoplasm. According to a general assumption the level of protein tyrosine phosphorylation affects epithelial-mesenchymal transition (Volberg *et al.* 1991, 1992, reviewed by Schmidt *et al.* 1993) and many kinases like Src, Neu, Erb B and Trk are mutated to oncogenes (Lodish *et al.* 2000). The src-transformed MDCK cells acquire more mesenchymal morphology (Behrens *et al.* 1993). Moreover, the cells are flattened and thereby have weakened intercellular adhesion, in addition to which their actin cytoskeleton and adhesion to substratum are changed and actin-associated proteins are redistributed (reviewed by Frame *et al.* 2002). The direct linkage from Src activation, phosphorylation of junctional components and epithelial-mesenchymal transition is, however, still missing. The loose contact to extracellular matrix (ECM) may be a result of increased secretion of proteases

that degrade the matrix components (reviewed by Kurchat & Mauch 2000) or alterations in the expression levels of integrins. The Ras family is another group of oncogenes that affect the cytoskeleton. The Ras-transformed cells are very invasive *in vivo* and display enhanced migratory activity in chemotaxis and chemokinesis, a response of motile cell to a soluble chemical (Ochieng *et al.* 1991, reviewed by Akhurst & Derynck 2001).

In normal epithelial cells E-cadherin is a prominent component of adherens junction, whereas in transformed cells the expression of E-cadherin on their cell surface is disturbed, probably increasing their invasion and metastasis (Mareel *et al.* 1991, reviewed by Birchmeier & Behrens 1994). The expression of E-cadherin and α-catenin is reduced in lobular type of mammary carcinomas as well as in primary and metastatic colorectal carcinomas (Zschiesche al. 1997, Ghadimi *et al.* 1999). The role of E-cadherin in invasion is still unclear, since the invasive properties of carcinomas correlate rather to expression of N-cadherin than loss of E-cadherin (Nieman *et al.* 1999).

Tumour cell invasion is a three-dimensional process. In order to evaluate the differentiation and invasion potency of the cells in relation to basement membrane, the cells are cultured in gels typically composed of collagen I, mixtures of different ECM components or extract of Engelbreth-Holm-Swarm murine sarcoma also called Matrigel (Santos & Nigam 1993, Kuzuya & Kinsella 1994, Price-Schiavi *et al.* 1998). Collagen is useful for *in vitro* studies concerning the heterogeneity of tumour cells as well as their invasive ability (Kusunoki *et al.* 2002). In collagen MDCK cells form spherical cysts composed of polarised epithelial cells with intrinsic central lumen, but activation of mitogen-activated protein kinase 1/ERK kinase (MEK1) makes them invasive in collagen (Montesano *et al.* 1999). The studies by O'Brien and co-workers (2001) have given the evidence that active Rac1 and the basement membrane component laminin determine the orientation of apical pole during cyst development of MDCK cells. Inactive Rac in turn impairs laminin assembly, which leads to inversion of apical pole to the cyst periphery. V-src transformed MDCK cells at permissive temperature grow in collagen I as an irregular cluster, but in Matrigel they acquire a better polarity by recruitment of spectrin, β-catenin and cadherin to the lateral walls (Rahikkala *et al.* 2001). Matrigel has also been used for morphogenetic studies, such as the formation of tubules in cultures of mammary epithelial cells (Niemann *et al.* 1998).

2.7 Helicobacter pylori infection

2.7.1 Attachment of bacterium to gastric cells

For a long time it has been known that attachment of bacteria and viruses on the surface of epithelium initiates the infection process. The pathogens either enter the host, like Listeria (reviewed by Cossart 2002), or remain on the surface of the host, like Escherichia coli (reviewed by Cook & Young 2002). Helicobacter pylori (H. pylori), a coloniser of the human stomach, is a spiral, gram-negative rod-shaped pathogen that attaches to gastric epithelial cells and is a causative agent of chronic active gastritis, peptic ulcer and

gastric cancer. H. pylori is a common bacterium that is found in at least 30-50 % of the world's population, and individuals colonised with this organism in early age have later an increased risk of developing gastric cancer. (Reviewed by Blaser 1990.) Adherence of H. pylori to the gastric epithelium and secretion of interleukins are believed to be an important step in the induction of active inflammation of the mucosal layer. The attachment of H. pylori to the host leads to several changes in the binding site, such as destruction of microvillus cytoskeleton, actin rearrangement directly beneath the bacterium, and cup/pedestal formation to the site of attachment (Segal *et al.* 1992, 1996). Other reported effects of the bacteria are tyrosine phosphorylation of two host cell proteins (105 and 145 kD, Segal *et al.* 1996), vacuolation inside the susceptible cells induced by bacterial toxin Vac A (Hotchin *et al.* 2000) and increased expression of interleukin 8 (Rieder *et al.* 1997). One of the most interesting signalling pathways linked to infection of gastric adenocarcinoma (AGS) cells with H. pylori, is activation of EGF receptor that activates Ras GTPase and finally leads to phosphorylation of extracellular signal-regulated kinase, ERK1/2 (Keates *et al.* 2001). If activation of the receptor is blocked, Ras is inactivated and the expression of IL-8 is decreased.

Different strains of H. pylori attach to human gastric cells via soluble blood group antigens of the cells that typically exist on red blood cells, but they are also expressed on the surface of the epithelial cells as histo-blood group antigens (reviewed by Clausen & Hakomori 1989). Most of the strains bind fucosylated Lewis b (Le^b) histo-blood group antigen and H-1 antigen *in situ*, whereas binding to other identified antigens including Le^a, H-2, Le^X and Le^Y has not been demonstrated. Those bacterial strains that bind to Le^b antigen secrete CagA toxin. The bacterial cell surface Le^b-binding adhesin, BabA delivers the bacterial virulence factors that cause the damage to the gastric epithelium (Ilver *et al.* 1998).

2.7.2 Secretion of toxins

The adherent pathogens secrete toxins either into the extracellular medium or directly into the cytosol of the host and thereby change the cell signalling of target cells (reviewed by Cook & Young 2002). To date several strains of H. pylori have been identified which differ in virulence depending on the expression of cytotoxins. Strain 1, typically existing in individuals with peptic ulcer, expresses the vacuolating cytotoxin A, VacA and the cytotoxin-associated gene A protein, CagA (Covacci *et al.* 1993, Marchetti *et al.* 1995). Strain II is less virulent and neither of these markers is expressed.

VacA is a major virulence factor of H. pylori that forms transmembrane anion-specific channels to the susceptible cells (Iwamoto *et al.* 1999, Tombola *et al.* 1999) and contributes to the formation of vacuoles inside the cells. In acidic or basic pH, VacA is activated, and this enhances its binding to the receptor on the cell surface (Yahiro *et al.* 1999). This might interfere with signal transduction pathways such as epidermal growth factor (EGF)-mediated signal cascades that affect the cell proliferation and ulcer healing (Pai *et al.* 1998).

The antigen, named CagA, is a hydrophilic, surface-exposed protein of 128 kD produced by most bacterial strains. Phosphorylated CagA induces a hepatocyte

growth-factor (HGF)-like response of host cells characterised by elongation and spreading, including the production of filopodia and lamellipodia (Segal *et al.* 1999). SFA (stress fibre associated) is another cellular phenotype that is induced after attachment by H. pylori. It is CagA independent and is produced by type I and type II of H. pylori. The visible effects are production of numerous stress fibres, multinucleation and increased vacuolisation (Segal *et al.* 1999). Different toxins of H. pylori use small GTPases for triggering of cytoskeletal re-organisation that alters the paracellular permeability of the cells. Alternatively, small GTPases may regulate transporters like Na^+-H^+ exchangers (Hooley *et al.* 1996, Szaszi *et al.* 2000) through which they disturb the transport properties at the beginning of infection, later on likely resulting in gastric cancer.

3 Aims of the study

The development and maintenance of epithelial architecture requires stable adhesion between the neighbouring cells and the extracellular matrix. In epithelial cells, cell-cell adhesion is based on three structures: adherens junctions, desmosomes and tight junctions. Several kinases, phosphatases and small GTPases regulate these adhesion structures. The components of cell-cell contacts are largely known, but their mutual functions are still under intensive study. During embryogenesis the cell-cell junctions are able to open in a carefully controlled manner, whereas during carcinogenesis they are randomly disrupted. In some conditions the cells are fused to each other in order to form syncytia or tubular structures.

The targets of the present study are the regulators of cell-cell adhesion and actin cytoskeleton in epithelial cells. The aims of the present study can be briefly defined as follows:

1. To study how vinculin and α-actinin regulate the structure and integrity of adherens junctions in MDBK cells;
2. To analyse the interactions of injected vinculin at cell-cell contacts in MDBK cells and the contribution of Rac GTPase;
3. To analyse the targets of Src kinase in short time intervals after its activation in ts-src-MDCK cells and the contribution of Tiam1, a Rac GEF;
4. To analyse the cytoskeletal rearrangements caused by the attachment of Helicobacter pylori to gastric carcinoma AGS cells and the role of Rac GTPase in these alterations.

4 Materials and methods

Detailed descriptions of the materials and methods are presented in the original papers I-IV. The cells and reagents used in cell studies are summarised in Table 1. Antibodies and actin indicators are presented in Table 2.

Table 1. The cells and reagents used in the original publications (I-IV).

Cells / Reagents for cell studies	Source	Used in
Cells		
MDBK	Bovine kidney	I, II
ts-src MDCK	Canine kidney, transformed	III
Tiam1 ts-src MDCK	Canine kidney, transformed	III
AGS	Human gastric cancer	IV
Helicobacter pylori	Human gastric mucosa	IV
Reagents for cell studies		
Human recombinant Rac	Cytoskeleton, Inc	I, II, III, IV
Human recombinant Cdc42	Cytoskeleton, Inc	I, II, IV
Wortmannin	Sigma Chemical Co	I
Nigericin	Sigma Chemical Co	II
PP2	Calbiochem	III
Actinomycin D	Sigma Chemical Co	IV
Cycloheximide	Sigma Chemical Co	IV

MDBK cells were used for microinjection of vinculin, α-actinin, Rac and Cdc42 GTPases. The functional targets of Src-kinase were sought with src-MDCK cells. The mutual relationship between Src and Rac was studied in Tiam1 src-MDCK cells. AGS cells were used for Helicobacter pylori infection. Wortmannin is an inhibitor of PI3-kinase and Src kinase is inhibited by PP2. Nigericin as a K^+-H^+ exchanger equilibrates intra- and extracellular pH of the cells in KCl buffer. Actinomycin D inhibits RNA synthesis and cycloheximide protein synthesis.

Table 2. The following primary antibodies were used in the original publications (I-IV). Phalloidins and phallacidin were used to visualise actin.

Primary antibody/Actin indicator	Source/Reference	Used in
E-cadherin mouse mAb (C20820)	Transduction Laboratories	I, II
E-cadherin mouse mAb (rr1)	Development Studies Hybridoma Bank	III
β-catenin mouse mAb (C19220)	Transduction Laboratories	I, III
γ-catenin (plakoglobin) mouse mAb (C26220)	Transduction Laboratories	II
α-catenin mouse mAb (IG5)	Johnson *et al.* 1993	III
p120ctn mouse mAb (P17920)	Transduction Laboratories	III
Vinculin mouse mAb (F9B11B4)	Glukhova *et al.* 1990	I, II
α-actinin mouse mAb (BM75.2)	Sigma	I
ZO-1 rat mAb	Chemicon International Inc.	I, III
Occludin rat mAb (MOC37)	Furuse *et al.*1993	I
Claudin rabbit pAb (71-7800)	Zymed Laboratories Inc.	I, III
Desmoplakin mouse mAb (DP 2.15)	Hesse *et al.* 2000	II
Eps8 mouse mAb (E18220)	Transduction Laboratories	III
Phosphotyrosine mouse mAb (clone PT-66)	Sigma	III
Phosphotyrosine rabbit pAb (06-427)	UBI	III
Alexa 488 phalloidin	Molecular Probes	I, II, III
Alexa 568 phalloidin	Molecular Probes	I, II, III
Bodipy FL phallacidin	Molecular Probes	IV
Rhodamine phalloidin	Molecular Probes	IV

4.1 Cell culture (I-IV)

4.1.1 Culture of epithelial cells (I-IV)

4.1.1.1 Two-dimensional culture (I-IV)

MDBK cells were obtained from American Type Culture Collection (ATCC, Rockville, MD, USA). The cells were cultured in D-MEM with high glucose supplemented with 2 mM glutamine, 10% FCS, antibiotics and antimycotics (I, II). The ts-src MDCK cells were provided by Professor Walter Birchmeier and Doctor Jürgen Behrens (Max Delbrück Center for Molecular Medicine, Berlin, Germany, Behrens *et al.*, 1993). Ts-src transformed MDCK cells (originally from Dr. Behrens) carrying a C1199 Tiam1-neo construct were obtained from Professor John Collard (The Netherlands Cancer Institute, Division of Cell Biology, Amsterdam, the Netherlands, Habets *et al.* 1994). Both cell lines were grown in D-MEM supplemented with 2 mM glutamine, 10% FCS and antibiotics/antimycotics at 40.5^{o}C (III). Human gastric adenocarcinoma (AGS) cells were purchased from American Type Culture Collection (ATCC, Rockville, MD, USA) and

cultured in Ham's F12 medium supplemented with L-glutamine, 10% FCS, antibiotics and antimycotics (IV).

4.1.1.2 Three-dimensional culture (III)

Tiam1 src-MDCK cells were cultivated in collagen I, in collagen I-laminin mixture and in Matrigel as described by Rahikkala *et al.* (2001). The culture medium was D-MEM supplemented with 2 mM glutamine, 10% FCS (for the culture in collagen I or in collagen-laminin mixture) or 10% horse serum (for the culture in Matrigel) and antibiotics/antimycotics.

4.1.2 Culture of bacterial cells (IV)

H. pylori strain CCUG17874 (Culture Collection, University of Gothenborg, Sweden), and two local strains (581/96 and 523/96) isolated from dyspeptic children were cultured on Mueller-Hinton plates supplemented with 5% sheep blood and antibiotics. CCUG17874 was cagA + vacA (toxin positive) strain. 581/96 and 523/96 represented cagA – vacA (toxin negative) strains (14).

4.2 Purification of vinculin (I, II) and α-actinin (I)

Vinculin and α-actinin were purified from chicken gizzard smooth muscle as described by Feramisco & Burridge (1980), Geiger (1979), O'Halloran *et al.* (1986). After tissue homogenisation and extraction, the cytoskeletal proteins were precipitated by 10 mM $MgCl_2$ and 20% $(NH_4)_2SO_4$. Vinculin and α-actinin were fractioned in DE (diethylaminoethyl)-53 (Whatman) column by using 10 to 380 mM NaCl gradient. The precipitated α-actinin fractions were purified further on Sepharose Cl-6B (Pharmacia) column equilibrated in 20 mM NaCl, 0.1 mM EDTA, 15 mM 2-mercaptoethanol, 20 mM Tris/acetate, pH 7.6. The purified proteins were stored at –70°C.

4.3 Digestion of vinculin and purification of vinculin fragments (I)

For digestion endoproteinase Glu-C (V8 from Staphylococcus aureus, ICN Biomedicals Inc.) was coupled to NHS-activated HiTrap (Amersham Pharmacia Biotech) column. Vinculin (0.8 mg/ml) was digested with about 30 µg of immobilised V8 protease for 90 minutes at 37°C. The head and tail fragments of vinculin were separated on a CM (carboxymethyl) 52 (Whatman) column according to Groesch & Otto (1990). Purification

of vinculin tail was further continued on a HA (hydroxylapatite, Bio-Rad Laboratories) column as described by Groesch & Otto (1990).

4.4 Labelling of vinculin (I, II), vinculin head (I) and α-actinin (I)

4.4.1 FITC-labelling (I)

Purified vinculin (1.6 mg/ml), vinculin heads (1.1 mg/ml) and α-actinin (2.7 mg/ml) were labelled with fluorescein 5-isothiocyanate (FITC, Molecular Probes), 10% on celite (2 mg dye/mg protein) in 0.25 M Tris-HCl, pH 9.6 for 30 minutes at room temperature. The mixture was loaded into a Sephadex G-50 (Pharmacia) column in 100 mM KCl, 5 mM HEPES, pH 7.25 and the labelled proteins eluting in the void volume were collected. Aggregation of the labelled α-actinin was prevented by its dialysis in 1 mM KCl, 1 mM $NaHCO_3$, 5 mM HEPES, pH 7.25 (Masaki & Takaiti 1969).

4.4.2 TAMRA-labelling (II)

Vinculin (1 mg/ml) was labelled with 5-carboxytetramethylrhodamine succinimidyl ester (5-TAMRA, SE, Molecular Probes) in 0.1 M $NaHCO_3$ (pH 8.3). The ratio was 30 μg dye/ mg vinculin. The labelling procedure followed the instructions of Molecular Probes. The conjugated protein was separated from the free label on a Sephadex G-25 (Pharmacia) column equilibrated with 100 mM KCl, 5 mM HEPES, pH 7.25.

4.5 Microinjection (I-IV)

Microinjections were carried out using an Eppendorf micromanipulator 5171 and microinjector 5246 (Hamburg, Germany) installed on an Axiovert 405 M inverted microscope with a heating stage (Zeiss, Oberkochen, Germany). MDBK cells and Tiam1 src-MDCK cells were injected in medium 199 with Hanks' salts. AGS cells were injected in their growth medium and transferred to medium without FCS and antibiotics/ antimycotics for 15 minutes before adding H. pylori bacteria.

4.6 Manipulation of intracellular pH and membrane potential (II)

The intracellular pH of microinjected MDBK cells was lowered with 5 μM nigericin in isotonic KCl buffer (140 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 0.5 mM KH_2PO_4, 20

mM HEPES, pH 5.5) as described by Eskelinen *et al.* (1991, 1992). The cells were depolarised with nigericin in KCl buffer, pH 7.5 and hyperpolarised by incubating them in 130 mM tetramethylammonium chloride solution as described by Vääräniemi *et al.* (1997).

4.7 Protein phosphorylations by Src (III)

Temperature sensitive src-MDCK cells were used to study phosphorylation of proteins and its effect on the integrity of junctions. Src kinase was expressed and activated in cells shifted from 40.5°C to 35°C for different time periods. The phosphorylation of proteins was analysed by immunoprecipitation and immunoblotting. Redistribution of junctional proteins and their colocalisation with tyrosine-phosphorylated proteins were studied with confocal microscopy.

4.8 Infection assay (IV)

AGS cells were infected with different strains of Helicobacter pylori in Ham's F12 medium without FCS and antibiotics/antimycotics. For infection the concentration of bacteria was optimised by absorbance measurement (A_{565} = 0.5). After two-hour incubation at 37°C the cells were fixed and stained for actin.

4.9 Fluorescence and microscopy techniques (I-IV)

4.9.1 General principles (I-IV)

The cells, grown on glass coverslips, were fixed after the experiments using the following optimised procedures for each antibody and cell line. In order to preserve two or three dimensional structure of the cells and the stability of actin cytoskeleton, the cells were usually fixed with 4% formaldehyde (FA), 0.2% Triton X-100 (TX-100) in cytoskeleton-stabilising buffer (PEM-buffer; 100 mM PIPES, 5 mM EGTA, 2 mM $MgCl_2$, pH 6.8) for 10 minutes at room temperature. The penetration of the antibodies was enhanced by post-fixation with methanol for 5 minutes at -20°C or with ethanol for few seconds at -20°C. Alternatively, the cells were fixed with 1% formaldehyde in PBS for 15 minutes and thereafter permeabilised with 0.2% Triton X-100 in PBS for 15 minutes. For some antibodies it was necessary to use methanol fixation (10 minutes at -20°C) that permeabilises the cells, but flattens their three-dimensional structure.

After fixation the unspecific protein binding sites were usually blocked with either 10% FCS or 1% BSA. Antibodies were diluted with either 5% FCS in PBS-glycine solution

(0.01 M NaH_2PO_4/ Na_2HPO_4, 145 mM NaCl, 0.02 M glycine, pH 7.2), or 1% BSA, 0.1% TX-100 in PBS. In stainings with anti-phosphotyrosine antibody, the blocking was omitted and the antibodies were diluted in NaCl-HEPES buffer (140 mM NaCl, 10 mM HEPES, pH 7.0). This way the addition of new phosphorylation sites to the specimen was avoided.

4.9.2 Conventional immunofluorescence microscopy of fixed and living cells (I, IV)

The fixed cells were incubated with the primary and secondary antibodies for 30-60 min either at 4°C or at room temperature. Actin was stained with bodipy phallacidin or with Alexa- or rhodamine phalloidins for 30 min at +4°C. The specimens were mounted in a Shandon mounting liquid (Immu-Mount, Pittsburg, PA, USA) and viewed by Zeiss Axiovert 405M microscope and 100x objective.

The fluorescence and phase contrast images were photographed using KODAK TMAX 3200 ASA film. The weak fluorescence signals were collected with a low-light-level Extended Isis video camera (Photonic Sciences, Mountford, UK) and digitised using a DT3851 frame grabber from Data Translation (Marlboro, MA, USA) and a microcomputer. The digitised images were displayed and analysed with the aid of a Global Lab Image program (Data Translation). The processed images were photographed from screen using Agfapan 100 ASA film. The same equipment was used for visualisation of injected fluorescent proteins within the living cells.

4.9.3 Confocal microscopy (I-III)

4.9.3.1 Two-dimensional analysis (I-III)

The cells were stained as described for immunofluorescence microscopy and viewed by Zeiss 510 confocal laser scanning microscope, equipped with an Argon laser (488 nm) and HeNe laser (543 nm). Scanning was performed in an XY-plane with 0.36 μm intervals using 63x oil objective, a 505-530 nm band pass filter for green emission and a 560 nm or 630 nm low pass filter for red emission.

4.9.3.2 Processing the cells for three-dimensional analysis (III)

Tiam1 src-MDCK cells grown in 3D gels were enzymatically permeabilised (0.05% collagenase A for collagen I and collagen I-laminin mixture, a coctail of collagenase A and 0.56% hyaluronidase for Matrigel) before fixation with 4% FA/0.2% Triton X-100 in PEM-buffer. After additional 0.1% Triton X-100 treatment, the gels were incubated with

the primary antibody overnight and secondary antibody for two hours at +4^{o}C as described by Rahikkala *et al.* (2001). The specimens, mounted on microscopic slides, were scanned through the whole cell complex in x-y planes with 0.4 µm to 1.5 µm intervals.

4.9.4 Immunoelectron microscopy of 3D cells (III)

Tiam1 src-MDCK cells, grown in 3D gels, were pre-treated with Matrisperse solution in order to dissolve the matrix components (Becton Dickinson). Thereafter, the cells were fixed in 4% paraformaldehyde, immersed in sucrose and frozen in liquid nitrogen. Ultrathin cryosections were cut with a Leica Ultracut UCT microtome. For immunolabelling, the sections were blocked with 5% BSA and 0.1% gelatin. They were then incubated with primary and secondary antibodies, followed by protein A-gold complex (size 5 nm). The sections were examined in a Philips CM100 transmission electron microscope.

4.10 Solubility assay (III)

In order to study the soluble and insoluble proteins in src-MDCK cells separately, the cells grown on plastic cell culture plates were scraped into RIPA buffer (50 mM Tris, pH 7.4, 150 mM NaCl, 10 mM NaF, 1 mM Na_3VO_4, 10 mM $Na_4P_2O_7$, 4 mM EDTA, 1 mM PMSF, 1% Nonidet P-40, 0.1% SDS). The supernatant ('soluble fraction') was removed and the residue ('insoluble cytoskeletal fraction') was boiled with SDS-buffer (15 mM Tris, 5 mM EDTA, 2.5 mM EGTA, 1% SDS, pH 7.5) as described by Hinck *et al.* (1994). The mixture was sonicated and diluted with RIPA buffer. After measurement of protein concentration, the cell fractions were used for immunoprecipitation and immunoblotting.

4.11 SDS-PAGE and immunoblotting (I, III)

The proteins, prepared in Laemmli's sample buffer, were separated on either 7.5 or 12% SDS-PAGE gels. The gels were stained with either Coomassie brilliant blue R-250 or silvernitrate. For immunoblotting the proteins were electrophoretically transferred to nitrocellulose as described by Towbin *et al.* (1979). After blocking, the nitrocellulose sheet was incubated with primary antibody and then with biotin or horseradish peroxidase (HRP)-conjugated secondary antibodies. The proteins were detected either with diaminobenzidine (DAB) in the presence of H_2O_2 and $NiCl_2$, or treated with luminol-coumaric acid-H_2O_2 detection solution. In the latter case the proteins were exposed to Hyperfilm ECL X-ray film (Amersham Pharmacia Biotech).

4.12 Immunoprecipitation (III)

Tyrosine phosphorylation of proteins in src-MDCK cells was analysed by immunoprecipitation and immunoblotting. For immunoprecipitation, the insoluble and soluble fractions of src-MDCK cells were first precleared with protein G Sepharose beads (Amersham Pharmacia Biotech) and then incubated with BSA blocked antibody (IgG 1-2 μg) beads. After overnight precipitation at 4^{o}C, the beads were washed with RIPA buffer and boiled in SDS sample buffer. The supernatants were submitted to SDS-PAGE and immunoblotting.

5 Results

5.1 Vinculin and α-actinin purified from chicken gizzards incorporate into native adhesion structures of cultured MDBK cells and induce cell fusions (I, II)

Microinjection of fluorophore-tagged cytoskeletal proteins has been a useful tool in studies of formation of focal adhesions (Burridge & Feramisco 1980, Pavalko *et al.* 1995). The same method was used in this thesis work to study the dynamics of adhesion structures in Madin-Darby bovine kidney (MDBK) epithelial cells. These cells are suitable for microinjection due to their well-developed epithelial cell - cell junctions and flat morphology. The flat shape facilitates the injection and prevents the damage of the contacts caused by injecting pressure. The primary aim of this study was to analyse how junctional components behave in response to disturbance in the amount of free proteins. Vinculin and α-actinin were selected as target proteins, because they are well-known structural components both at adherens junctions and focal adhesions. Moreover, their interactions are well characterised (reviewed by Jockusch & Rüdiger 1996) and they have functional roles in the assembly of these structures. Vinculin and α-actinin were purified from chicken gizzards as single polypeptides of 115 kD and 100 kD (I; Fig. 1), respectively, and labelled with FITC or TAMRA fluorophores for microinjection experiments. The microinjected proteins incorporated very rapidly (within 5 minutes) to focal adhesions and adherens junctions of the cells, showing their acceptance as part of the structural elements of the cells. In addition, vinculin was visible in nuclei of the cells (vinculin: I; Fig. 2). A surprising feature of the proteins was their ability to induce cell-cell fusions during the follow-up time. Within two hours the injected confluent cells formed polykaryons having as many as 5 to 13 nuclei accumulated in the centre of the polykaryon. The injected proteins delineated the fused cells but were also found at focal adhesions (vinculin: I; Fig. 2). Inside the polykaryons they localised to membrane fragments and sometimes the whole cell-cell contact site was cut into small pieces that remained *in situ* (I; Figs. 3-5 and II; Figs. 1, 2). Since the cells kept their contact with the

substratum it can be concluded that the excess amount of vinculin or α-actinin had hardly any effect on the function of focal adhesions.

In solution vinculin exists in both open and closed form, and the binding properties of vinculin are strongly dependent on its conformation. Therefore we analysed separately the ability of head and tail domains to incorporate into adhesion structures. The head domain, digested from vinculin with V8 protease as a 95 kD polypeptide (I; Fig. 1), was labelled with FITC and microinjected into the cells. It incorporated rapidly into focal adhesions and adherens junctions but in contrast to intact vinculin it induced disappearance of focal adhesions within 30 minutes after injection and detached the cells from substratum (I; Fig. 6). The ability of the purified tail domain (30/27 kD, I; Fig. 1) to incorporate into adhesion structures remained unsolved because it was probably rapidly fragmented inside the cells. Hence, it seems that incorporation of vinculin into adherens junction takes place via head domain, but additional binding sites cannot be excluded. Since PI3-kinase regulates the polymerisation of actin and activity of small GTPases we studied its effects on vinculin interactions. Therefore we blocked the activity of enzyme with wortmannin. However, this treatment had no effect on vinculin-membrane association, showing that PI3-kinase is not critical in the delivery of vinculin to its destination (I; Fig. 6). Since acidic pH affects the conformation of vinculin (Miller & Ball 2001), the injected cells were acidified to pH 5.5 for 2 hours with the aid of K^+-H^+ exchanger, nigericin in KCl buffer. Surprisingly, injected vinculin was found as thick layers along the membranes, but not at focal adhesions or in nuclei (II; Fig. 4). Also cell fusions were absent in nigericin-treated specimens. However, the critical factor for cell fusion seemed to be membrane potential, because also nigericin treatment at neutral pH (depolarising conditions) inhibited fusion (II; Fig. 4). The hyperpolarised conditions (TMACl buffer) allowed cell fusions (II; Fig. 4). These results clearly showed that lipid and vinculin conformation as well as membrane charge regulate the integrity of epithelium. We also studied the behaviour of endogenous vinculin (II; Fig. 4), cadherin and actin in these conditions. The depolarised conditions weakened the cell-cell contacts, cadherin and actin delineated the membranes in fragments, whereas stress fibres and focal adhesions vanished completely. Vinculin staining was very faint at membranes. In hyperpolarised cells the amount of stress fibres and focal adhesions was prominent. In these cells vinculin incorporated mainly into focal adhesions, but faint lateral staining was also seen. Actin and cadherin stained the membranes as in control cells.

5.2 Disintegration of junctional complexes is a prerequisite for cell fusions (II)

Confocal microscopy turned out to be an ideal tool for the analysis of localisation of membrane fragments within the polykaryons and the mutual distribution of adhesion proteins in these fragments. The analysis of adhesion complexes in vinculin-injected cells also gave important information about the sequential release of components from adherens junctions. For detailed confocal analysis of vinculin interactions during disintegration process, vinculin was labelled with TAMRA and the injected cells were fixed and stained with specific antibodies against adhesion proteins. The optical

sectioning of the cells showed that injected vinculin was incorporated into the lateral membranes close to the basal part of the cells and was occasionally colocalised there with cadherin, actin, plakoglobin and desmoplakin (II; Figs 1, 2). Moreover, especially cadherin and plakoglobin were seen in clusters or vesicles in cytoplasm (II; Figs. 1, 2 and in thesis; Fig. 5B), whereas desmoplakin remained at membranes (II; Figs. 1, 2). The most apical actin delineated membranes apart from vinculin (II; Fig. 1), whereas in the middle part of the cells vinculin colocalised with actin (in thesis; Fig. 5C). In large polykaryons formed within 60 min after injection, TAMRA-vinculin clearly colocalised with actin (II; Fig. 2) and desmoplakin in membrane fragments inside the polykaryons close to the basal surface of the cells. However, cadherin and plakoglobin were still in cytoplasmic clusters close to vinculin-stained membrane fragments (II; Fig. 2). Thus, vinculin remained at membranes even in cases where actin, cadherin and plakoglobin had been removed to cytoplasm of the cells, most likely due to disintegration of the membrane skeleton and subsequent endocytosis of membraneous cadherin and plakoglobin. This indicates that vinculin at lateral walls binds either directly to lipids or to other proteins, such as ponsin (Mandai *et al.* 1999).

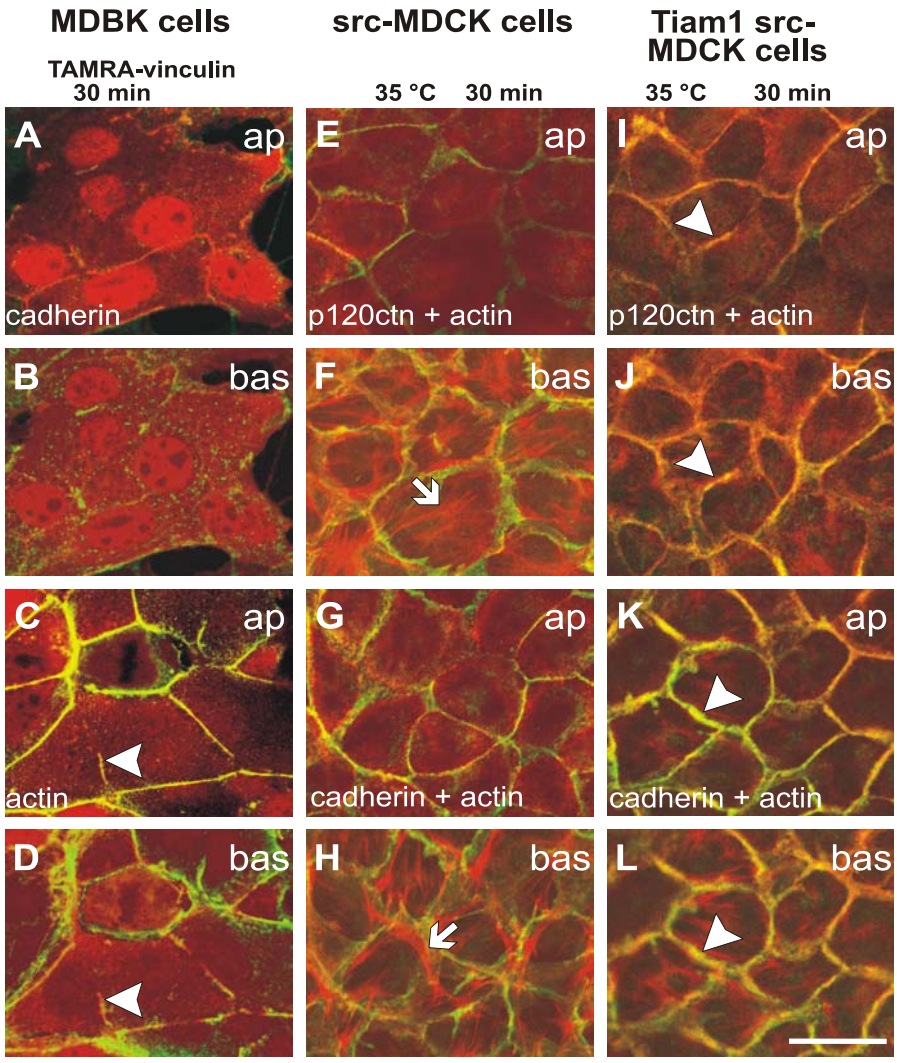

Fig. 5. A-D: Confocal images showing the localisation of TAMRA-vinculin with E-cadherin (A, B) and with actin (C, D) at apical and basal sections of MDBK cells fixed 30 min after injection of TAMRA-vinculin. TAMRA-vinculin is seen in red, cadherin and actin in green and their co-localisation in yellow colour. The arrowhead in C and D indicates the fragment of cell-cell wall in which vinculin at the basal side is in a broad band partially colocalising with actin (D), whereas on the apical side of the cells the proteins are completely colocalised (C). E-L: Confocal images showing double stainings of Alexa 568 phalloidin with anti-p120ctn (E, F, I, J) and with anti-E-cadherin (G, H, K, L) in src-MDCK cells (E-H) and Tiam1 src-MDCK cells (I-L) fixed 30 min after shifting the cells from non-permissive temperature (40.5°C) to permissive temperature (35°C). Actin is seen in red, p120ctn and E-cadherin in green and colocalisation in yellow colour. Arrow in F and H shows bundles of stress fibres on the basal surface of the cells. Arrowhead in I-L indicates accumulation of p120ctn and cadherin to lateral walls induced by active Rac GTPase. Bar 20 μm

5.3 Active Rac GTPase increases the amount of cadherin to membranes and thereby diminishes formation of polykaryons induced by vinculin (I, II)

Because Cdc42 and Rac GTPases are known to regulate the cell-cell adhesion, the behaviour of injected vinculin was analysed in the presence of active or inactive GTPases (I; Figs. 7, 8 and II; Fig. 3). The cell junctions were compared with the ones in cells injected with GTPases alone. In the presence of active Rac, TAMRA-vinculin incorporated into adherens juntion together with cadherin (II; Fig. 3). Moreover, colocalisation of vinculin and cadherin increased at the apical and basal sides of the cells. Only few polykaryons were formed. Thus, accumulation of cadherin protected the cells from vinculin-induced polykaryons. Active or inactive Rac alone had no remarkable effect on cadherin-based adhesion. The effects of active Cdc42 with or without TAMRA-vinculin were less evident and the cells clearly suffered from the simultaneous injection of these proteins. On the other hand, microinjection of inactive Rac or Cdc42 together with vinculin neither inhibited nor promoted formation of polykaryons (I; Fig. 8 and II; Fig. 3). However, the cell walls were very thin, vinculin was weakly visible at cell-cell contacts and there was no colocalisation between cadherin and vinculin (II; Fig. 3). The results showed that vinculin-membrane associations were independent of cadherin complex, but formation of polykaryons was prevented by a large amount of cadherin at membranes.

5.4 Activation of Src kinase changes the adhesion properties of src-transformed MDCK cells (III)

5.4.1 Distribution and phosphorylation of junctional proteins (III)

Activation of Src kinase weakens cellular adhesion and elevates tyrosine phosphorylation of junctional proteins, such as β-catenin (Behrens *et al.* 1993). We tested this hypothesis by shifting ts-src MDCK cells from non-permissive to permissive temperature and analysing the colocalisation of junctional proteins with tyrosine phosphorylated ones. Our study showed that when Src kinase is inactivated at nonpermissive temperature, adherens junctional proteins, E-cadherin (III; Fig. 1), p120ctn, α -and β-catenin lined the lateral membranes colocalising apically with tyrosine-phosphorylated proteins. Already within 15 minutes after Src activation at permissive temperature cadherin clustered at the basal surface and staining at lateral walls was broader (III; Fig. 1). In 30 min at 35°C also p120ctn (in thesis; Fig. 5F), α -and β-catenin were seen in clusters of basal surfaces. In 120 min all adherens junctional proteins were localised to basal clusters partially colocalising with anti-phosphotyrosine antibody and only a few remnants of proteins remained at lateral membranes (III; Fig. 2). The cells were concomitantly flattened and lost their epithelial morphology. Among the tight junctional components especially

claudin remained at the lateral membranes at 35^{o}C. Thus, adherens junctions were completely disintegrated due to activation of Src, whereas tight junctions remained apparently intact. The epidermal growth factor receptor (EGFR) substrate and actin-binding protein Eps8 was seen at microvilli and lateral membranes, but did not translocate by Src activation.

Staining of junctional proteins with anti-phosphotyrosine antibody does not give reliable evidence about tyrosine phosphorylation of a single protein in fluorescence microscopy. Thus, the phosphorylation of proteins was studied by immunoprecipitation and immunoblotting. The individual proteins (E-cadherin, β-catenin, p120ctn, Eps8, ZO-1) were immunoprecipitated from soluble and insoluble fraction of src-MDCK cells and the precipitated proteins were stained with anti-phosphotyrosine antibody on blot. At non-permissive temperature only β-catenin was weakly phosphorylated in soluble fraction of src-MDCK cells (III; Table 1). Soluble p120ctn and ZO-1 were phosphorylated within 15-30 min after the temperature shift to 35°C (III; Fig. 3 and Table 1). Eps8 showed hardly detectable phosphorylation at 35°C (III; Table 1). When the proteins were precipitated with anti-phosphotyrosine antibodies and stained on blot with specific antibodies against E-cadherin, β-catenin, p120ctn, Eps8 and ZO-1, the intensity of the protein bands increased (data not shown). In this case anti-phosphotyrosine antibody very likely co-precipitated phosphorylated proteins with non-phosphorylated ones and thereby led to increased signal on blot. Thus, precipitation of proteins with specific antibodies and subsequent analysis of their tyrosine staining on blot seemed to be a reliable method. In addition, the sensitivity of anti-phosphotyrosine antibodies to detect small amounts of tyrosine phosphorylation on blot varied. In our study, polyclonal anti-phosphotyrosine gave no or only a weak signal on blot, whereas monoclonal anti-phosphotyrosine clearly detected very low levels of phosphorylation.

5.4.2 Distribution of actin cytoskeleton (III)

To find out the behaviour of actin cytoskeleton during activation of Src kinase, the cells were stained with Alexa 568 phalloidin. At non-permissive temperature actin was at the lateral membranes and assembled in short stress fibres at basal surfaces of the cells. In 15 min at 35°C membraneous actin staining decreased and stress fibres started to gather on the basal surface of the cells. Large basal bundles of fibres were seen 30-120 min after shift to 35°C (in thesis; Fig. 5F, H and III; Fig. 2). Staining at lateral walls diminished gradually and finally vanished (III; Fig. 2). Hence, activation of Src stimulates polymerisation of actin to stress fibres.

5.5 Inhibition of Src blocks the epithelial-mesenchymal transition in src-MDCK cells (III)

Distribution of junctional proteins was also studied in cells treated with Src-specific inhibitor, PP2. The cells were pre-incubated with PP2 for 15 min at 40.5°C and thereafter 15 min to 2 hours at 35°C. In the presence of inhibitor the cells assumed the cubic morphology at 35°C. Cadherin, α- and β-catenin seemed to remain at lateral walls, but inhibitor was unable to prevent accumulation of p120ctn and actin on basal surface (III; Fig. 2). In addition, soluble p120ctn was still phosphorylated at 35°C (III; Fig. 3). The results show that activation of Src changes the morphology of the cells and induces disintegration of adherens junctions. Simultaneously, p120ctn becomes phosphorylated and is released from the lateral walls.

5.6 Activation of Rac signalling pathway accumulates cadherin on lateral membranes in Tiam1 src-MDCK cells (III)

Small GTPases of the Rho family play an important role in the control of actin cytoskeleton. Src family tyrosine kinases are part of the signalling pathway upstream from the Rho family (reviewed by Abram & Courtneidge 2000). To study the mutual relationships between small GTPases and Src kinase, the experiments were repeated with Tiam1-transfected ts-src-transfected MDCK cells. In these cells Rac GTPase is constitutively active and activation of Src depends on the ambient temperature. Contrary to src-transformed MDCK-cells, the morphology of Tiam1 src-MDCK cells remained practically normal after the shift to permissive temperature (III; Fig. 4 and in thesis; Fig. 5I-L). In these cells cadherin stayed at lateral membranes during time periods of 30 to 120 min at 35°C (III; Fig. 4 and in thesis; Fig. 5K, L). Similarly to src-MDCK cells, the number of actin filaments increased at basal surfaces (III; Fig. 4 and in thesis; Fig. 5J, L). Microinjection of inactive Rac into Tiam1 src-MDCK cells before their shift to 35°C gave a discontinuous staining pattern of cadherin and p120ctn at the lateral walls as well as clustering them to cytoplasm at 35°C (III; Fig. 4). Thus, activation of Rac by Tiam1 can antagonise Src-induced epithelial-mesenchymal transition by keeping cadherin at lateral membranes. However, it was unable to prevent formation of stress fibres.

5.7 Tiam1 promotes differentiation of src-MDCK cells in Matrigel (III)

Behaviour of src-MDCK cells varies according to their culture temperature and environment. In three-dimensional environment and at permissive temperature they form an irregular cluster in collagen I, invasive extensions in the mixture of laminin and collagen I, and a polarised cell cyst in Matrigel (Rahikkala *et al.* 2001). Tiam1 in turn induces invasion of lymphoma cells and suppresses invasion of Ras-transformed MDCK cells to collagen (Hordijk *et al.* 1997). In order to study the role of Tiam1 in invasion and

differentiation of the cells, Tiam1 src-MDCK cells were cultured in three-dimensional gels at non-permissive temperature where Src is inhibited but Tiam1 is active. In collagen I or in the laminin-collagen I-mixture the cells formed an irregular cluster with some signs of lumen but void of clear apico-basal axis, whereas in Matrigel they formed a perfect cell cyst with a clear lumen (III; Fig. 5). At permissive temperature the cells formed a non-differentiated cluster in all matrices. Since untransformed MDCK cells form a differentiated cyst also in collagen I we can conclude that Tiam1 sensitises MDCK cells to their environment and promotes differentiation and formation of apico-basal axis only in Matrigel composed of growth factors and basal lamina components. Ultrastructural analysis of the cell-cell contacts by immunoelectron microscopy supported the hypothesis. In cells grown in collagen at 40.5°C cadherin formed a loose adhesion between the cells without p120ctn that remained in cytoplasm (III; Fig. 6). Matrigel, in turn, promoted formation of well-developed and tight adhesion structure where both cadherin and p120ctn were present.

5.8 Serum starvation depolymerises actin in AGS cells (IV)

In many tissue diseases inflammation is a pre-stage to progression of cancer. Small GTPases play a central role during the process of infection since inflammatory bacteria use them as their down-stream effectors. We used human cultured gastric adenocarcinoma (AGS) cells to study the effects of H. pylori infection on actin cytoskeleton since they resemble epithelial cells and respond to serum starvation. In medium with serum they have stress fibres and some lamellipodia quite comparable to those in non-transformed fibroblasts, whereas serum starvation induced depolymerisation of actin (IV; Fig. 1). The phenomenon was reversible after the cells were returned to medium with serum. Thus, AGS cells seemed to be extremely sensitive to signals in serum affecting depolymerisation/polymerisation of actin.

5.9 Helicobacter pylori induces morphological changes and cytoskeletal rearrangements in AGS cells (IV)

Helicobacter pylori triggers inflammation of gastric cells and induces cytoskeletal changes, but the signalling mechanisms involved in these events are largely unknown. Infection studies with H. pylori were made with serum-starved AGS cells to ensure that the effects are solely due to the adherence of bacteria and not caused by a combined effect of bacteria and some components in the foetal calf serum. In H. pylori infected cells the lamellipodia-like structure developed especially on the edges of spread cells where bacteria were clustered. The phenomenon was time dependent and the best actin structures were obtained within 2 hours after infection (IV; Fig. 2). The significance of toxicity in the induction of cytoskeletal changes was analysed using cagA and toxin positive (cag A + vacA) or cagA and toxin negative (cag A – vacA) strains of H. pylori. Toxicity of bacterium was a nonessential factor for changes, since both toxin positive and

negative strains induced strong polymerisation of actin, including development of stress fibres and formation of a meshwork of actin around the cell edges (IV; Fig. 2) resembling lamellipodia in fibroblasts.

5.9.1 Actinomycin D prevents actin polymerisation in H. pylori-infected cells (IV)

The role of RNA and protein synthesis in the actin polymerisation process of H. pylori-treated cells was studied by inhibiting RNA synthesis with actinomycin D and protein synthesis with cycloheximide 15 minutes before H. pylori infection. Actinomycin D at a concentration of 2 μg/ml prevented actin polymerisation in H. pylori infected AGS cells and only some microvilli were seen (IV; Fig. 5). In more diluted solutions (1 and 0.2 μg/ml) some focal adhesions and stress fibres became visible. Instead, actin polymerisation was only partially prevented in cells treated with cycloheximide (10 μg/ml, IV; Fig. 5). The results give evidence that RNA synthesis is a prerequisite for induction of actin assemblies to H. pylori infected cells, and that new proteins are expressed during inflammation process.

5.9.2 Inactive Rac prevents H. pylori induced effects on actin assemblies (IV)

In Swiss 3T3 fibroblasts activation of Cdc42, Rac, and Rho leads to formation of filopodia, lamellipodia, membrane ruffles and stress fibres, respectively (Ridley & Hall 1992, Nobes *et al.* 1995). To find out the role of GTPases in H. pylori infection, inactive Rac and Cdc42 proteins were microinjected into the AGS cells before bacteria infection. Formation of H. pylori induced actin ruffles and lamellipodia-like network was clearly prevented by inactive Rac, and in some cells microvilli were seen as aggregates (IV; Fig. 3). In contrast, inactive Cdc42 could not prevent the formation of membrane ruffles and the cells were similar to controls. Hence, Rac is necessary for H. pylori induced effects.

5.10 Active Rac induces similar actin structures as H. pylori (IV)

In order to compare the effect of H. pylori to activation of Rac and Cdc42 in AGS cells, active Rac and Cdc42 were injected into serum-starved and antibiotics-depleted cells. In these cells Rac induced a very thin network of actin filaments and ruffles to the edges of the cells within 1 hour after microinjection, but the response was not as strong as with H. pylori. Cdc42 induced only small microspikes and spreading of the cells (IV; Fig. 4). H. pylori seems to stimulate the signalling cascade that activates Rac and leads to formation of lamellipodia-like network in serum-starved cells.

6 Discussion

Growth factor receptors (GFRs), particularly their tyrosine phosphorylated cytoplasmic domains are the key elements in transmission of extracellular signals to the appropriate sub-membrane locations of the cells. Under resting conditions different signalling molecules, such as Sos-1 (GEF for Ras), protein kinase C and small GTPases, Ras and Rac exist in an inactive state inside the cells. Binding of growth factor to the receptor induces immediate activation of several signalling cascades that trigger activation of small GTPases of the Rho family (reviewed by Scita *et al.* 2000). It is likely that Src family tyrosine kinases communicate with several downstream signalling molecules and adhesion components, i.e. PI3-kinase and p120ctn (reviewed by Thomas & Brugge 1997). For promotion of cytoskeletal changes via Rho family GTPases, Src uses its downstream effectors, GAPs and GEFs. Ras and Rho family of proteins are involved in signalling pathways that promote cell proliferation, motility, differentiation, transformation and apoptosis. In transformed cells many signalling pathways are constitutively active, resulting in alterations in the cell cytoskeleton, adhesions and proliferation. This thesis was designed to analyse the mechanisms that induce phenotypical changes of the epithelial cells during differentiation, morphogenesis, infection and tumourgenesis and the regulatory roles of vinculin, Rac GTPase and Src. The results are summarised in the following schematic drawing (Fig. 6) and discussed below.

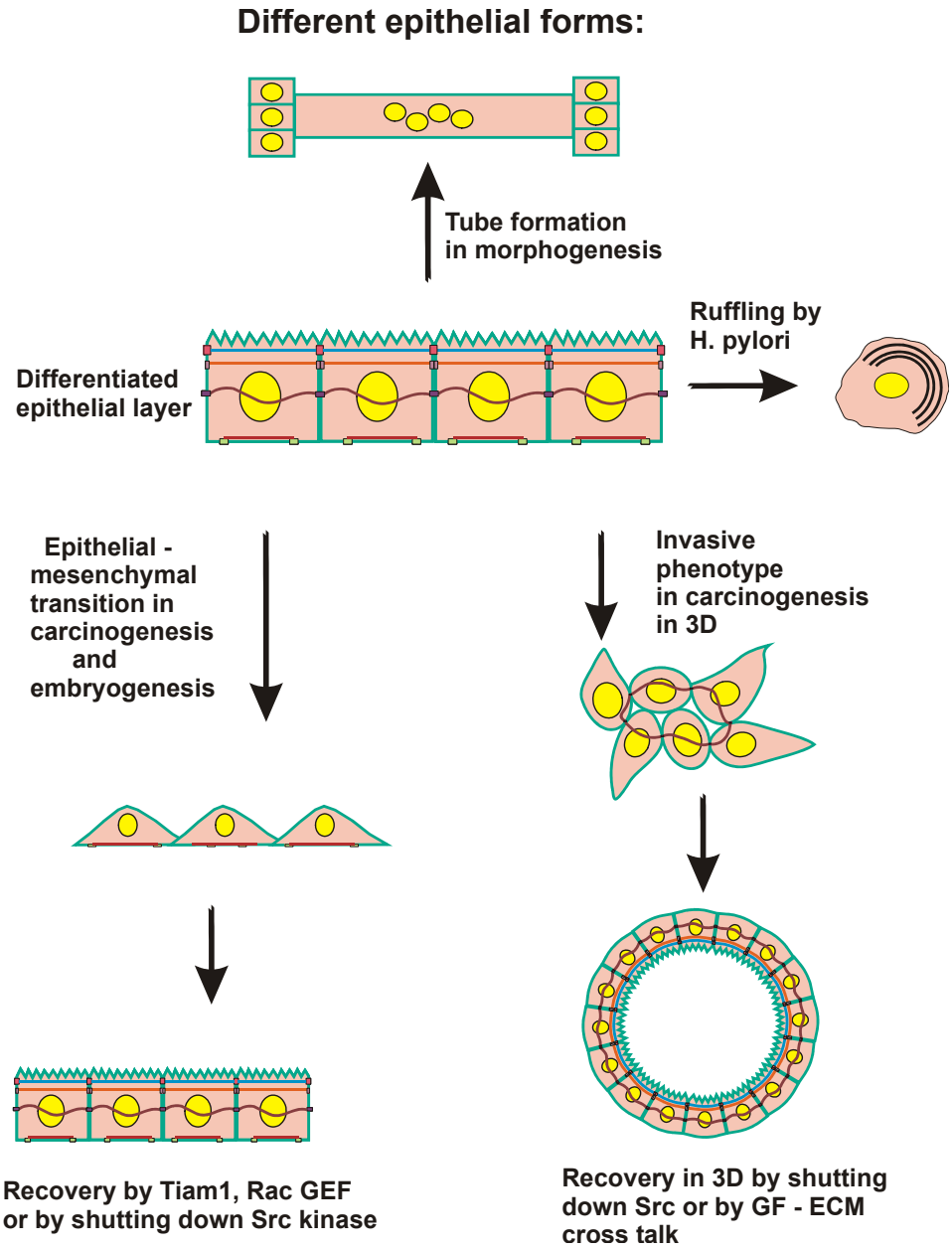

Fig. 6. Summary of morphological alterations in cultured epithelial cells under various stimuli.

6.1 Association of vinculin to adhesion complexes

Epithelial cells adhere to each other with intercellular junctions. Focal adhesions connect them to the extracellular matrix. In these structures numerous proteins form a network of interactions in which single proteins such as vinculin or β-catenin can build a structural complex with several proteins, but also function as a signalling component (reviewed by Yamada & Geiger 1997). Vinculin is an actin-binding protein that is composed of a head and tail domain. Besides actin, vinculin also associates with α-catenin (Weiss *et al.* 1998), acidic phospholipids (Johnson & Craig 1995b, Johnson *et al.* 1998), α-actinin (Belkin & Koteliansky 1987), ponsin (Mandai *et al.* 1999), talin (Otto 1983), VASP (Brindle *et al.* 1996, Hüttelmaier *et al.* 1998), paxillin (Turner *et al.* 1990), vinexin (Kioka *et al.* 1999) and raver 1 (Hüttelmaier *et al.* 2001). In cells vinculin exists both in soluble form and associated to the components of adhesion structures (Schlessinger & Geiger 1983). Its incorporation into adhesion sites is predominantly determined by the interactions with other proteins which in turn depend on conformation of vinculin (Ball *et al.* 1986, reviewed by Jockusch & Rüdiger 1996, reviewed by Rüdiger 1998). In the first and second work of the present PhD thesis we used microinjection to study the effect of excess amount of fluorophore-labelled vinculin on the existing adhesion structures of MDBK cells. Vinculin was used as a marker for adhesion complexes, but also for analysis of its interactions at cell-cell junctions. Vinculin incorporated rapidly into focal adhesions and adherens junctions of the cells showing its acceptance to native adhesion structures. The fluorophore-labelled α-actinin showed identical localisation (I). The behaviour of these proteins in MDBK cells has not been previously described. Instead, intact talin and its large domain have been microinjected into these cells. A large talin fragment, responsible for vinculin binding, was detected both at focal adhesions and adherens junctions. Intact talin localised only to focal adhesions (Nuckolls *et al.* 1990). Our injected vinculin incorporated into the lateral membranes close to the basal part of the cells and colocalised there partially with actin, cadherin, plakoglobin and desmoplakin. At the apical side of the cells, vinculin was seen in close contact with cadherin and plakoglobin but there was no colocalisation (II). Also apical actin was seen apart from vinculin. To find out the role of vinculin conformation in incorporation to adhesion structures we used vinculin head and tail, digested from vinculin with V8 protease. Vinculin head incorporated into focal adhesions and adherens junctions, but detached the cells from substratum within 30 minutes after injection, likely due to its efficient binding to talin at focal adhesions. Previously it has been shown that the talin-binding domain of vinculin head binds talin four times better than intact vinculin (Groesch & Otto 1990, Johnson & Craig 1994). On the other hand, the binding of vinculin to talin weakens the direct interaction between talin and integrin (Goldmann 2000). Since talin is linked to F-actin either directly (Schmidt *et al.* 1999) or via vinculin this association is also likely disrupted when excess vinculin or vinculin head is targeted to talin. There are, however, several other binding possibilities that may contribute to the detachment of cells from substratum, such as association of vinculin to α-actinin (Kroemker *et al.* 1994). The effect of the tail domain of vinculin remained obscure, most likely due to its rapid breakdown within the cells (Strasser *et al.* 1993).

The vinculin head domain also associated to adherens junctions that still remained intact. This suggests that the interacting partners of vinculin at adherens junctions differ from focal adhesions. The head domain of vinculin associates to adherens junctions by binding to tail domain of α-catenin (Weiss *et al.* 1998), which in turn forms a linkage between the cadherin, β-catenin and actin-based cytoskeleton. Besides α-catenin, vinculin interacts with α-actinin (Belkin & Koteliansky 1987) and ponsin (Mandai *et al.* 1999) at adherens junctions. In addition to protein interactions, vinculin associates to acidic phospholipids (Johnson & Craig 1995b, Johnson *et al.* 1998), such as phosphatidylinositol 4,5-bisphosphate, PIP_2, responsible for opening the vinculin molecule (Gilmore & Burridge 1996, Weekes *et al.* 1996, Bakolitsa *et al.* 1999). This is important for incorporation of vinculin into an adherens junction, since opening of the molecule reveals a site that is required for actin filament attachment (Johnson & Craig 1995a). In solution vinculin exists both in open and closed conformation (Eimer *et al.* 1993). Our studies proved that vinculin and vinculin head incorporated rapidly into adherens junctions of MDBK cells (I, II). The whole molecule lined the membranes even during disturbance of actin polymerisation with an inhibitor of PI3-kinase, wortmannin (I), as well as during acidification of intracellular pH that alters the lipid charges and vinculin conformation (II). This showed that incorporation of vinculin to adherens junctions was largely independent of its conformation.

6.2 Membrane potential and endogenous vinculin

We also analysed the behaviour of endogenous vinculin during manipulation of intracellular pH and membrane potential. We depolarised the cells by incubating them in nigericin-KCl buffer at pH 7.5 which weakened the junctional structures besides leading to disappearance of stress fibres and focal adhesions. Vinculin still associated to lateral walls, but staining was very faint. In hyperpolarised conditions the stress fibres and focal adhesions were clearly detectable. In these cells vinculin incorporated mainly into focal adhesions but faint lateral staining was also seen. The results showed that depolarised conditions have dramatic effects on actin cytoskeleton and junctional structures in MDBK cells. Whether vinculin reacted to depolarisation directly or whether it had lost its binding partners, remains to be shown.

Similar experiments have previously been made with MDCK cells originating from the distal tubulus of dog kidney. These cells behaved normally at neutral KCl buffer in the presence of nigericin, whereas lowering intracellular pH below 6.5 changed the cell morphology, increased the apical surface area and un-stabilised the lateral walls (Eskelinen *et al.* 1992). In these conditions basolateral spectrin and actin were translocated to cytoplasm. The origin of MDBK cells is not known, but they seem to be more sensitive to membrane potential and distorted function of K^+ and Na^+ transporters than MDCK cells.

6.3 Cell fusion

Cell-cell fusion is a central event during differentiation of the muscle, bone and trophoblast cells. Fusions also occur during embryogenesis and morphogenesis. In Drosophila, as in vertebrates, each muscle is a syncytium and arises from mesodermal cells by successive fusion (reviewed by Paululat *et al.* 1999). Differentiation of the bone cells proceeds through fusion of mononuclear osteoclast precursors to multinucleated cells (Mbalaviele *et al.* 1995). In placenta trophoblasts fuse to syncytiotrophoblasts (Getsios *et al.* 1998) and myotubes of skeletal muscle are formed by fusion of myoblasts (Kaufmann *et al.* 1999). In several organisms fusions of somatic cells are a prerequisite for the development of tissues and organs. This can be followed at cellular level in C. elegans, in which numerous epithelial fusions occur in the hypodermis, vulva, uterus, and excretory gland cells (Sulston *et al.* 1983). Fusion of the epithelial cells may cause rearrangements of the adherens junctions between the cells, and the spatial order of the cells to fuse is strictly determined (Podbilewicz & White 1994). Fusions of the epithelial tubes are required for development of tubular networks. Fusions of this kind exist in the mammalian vascular system and kidney as well as in the tracheal system of Drosophila (Samakovlis *et al.* 1996).

The specific contribution of vinculin to cell fusions has not been described in the literature, although it has an essential role in cellular processes that provide rapid disassembly and reassembly of adhesion structures. This is facilitated by its easy, reversible modulation of conformation. Vinculin, actin bundles, and α-actinin have been found in ring-like structures on the ventral surface of actively fused avian myoblasts (Hirayama *et al.* 2000), whereas m-calpain has been shown to modulate the cytoskeletal/plasma membrane interactions during fusions of rat satellite cells (Temm-Grove *et al.* 1999). In our experiments the surprising feature of microinjected vinculin was not only its incorporation into adherens junctions but also its ability to induce cell-cell fusions already 30 minutes after injection into MDBK cells (I, II). α-actinin behaved identically, although the fusions were smaller and their induction took a longer time. During fusions of the cells nuclei were accumulated into the centre of the fused cells (polykaryons) and short membrane fragments appeared in the cytoplasm. The existence of cadherin, β-catenin, plakoglobin, ZO-1, occludin, claudin and actin in these fragments showed that both adherens and tight junctions were fragmented to pieces. Microinjected vinculin surrounded the fused cells and was also localised to membrane remnants. We assume that microinjected vinculin caused an imbalance in the protein interactions at adherens junctions, leading to a controlled disintegration of the cell adhesion complex. The phenomenon was strictly local, since the neighbouring cells remained attached to each other. On the other hand, disintegration of adhesion structures might reveal the intrinsic fusogen(s) of the membrane. The fusions of MDBK cells have also been described in conditions in which the function of fodrin has been blocked with anti-fodrin antibodies or by activating all intracellular GTPases with GTPγS or inactivating intracellular signalling pathways by EGTA (Eskelinen & Lehto 1994). Since fodrin is linked to ZO-1 (Itoh *et al.* 1991) and some of Rab GTPases are localised to tight junctions (Zahraoui *et al.* 1994), one may speculate that tight junctions may be involved in the fusion process of these cells.

Interestingly, the vinculin-induced fusions were prevented when the intracellular pH of the cells was lowered to 5.5 with nigericin-KCl treatment. In this condition microinjected vinculin and endogenous cadherin lined the membranes of the individual cells (II). There are several alternatives as to why vinculin was unable to induce fusions of MDBK cells at low pH. Firstly, endocytosis and intracellular transport of the cells are inhibited (Cosson *et al.* 1989, Eskelinen *et al.* 1991). The association between Hakai protein and E-cadherin has been shown to enhance endocytosis of cadherin and thereby disruption of cell-cell contacts (Fujita *et al.* 2002). The protein complex is likely packed to clathrin-coated pits that as uncoated fuse to early endosomes and thereafter E-cadherin is either transported back to cell surface or degraded in lysosomes. The second alternative is alterations of lipid conformation that affect vinculin interactions at plasma membrane. Third, low pH affects vinculin conformation, which may explain the phenomenon (Miller & Ball 2001). The fact that fusion was also inhibited in depolarised conditions favours the lipids and lipid-vinculin interactions as the key player in fusions.

6.4 Targets of Src in cell adhesion sites

Src kinase is associated to adherens junctions and focal adhesions of epithelial cells (Tsukita *et al.* 1991). Hence, one can expect that it participates in the cell adhesion. The characteristic features of transformed epithelial cells are their fibroblastic or mesenchymal morphology and altered cell adhesion (reviewed by Frame *et al.* 2002). MDCK cells transformed by a temperature-sensitive v-Src tyrosine kinase are widely used as a model for adhesion and phosphorylation studies. Activation of Src kinase can be triggered by shifting the cells from the restrictive (40.5^{o}C) to the permissive temperature (35^{o}C, Behrens *et al.* 1993). In src-transformed MDCK cells cell-cell adhesion, especially the cadherin-based adhesion, is weakened, probably due to increased phosphorylations of junctional proteins that might also enhance their solubility (Behrens *et al.* 1993, Takeda *et al.* 1995, Takeda & Tsukita 1995). Activated v-Src kinase phosphorylates its protein substrates exclusively on tyrosine residues (Hunter & Sefton 1980). Most of them, found at focal adhesions, are the key components in the integrin-mediated signal transduction and bound to actin or integrin, such as vinculin, cortactin, talin, paxillin, FAK, tensin, ezrin and p130cas. Furthermore, Src-kinase induces tyrosine phosphorylation of junctional proteins, such as β- and γ-catenin, p120ctn, ZO-1, occludin, connexin 43 and nectin-2 delta (reviewed by Parsons & Parsons 1997, reviewed by Thomas & Brugge 1997, Tsukamoto & Nigam 1999, reviewed by Abram & Courtneidge 2000, Lin *et al.* 2001, Kikyo *et al.* 2000). The other known targets of Src are enzymes involved in phospholipid metabolism, such as PLC-γ, p85 subunit of PI3-kinase and the signalling molecules p190RhoGAP, p120rasGAP and EGF receptor substrate, Eps8 (reviewed by Thomas & Brugge 1997, reviewed by Abram & Courtneidge 2000, Gallo *et al.* 1997). In our studies we followed the sequential release of cell-cell adhesion components in MDCK cells and tried to find correlations to their phosphorylation at different time periods after activation of v-Src at 35^{o}C (III). Within 15 min at 35^{o}C, the cell-cell contacts opened and the components of E-cadherin complex released from the

lateral walls onto basal surfaces, whereas the tight junctional components, especially claudin, kept their position. Our results are in agreement with the observations made with vanadate-treated MDCK cells, in which inhibition of phosphotyrosine phosphatases affected directly adherens junctions and associated actin, whereas the effects to desmosomes and tight junctions were insignificant (Volberg *et al.* 1992, Collares-Buzato *et al.* 1998). We selected E-cadherin, β-catenin, p120ctn, Eps8 and ZO-1 for our tyrosine phosphorylation studies of src-MDCK cells since these proteins, except for E-cadherin, have been identified as Src substrates and have an important role in cell adhesion. In addition, the roles of β-catenin, p120ctn and E-cadherin in the integrity of adherens junctions have been studied in many connections. Activation of Src clearly enhanced tyrosine phosphorylation of soluble p120ctn and ZO-1 but only slightly that of Eps8. Phosphorylation of β-catenin was difficult to analyse since it turned out to be already phosphorylated at non-permissive temperature. Hence, the straight connection between elevated phosphorylation of proteins and their simultaneous redistribution was valid only in the case of soluble p120ctn, which phosphorylated and translocated from lateral onto basal surfaces at the same time interval. However, p120ctn behaved similarly in the presence of Src inhibitor, PP2. This indicates that Src is not maximally inhibited by PP2 and/or other kinases are responsible for phosphorylation of p120ctn at 35^{o}C.

6.5 Regulatory role of p120ctn at adherens junctions

p120ctn (or p120cas) was originally found in transformed cells as a major substrate of Src kinase (Kanner *et al.* 1991). In adherens junction p120ctn associates directly with the cytoplasmic tail of E-cadherin (Finnemann *et al.* 1997). In src-transformed cells p120ctn is tyrosine-, serine -and threonine phosphorylated (Kanner *et al.* 1991). Src tyrosine kinase phosphorylates eight tyrosine residues of p120ctn (Mariner *et al.* 2001), but several growth factors also induce its tyrosine phosphorylation (Downing & Reynolds 1991, Kanner *et al.* 1991).

The exact function of p120ctn is not known. Its existence in cadherin complex may either strengthen or weaken the adhesion. The mutations in p120ctn binding site of E-cadherin cause uncoupling of cadherin-p120ctn interaction and formation of weak adhesion. On the other hand, aggregated cells, expressing p120-uncoupled cadherins dissociate readily from each other and as a consequence the insertion of actin cytoskeleton to E-cadherin plaque is blocked (Thoreson *et al.* 2000). Thus, the p120ctn binding region of E-cadherin and p120ctn play a central role in cadherin clustering and induction of strong adhesion (Thoreson *et al.* 2000, Anastasiadis & Reynolds 2000). Phosphorylation of p120ctn is in turn connected to disassembly of adherens junctions. For instance, in Ras-transformed breast epithelial cells (MCF) high tyrosine phosphorylation of p120ctn increases its association to E-cadherin in cytoplasm and cell-cell adhesion is weakened (Kinch *et al.* 1995). We found that inhibition of Src by PP2 restored the epithelial phenotype of the cells, but p120ctn remained phosphorylated in cytoplasm. Thus it was not a critical component for epithelial morphology. In Matrigel, well-polarised Tiam1 src-MDCK cells had mature adherens junctions where both p120ctn and cadherin were

accumulated at the junction site. Hence, it seems that when the cell-cell contact sites have time to develop both proteins are present there. We assume that phosphorylation of p120ctn may have a role in the signalling pathway regulating the activity of Rho, but the mechanisms are outside the scope of this work. In this respect it is noteworth that in the presence of PP2 p120ctn remained phosphorylated and stress fibres were present. The mechanism of how Src induced disintegration of adherens junctions remained unresolved, but it is likely that small GTPases play a role.

6.6 Small GTPases in cell adhesion

Rho GTPases including RhoA, Rac1 and Cdc42 are the key regulators of cadherin-based cell-cell adhesion. Rac GTPase has a central role in the maintenance of cell adhesion in epithelial cells since when activated it increases the accumulation of actin filaments and cadherin to lateral walls of cells (Takaishi *et al.* 1997, Yasuda *et al.* 2000). A target of Cdc42 and Rac1 small GTPases, IQGAP1, binds directly to β-catenin and negatively regulates E-cadherin-mediated cell-cell adhesion by dissociating α-catenin from the cadherin-catenin complex *in vivo*. Binding of active Rac to IQGAP1 in turn releases β-catenin from the complex and allows its association to α-catenin (Kuroda *et al.* 1998, Fig. 7). In epithelial cells the mechanisms that shuttle Rac to membranes are largely unknown.

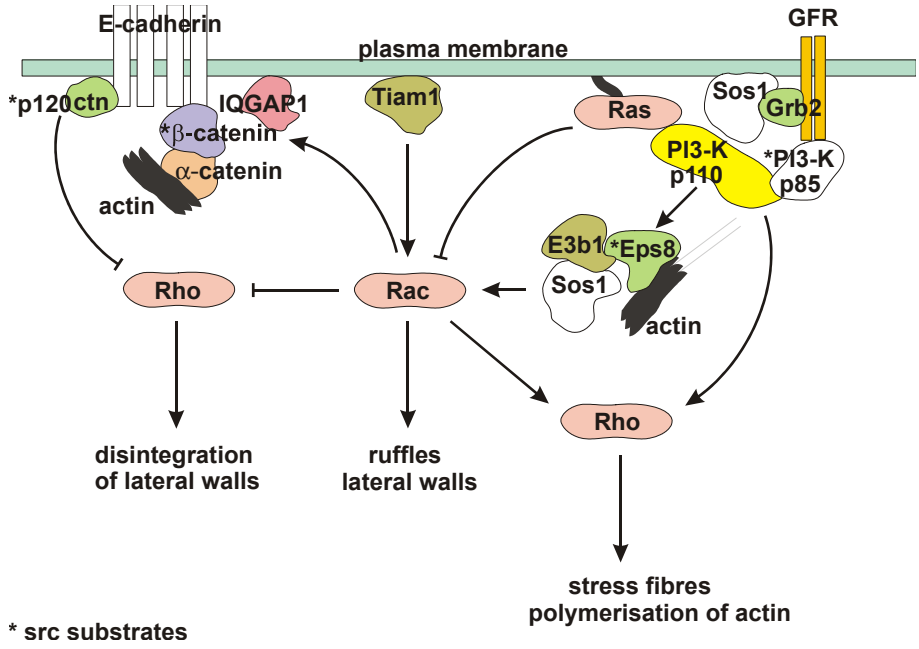

Fig. 7. Signalling pathways from growth factor receptor (GFR), IQGAP1 and Tiam1 to small GTPases of the Rho family.

Rac1 is recruited to cell-cell contact sites together with E-cadherin, and formation of adhesion induces activation of Rac1 (Nakagawa *et al.* 2001). Disruption of cadherin-based adhesion by Ca^{2+} chelation leads to translocation of Rac and E-cadherin to cytosol. Our experiments indicated that active Rac GTPase induced accumulation of cadherin to the lateral walls in vinculin-injected MDBK cells and thereby diminished the formation of polykaryons (I, II). Hence, active Rac GTPase clearly strengthened the assembly of cadherin-based adhesion. Inactive Rac had an opposite effect on cadherin recruitment, but injected vinculin still remained at lateral membranes, supporting the view that at least part of vinculin associates directly to lipids.

We also studied the role of Rac GTPase in the tranformation process by using src-MDCK cells double transfected with Rac GEF, Tiam1 (III). In these cells Rac GTPase is always activated due to Tiam1. The effecs of Tiam1 are cell-specific so that it might prevent or increase migration of the cells as well as promote or suppress the invasion of the cells (Sander *et al.* 1998, Michiels *et al.* 1995). In our experiments Tiam1 increased accumulation of cadherin to the membrane at permissive temperature. Similar to src-MDCK cells, stress fibres were formed at basal surface of these cells, suggesting that Rho GTPase is activated in both cell lines after Src activation independently of the activation status of Rac.

6.7 Small GTPases and actin cytoskeleton

Rho GTPases regulate the actin cytoskeleton during cell spreading, chemotaxis, or invasion (reviewed by Hall *et al.* 1998). Tyrosine kinases, in turn, are their upstream modulators (reviewed by Abram & Courtneidge 2000). We followed the behaviour of actin in src-MDCK cells at non-permissive and permissive temperature (III). Activation of Src kinase clearly promoted depolymerisation of actin at the lateral membranes and bundling of stress fibres on basal surface of the cells. As a result the cells were flattened. The appearance of prominent bundles of stress fibres to the basal surface gave evidence that Rho GTPase is activated in these cells followed by activation of Src. It is known that p120ctn is able to inhibit Rho activity (reviewed by Anastasiadis & Reynolds 2001). We speculate that in MDCK cells p120ctn in its phosphorylated form may lose its inhibitory effect on Rho and in this way promote formation of stress fibres. On the other hand, a potent Rho regulator protein, p190RhoGAP (GTPase-activating protein), reorganises actin and is a Src substrate (Brouns *et al.* 2001). However, its activation should result in loss of stress fibres instead of their formation. Therefore, it is an unlikely functional target of Src in MDCK cells. We believe that the primary targets of active Src are signalling proteins that regulate lateral actin through depolymerisation and bundling of stress fibres on basal surface of the cells.

We studied the mutual relationships of Src, Rac and Rho GTPases in Tiam1 src-MDCK cells. Expression of Rac GEF, Tiam1 changed the morphology of src-MDCK cells more epithelial-like and promoted accumulation of cadherin on the lateral walls during activation of Src. Tiam1 also modulates the behaviour of the cells by inhibition or promotion of invasion (Michiels *et al.* 1995, Hordijk *et al.* 1997, Sander *et al.* 1998). We studied the

behaviour of Tiam1 src-MDCK cells in three-dimensional environment at non-permissive temperature (III). The results showed that the cells grown in collagen I had non-polarised phenotype, whereas in Matrigel they were differentiated. The ultrastructural analysis of the cells by immunoelectron microscopy revealed that cadherin was a critical component in the formation of cell-cell contact both in collagen and Matrigel. p120ctn in turn was present at cell-cell contact sites only in Matrigel. This suggests that p120ctn is required for stabilisation of cadherin-based adhesion in 3D environments. Moreover, we suggest that soluble growth factors of the Matrigel or the cross talk between adhesion proteins, such as cadherin and integrins, promote the polarity of Tiam1 src-MDCK cells.

6.8 Small GTPases and inflammation

The morphological changes of the cells and cytoskeletal rearrangements are typical events during bacterial infection. However, the signalling transduction pathways that are activated after attachment of bacterium to the receptor(s) are poorly known. Helicobacter pylori (H. pylori), a once-ubiquitous coloniser of the human stomach, is a causative agent of chronic active gastritis, peptic ulcer and gastric cancer (reviewed by Blaser 1990). Adherence of H. pylori to the gastric epithelium and secretion of interleukins are believed to be an important step in the induction of active inflammation of the mucosal layer. We used H. pylori infected gastric adenocarcinoma (AGS) cells as a model to study the response of infection on actin cytoskeleton (IV). In the literature H. pylori has had either no effects on gastric cells (Dytoc *et al.* 1993, Ismaili *et al.* 1995), or it has induced actin polymerisation at the attachment site (Smoot *et al.* 1993, Segal *et al.* 1996). The discrepancies might be explained by the variable conditions used in experiments. The presence of serum components leads to activation of small GTPases and thereby hampers analysis of the effect of H. pylori (Ridley & Hall 1992). On the other hand, the culture medium without foetal serum increases accumulation of actin beneath adhesion sites of H. pylori, whereas serum proteins mediate the entry of H. pylori into AGS cells (Petersen *et al.* 2000). Since bacterial toxins use small GTPases as targets (Lerm *et al.* 1999) and induce their activation (reviewed by Mackay & Hall 1998), we were interested in analysing the role of GTPase signalling in Helicobacter pylori infected AGS cells. We found that in H. pylori infected and serum-starved cells actin was polymerised into lamellipodia-like structures and membrane ruffles at the edge of the cells. The assemblies were similar to those obtained with microinjected active Rac. We propose that the Rac signalling pathway is involved in the reorganisation of actin in H. pylori infected cells. Churin and co-workers (2001) have made an identical observation, but in their work also Cdc42 was activated. In fact, we also found that infected cells formed small microspikes and the cells were spread. These signs refer to activation of Cdc42. Rac1 is also involved in the regulation of VacA activity, a cytotoxin, secreted by H. pylori and responsible for the formation of acid vacuoles inside the infected cells (Hotchin *et al.* 2000). Expression of dominant negative Rac1 inhibits vacuole formation, whereas active Rac1 promotes it.

We found that both cagA toxin positive (cagA + vacA) and cagA toxin negative (cagA – vacA) strains of H. pylori induced polymerisation of actin. However, actin polymerisation

by active Rac was not as strong as with H. pylori. This suggests that other factors are also included in the infection process. We studied the role of gene expression and protein synthesis by inhibiting RNA synthesis with actinomycin and protein syntesis with cycloheximide during bacterium infection. Actinomycin clearly blocked the formation of lamellipodia-like structure in these cells, showing that both gene expression and protein synthesis are linked to reorganisation of actin filaments. In fact, Chiou and co-workers (2001) have identified 21 highly expressed genes in H. pylori infected AGS cells, including transcription factors such as c-jun and the genes involved in signal transduction pathways, such as mitogen-activated protein kinase (MAP kinase), interleukin 5 and insulin-like growth factor. Moreover, the genes that regulate cell cycle and differentiation were also involved. Besides Cdc42 and Rho, Rac induces efficient activation of transcription factors, such as nuclear factor, NF-κB that may increase the induction of inflammatory genes. Recent studies have demonstrated that CagA changes the morphology of infected cells and is phosphorylated by c-Src and Lyn kinases inside the host (Stein *et al.* 2002). This may in turn lead to changes in actin cytoskeleton, increased motility and elongation of the cells. Thus, CagA likely uses Rac GTPase for induction of these responses. The small GTPases might have a crucial role in the development of ulcer and consequently gastric carcinoma.

7 Summary and conclusions

The present study was designed to analyse the regulatory factors of cell-cell adhesion and actin cytoskeleton in non-transformed and transformed epithelial cells. The experiments were made with fully polarised kidney epithelial cells (MDBK), src-transformed and Tiam1 src-transformed MDCK cells and gastric adenocarcinoma (AGS) cells.

In the first and second part of the thesis work the aim was to find out the role of vinculin, α-actinin and Rac GTPase in the regulation of adherens junctions of MDBK cells. The cell-cell adhesion of MDBK cells seemed to be a very dynamic structure in which microinjected vinculin not only functioned as a structural component of the cells, but was able to disintegrate the cell-cell contact sites in a way which ultimately led to cell fusions. Vinculin was tightly bound to its target since it remained at lateral membranes even after cadherin, plakoglobin and actin were clustered into cytoplasm of the cells. Injection of active Rac GTPase reduced formation of polykaryons and strengthened the cell-cell adhesion by accumulating cadherin onto adhesion sites. Inhibition of endocytosis and alteration of membrane potential with low pH or KCl-nigericin treatment prevented cell fusion, but not vinculin-membrane association. Due to its responsiveness to lipids and multiple interacting partners, vinculin may be a key regulatory component of cell-cell adhesion during embryogenesis when cellular junctions are formed and disintegrated in a controlled manner. In favourable circumstances the cells fuse.

In carcinomas cell-cell junctions are also disrupted and in this context phosphorylation of proteins has a central role. We used src-transformed MDCK cells for identification of the targets of Src kinase at cell junctions. The instability of cell-cell contact in Src activated cells was clearly connected to increased and rapid release of adherens junctional components from lateral walls to basal surface of the cells. Still, only phosphorylation of p120ctn correlated with its release from membranes. Inhibition of Src by a specific inhibitor PP2 restored cubic morphology of MDCK cells and kept cadherin, α- and β-catenin at lateral membranes. However, p120ctn was phosphorylated and remained in cytoplasm. Hence, it was not necessary for restoration of the epithelial morphology. Active Src promoted formation of stress fibres to the basal surface of the cells, suggesting that Src is an upstream regulator of Rho GTPase. Expression of Rac GEF, Tiam1, in these cells accumulated cadherin back to membranes and restored cubic morphology. This showed the ability of Rac GTPase to antagonise the Src-induced epithelial-mesenchymal transition.

Hence, Rac GTPase and cadherin seem to control the maintenance of epithelial morphology, whereas phosphorylated p120ctn and Rho GTPase are involved in mesenchymal transition of the cells.

The significance of small GTPases was also studied during infection of gastric AGS cells with H. pylori. This bacterium, when attached to cultured AGS cells, changed the morphology and actin cytoskeleton of the cells in the same way as injected active Rac GTPase. As a result lamellipodia-like structures were formed on the edges of infected cells. Whether activation of Rac GTPase has a role in the progression of gastric ulcer *in vivo*, remains to be shown.

In summary, the results show that in epithelial cells there is a very delicate balance between cell adhesion on one hand and reformation of tissues on the other. In the maintenance of this balance, cytoskeletal elements such as actin, cadherin and vinculin, small GTPases such as Rac and various tyrosine kinases play a central role.

8 References

Abercrombie M, Heaysman JE & Pegrum SM (1971) The locomotion of fibroblasts in culture. IV. Electron microscopy of the leading lamella. Exp Cell Res 67: 359-367.

Abram CL & Courtneidge SA (2000) Src family tyrosine kinases and growth factor signaling. Exp Cell Res 254: 1-13.

Akhurst RJ & Derynck R (2001) TGF-beta signaling in cancer--a double-edged sword. Trends Cell Biol 11: S44-S51.

Aktories K (1997) Rho proteins: targets for bacterial toxins. Trends Microbiol 5: 282-288.

Amano M, Chihara K, Kimura K, Fukata Y, Nakamura N, Matsuura Y & Kaibuchi K (1997) Formation of actin stress fibers and focal adhesions enhanced by Rho- kinase. Science 275: 1308-1311.

Anastasiadis PZ, Moon SY, Thoreson MA, Mariner DJ, Crawford HC, Zheng Y & Reynolds AB (2000) Inhibition of RhoA by p120 catenin. Nat Cell Biol 2: 637-644.

Anastasiadis PZ & Reynolds AB (2000) The p120 catenin family: complex roles in adhesion, signaling and cancer. J Cell Sci 113 (Pt 8): 1319-1334.

Anastasiadis PZ & Reynolds AB (2001) Regulation of Rho GTPases by p120-catenin. Curr Opin Cell Biol 13: 604-610.

Anderson JM, Balda MS & Fanning AS (1993) The structure and regulation of tight junctions. Curr Opin Cell Biol 5: 772-778.

Anderson WB, Estival A, Tapiovaara H & Gopalakrishna R (1985) Altered subcellular distribution of protein kinase C (a phorbol ester receptor). Possible role in tumor promotion and the regulation of cell growth: relationship to changes in adenylate cyclase activity. Adv Cyclic Nucleotide Protein Phosphorylation Res 19: 287-306.

Ando-Akatsuka Y, Yonemura S, Itoh M, Furuse M & Tsukita S (1999) Differential behavior of E-cadherin and occludin in their colocalization with ZO-1 during the establishment of epithelial cell polarity. J Cell Physiol 179: 115-125.

Arpin M, Algrain M & Louvard D (1994) Membrane-actin microfilament connections: an increasing diversity of players related to band 4.1. Curr Opin Cell Biol 6: 136-141.

Asakura T, Nakanishi H, Sakisaka T, Takahashi K, Mandai K, Nishimura M, Sasaki T & Takai Y (1999) Similar and differential behaviour between the nectin-afadin-ponsin and cadherin-catenin systems during the formation and disruption of the polarized junctional alignment in epithelial cells. Genes Cells 4: 573-581.

Aspenström P (1999) Effectors for the Rho GTPases. Curr Opin Cell Biol 11: 95-102.

Bacallao R, Antony C, Dotti C, Karsenti E, Stelzer EH & Simons K (1989) The subcellular organization of Madin-Darby canine kidney cells during the formation of a polarized epithelium. J Cell Biol 109: 2817-2832.

Bagrodia S, Derijard B, Davis RJ & Cerione RA (1995) Cdc42 and PAK-mediated signaling leads to Jun kinase and p38 mitogen- activated protein kinase activation. J Biol Chem 270: 27995-27998.

Bakolitsa C, de Pereda JM, Bagshaw CR, Critchley DR & Liddington RC (1999) Crystal structure of the vinculin tail suggests a pathway for activation. Cell 99: 603-613.

Balda MS & Anderson JM (1993) Two classes of tight junctions are revealed by ZO-1 isoforms. Am J Physiol 264: C918-C924.

Balda MS & Matter K (1998) Tight junctions. J Cell Sci 111 (Pt 5): 541-547.

Ball EH, Freitag C & Gurofsky S (1986) Vinculin interaction with permeabilized cells: disruption and reconstitution of a binding site. J Cell Biol 103: 641-648.

Barbacid M (1987) ras genes. Annu Rev Biochem 56: 779-827.

Barry ST & Critchley DR (1994) The RhoA-dependent assembly of focal adhesions in Swiss 3T3 cells is associated with increased tyrosine phosphorylation and the recruitment of both pp125FAK and protein kinase C-delta to focal adhesions. J Cell Sci 107 (Pt 7): 2033-2045.

Barstead RJ & Waterston RH (1991) Vinculin is essential for muscle function in the nematode. J Cell Biol 114: 715-724.

Beck KA, Buchanan JA, Malhotra V & Nelson WJ (1994) Golgi spectrin: identification of an erythroid beta-spectrin homolog associated with the Golgi complex. J Cell Biol 127: 707-723.

Beckerle MC, Burridge K, DeMartino GN & Croall DE (1987) Colocalization of calcium-dependent protease II and one of its substrates at sites of cell adhesion. Cell 51: 569-577.

Behrens J, Vakaet L, Friis R, Winterhager E, Van Roy F, Mareel MM & Birchmeier W (1993) Loss of epithelial differentiation and gain of invasiveness correlates with tyrosine phosphorylation of the E-cadherin/beta-catenin complex in cells transformed with a temperature-sensitive v-SRC gene. J Cell Biol 120: 757-766.

Behrens J, von Kries JP, Kuhl M, Bruhn L, Wedlich D, Grosschedl R & Birchmeier W (1996) Functional interaction of beta-catenin with the transcription factor LEF-1. Nature 382: 638-642.

Belkin AM & Koteliansky VE (1987) Interaction of iodinated vinculin, metavinculin and alpha-actinin with cytoskeletal proteins. FEBS Lett 220: 291-294.

Bendori R, Salomon D & Geiger B (1989) Identification of two distinct functional domains on vinculin involved in its association with focal contacts. J Cell Biol 108: 2383-2393.

Bennett MK, Wandinger-Ness A & Simons K (1988) Release of putative exocytic transport vesicles from perforated MDCK cells. EMBO J 7: 4075-4085.

Bennett MV, Barrio LC, Bargiello TA, Spray DC, Hertzberg E & Sáez JC (1991) Gap junctions: new tools, new answers, new questions. Neuron 6: 305-320.

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

Bennett V & Gilligan DM (1993) The spectrin-based membrane skeleton and micron-scale organization of the plasma membrane. Annu Rev Cell Biol 9: 27-66.

Berryman M, Franck Z & Bretscher A (1993) Ezrin is concentrated in the apical microvilli of a wide variety of epithelial cells whereas moesin is found primarily in endothelial cells. J Cell Sci 105 (Pt 4): 1025-1043.

Beyer EC, Paul DL & Goodenough DA (1990) Connexin family of gap junction proteins. J Membr Biol 116: 187-194.

Bi E & Zigmond SH (1999) Actin polymerization: Where the WASP stings. Curr Biol 9: R160-R163.

Birchmeier C & Birchmeier W (1993) Molecular aspects of mesenchymal-epithelial interactions. Annu Rev Cell Biol 9: 511-540.

Birchmeier W & Behrens J (1994) Cadherin expression in carcinomas: role in the formation of cell junctions and the prevention of invasiveness. Biochim Biophys Acta 1198: 11-26.

Blanchard A, Ohanian V & Critchley D (1989) The structure and function of alpha-actinin. J Muscle Res Cell Motil 10: 280-289.

Blaser MJ (1990) Helicobacter pylori and the pathogenesis of gastroduodenal inflammation. J Infect Dis 161: 626-633.

Borrmann CM, Mertens C, Schmidt A, Langbein L, Kuhn C & Franke WW (2000) Molecular diversity of plaques of epithelial-adhering junctions. Ann N Y Acad Sci 915: 144-150.

Brady-Kalnay SM, Rimm DL & Tonks NK (1995) Receptor protein tyrosine phosphatase PTPmu associates with cadherins and catenins in vivo. J Cell Biol 130: 977-986.

Braga VM, Betson M, Li X & Lamarche-Vane N (2000) Activation of the small GTPase Rac is sufficient to disrupt cadherin- dependent cell-cell adhesion in normal human keratinocytes. Mol Biol Cell 11: 3703-3721.

Braga VM, Del Maschio A, Machesky L & Dejana E (1999) Regulation of cadherin function by Rho and Rac: modulation by junction maturation and cellular context. Mol Biol Cell 10: 9-22.

Braga VM, Machesky LM, Hall A & Hotchin NA (1997) The small GTPases Rho and Rac are required for the establishment of cadherin-dependent cell-cell contacts. J Cell Biol 137: 1421-1431.

Breitfeld PP, McKinnon WC & Mostov KE (1990) Effect of nocodazole on vesicular traffic to the apical and basolateral surfaces of polarized MDCK cells. J Cell Biol 111: 2365-2373.

Bretscher A, Reczek D & Berryman M (1997) Ezrin: a protein requiring conformational activation to link microfilaments to the plasma membrane in the assembly of cell surface structures. J Cell Sci 110 (Pt 24): 3011-3018.

Brindle NP, Holt MR, Davies JE, Price CJ & Critchley DR (1996) The focal-adhesion vasodilator-stimulated phosphoprotein (VASP) binds to the proline-rich domain in vinculin. Biochem J 318 (Pt 3): 753-757.

Brouns MR, Matheson SF & Settleman J (2001) p190 RhoGAP is the principal Src substrate in brain and regulates axon outgrowth, guidance and fasciculation. Nat Cell Biol 3: 361-367.

Brown D & Stow JL (1996) Protein trafficking and polarity in kidney epithelium: from cell biology to physiology. Physiol Rev 76: 245-297.

Brown MT & Cooper JA (1996) Regulation, substrates and functions of src. Biochim Biophys Acta 1287: 121-149.

Brugge JS & Erikson RL (1977) Identification of a transformation-specific antigen induced by an avian sarcoma virus. Nature 269: 346-348.

Burridge K & Chrzanowska-Wodnicka M (1996) Focal adhesions, contractility, and signaling. Annu Rev Cell Dev Biol 12: 463-518.

Burridge K, Fath K, Kelly T, Nuckolls G & Turner C (1988) Focal adhesions: transmembrane junctions between the extracellular matrix and the cytoskeleton. Annu Rev Cell Biol 4: 487-525.

Burridge K & Feramisco JR (1980) Microinjection and localization of a 130K protein in living fibroblasts: a relationship to actin and fibronectin. Cell 19: 587-595.

Burridge K, Turner CE & Romer LH (1992) Tyrosine phosphorylation of paxillin and pp125FAK accompanies cell adhesion to extracellular matrix: a role in cytoskeletal assembly. J Cell Biol 119: 893-903.

Brändli AW, Parton RG & Simons K (1990) Transcytosis in MDCK cells: identification of glycoproteins transported bidirectionally between both plasma membrane domains. J Cell Biol 111: 2909-2921.

Carlier MF (1998) Control of actin dynamics. Curr Opin Cell Biol 10: 45-51.

Carlier MF, Ducruix A & Pantaloni D (1999) Signalling to actin: the Cdc42-N-WASP-Arp2/3 connection. Chem Biol 6: R235-R240.

Carpenter CL, Tolias KF, Couvillon AC & Hartwig JH (1997) Signal transduction pathways involving the small G proteins rac and Cdc42 and phosphoinositide kinases. Adv Enzyme Regul 37: 377-390.

Carraway KL & Carraway CA (1989) Membrane-cytoskeleton interactions in animal cells. Biochim Biophys Acta 988: 147-171.

Cary LA, Klinghoffer RA, Sachsenmaier C & Cooper JA (2002) SRC catalytic but not scaffolding function is needed for integrin- regulated tyrosine phosphorylation, cell migration, and cell spreading. Mol Cell Biol 22: 2427-2440.

Castresana J & Saraste M (1995) Does Vav bind to F-actin through a CH domain? FEBS Lett 374: 149-151.

Chen Y, Lu Q, Schneeberger EE & Goodenough DA (2000) Restoration of tight junction structure and barrier function by down- regulation of the mitogen-activated protein kinase pathway in ras-transformed Madin-Darby canine kidney cells. Mol Biol Cell 11: 849-862.

Chiou CC, Chan CC, Sheu DL, Chen KT, Li YS & Chan EC (2001) Helicobacter pylori infection induced alteration of gene expression in human gastric cells. Gut 48: 598-604.

Chou YH, Rosevear E & Goldman RD (1989) Phosphorylation and disassembly of intermediate filaments in mitotic cells. Proc Natl Acad Sci U S A 86: 1885-1889.

Chrzanowska-Wodnicka M & Burridge K (1994) Tyrosine phosphorylation is involved in reorganization of the actin cytoskeleton in response to serum or LPA stimulation. J Cell Sci 107 (Pt 12): 3643-3654.

Chrzanowska-Wodnicka M & Burridge K (1996) Rho-stimulated contractility drives the formation of stress fibers and focal adhesions. J Cell Biol 133: 1403-1415.

Chuang TH, Hahn KM, Lee JD, Danley DE & Bokoch GM (1997) The small GTPase Cdc42 initiates an apoptotic signaling pathway in Jurkat T lymphocytes. Mol Biol Cell 8: 1687-1698.

Churin Y, Kardalinou E, Meyer TF & Naumann M (2001) Pathogenicity island-dependent activation of Rho GTPases Rac1 and Cdc42 in Helicobacter pylori infection. Mol Microbiol 40: 815-823.

Clausen H & Hakomori S (1989) ABH and related histo-blood group antigens; immunochemical differences in carrier isotypes and their distribution. Vox Sang 56: 1-20.

Collares-Buzato CB, Jepson MA, Simmons NL & Hirst BH (1998) Increased tyrosine phosphorylation causes redistribution of adherens junction and tight junction proteins and perturbs paracellular barrier function in MDCK epithelia. Eur J Cell Biol 76: 85-92.

Collett MS & Erikson RL (1978) Protein kinase activity associated with the avian sarcoma virus src gene product. Proc Natl Acad Sci U S A 75: 2021-2024.

Cook I & Young A (2002) Towards a physiology of epithelial pathogens. Pflugers Arch 443: 339-343.

Cossart P (2000) Actin-based motility of pathogens: the Arp2/3 complex is a central player. Cell Microbiol 2: 195-205.

Cossart P (2002) Molecular and cellular basis of the infection by Listeria monocytogenes: an overview. Int J Med Microbiol 291: 401-409.

Cosson P, Curtis I de, Pouyssegur J, Griffiths G & Davoust J (1989) Low cytoplasmic pH inhibits endocytosis and transport from the trans- Golgi network to the cell surface. J Cell Biol 108: 377-387.

Covacci A, Censini S, Bugnoli M, Petracca R, Burroni D, Macchia G, Massone A, Papini E, Xiang Z & Figura N (1993) Molecular characterization of the 128-kDa immunodominant antigen of Helicobacter pylori associated with cytotoxicity and duodenal ulcer. Proc Natl Acad Sci U S A 90: 5791-5795.

Cowin P, Kapprell HP, Franke WW, Tamkun J & Hynes RO (1986) Plakoglobin: a protein common to different kinds of intercellular adhering junctions. Cell 46: 1063-1073.

Crljen V, Volinia S & Banfic H (2002) Hepatocyte growth factor activates phosphoinositide 3-kinase C2 beta in renal brush-border plasma membranes. Biochem J 365: 791-799.

Cronier L, Herve JC, Deleze J & Malassine A (1997) Regulation of gap junctional communication during human trophoblast differentiation. Microsc Res Tech 38: 21-28.

Cross TG, Scheel-Toellner D, Henriquez NV, Deacon E, Salmon M & Lord JM (2000) Serine/ threonine protein kinases and apoptosis. Exp Cell Res 256: 34-41.

Davis LH & Bennett V (1994) Identification of two regions of beta G spectrin that bind to distinct sites in brain membranes. J Biol Chem 269: 4409-4416.

Davison MD & Critchley DR (1988) alpha-Actinins and the DMD protein contain spectrin-like repeats. Cell 52: 159-160.

De La Cruz EM, Ostap EM, Brundage RA, Reddy KS, Sweeney HL & Safer D (2000) Thymosin-beta(4) changes the conformation and dynamics of actin monomers. Biophys J 78: 2516-2527.

De Matteis MA & Morrow JS (2000) Spectrin tethers and mesh in the biosynthetic pathway. J Cell Sci 113 (Pt 13): 2331-2343.

Derick LH, Liu SC, Chishti AH & Palek J (1992) Protein immunolocalization in the spread erythrocyte membrane skeleton. Eur J Cell Biol 57: 317-320.

Dessev G, Iovcheva C, Tasheva B & Goldman R (1988) Protein kinase activity associated with the nuclear lamina. Proc Natl Acad Sci U S A 85: 2994-2998.

Dodane V & Kachar B (1996) Identification of isoforms of G proteins and PKC that colocalize with tight junctions. J Membr Biol 149: 199-209.

Doe CQ & Bowerman B (2001) Asymmetric cell division: fly neuroblast meets worm zygote. Curr Opin Cell Biol 13: 68-75.

Doornbos RP, Theelen M, van der Hoeven PC, van Blitterswijk WJ, Verkleij AJ & van Bergen en Henegouwen PM (1999) Protein kinase Czeta is a negative regulator of protein kinase B activity. J Biol Chem 274: 8589-8596.

Downing JR & Reynolds AB (1991) PDGF, CSF-1, and EGF induce tyrosine phosphorylation of p120, a pp60src transformation-associated substrate. Oncogene 6: 607-613.

Downward J (1998) Mechanisms and consequences of activation of protein kinase B/Akt. Curr Opin Cell Biol 10: 262-267.

Dubois A (1995) Spiral bacteria in the human stomach: the gastric helicobacters. Emerg Infect Dis 1: 79-85.

Dunham SP (1999) Cytokines and anti-cytokine therapy: clinical potential for treatment of feline disease. J Feline Med Surg 1: 7-14.

Dytoc M, Gold B, Louie M, Huesca M, Fedorko L, Crowe S, Lingwood C, Brunton J & Sherman P (1993) Comparison of Helicobacter pylori and attaching-effacing Escherichia coli adhesion to eukaryotic cells. Infect Immun 61: 448-456.

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

Eimer W, Niermann M, Eppe MA & Jockusch BM (1993) Molecular shape of vinculin in aqueous solution. J Mol Biol 229: 146-152.

Ellis B, Schneeberger EE & Rabito CA (1992) Cellular variability in the development of tight junctions after activation of protein kinase C. Am J Physiol 263: F293-F300.

Eskelinen S, Huotari V, Sormunen R, Palovuori R, Kok JW & Lehto VP (1992) Low intracellular pH induces redistribution of fodrin and instabilization of lateral walls in MDCK cells. J Cell Physiol 150: 122-133.

Eskelinen S, Kok JW, Sormunen R & Hoekstra D (1991) Coated endosomal vesicles: sorting and recycling compartment for transferrin in BHK cells. Eur J Cell Biol 56: 210-222.

Eskelinen S & Lehto VP (1994) Induction of cell fusion in cultured fibroblasts and epithelial cells by microinjection of EGTA, GTP gamma S and antifodrin antibodies. FEBS Lett 339: 129-133.

Fagotto F & Gumbiner BM (1996) Cell contact-dependent signaling. Dev Biol 180: 445-454.

Fanning AS, Mitic LL & Anderson JM (1999) Transmembrane proteins in the tight junction barrier. J Am Soc Nephrol 10: 1337-1345.

Felsenfeld DP, Schwartzberg PL, Venegas A, Tse R & Sheetz MP (1999) Selective regulation of integrin--cytoskeleton interactions by the tyrosine kinase Src. Nat Cell Biol 1: 200-206.

Feramisco JR & Burridge K (1980) A rapid purification of alpha-actinin, filamin, and a 130,000-dalton protein from smooth muscle. J Biol Chem 255: 1194-1199.

Finnemann S, Mitrik I, Hess M, Otto G & Wedlich D (1997) Uncoupling of XB/U-cadherin-catenin complex formation from its function in cell-cell adhesion. J Biol Chem 272: 11856-11862.

Flatau G, Lemichez E, Gauthier M, Chardin P, Paris S, Fiorentini C & Boquet P (1997) Toxin-induced activation of the G protein p21 Rho by deamidation of glutamine. Nature 387: 729-733.

Force T & Bonventre JV (1998) Growth factors and mitogen-activated protein kinases. Hypertension 31: 152-161.

Frame MC, Fincham VJ, Carragher NO & Wyke JA (2002) v-Src's hold over actin and cell adhesions. Nat Rev Mol Cell Biol 3: 233-245.

Fruman DA, Meyers RE & Cantley LC (1998) Phosphoinositide kinases. Annu Rev Biochem 67: 481-507.

Fuchs E (1996) The cytoskeleton and disease: genetic disorders of intermediate filaments. Annu Rev Genet 30: 197-231.

Fuchs E & Cleveland DW (1998) A structural scaffolding of intermediate filaments in health and disease. Science 279: 514-519.

Fujimoto K, Nagafuchi A, Tsukita S, Kuraoka A, Ohokuma A & Shibata Y (1997) Dynamics of connexins, E-cadherin and alpha-catenin on cell membranes during gap junction formation. J Cell Sci 110 (Pt 3): 311-322.

Fujita Y, Krause G, Scheffner M, Zechner D, Leddy HE, Behrens J, Sommer T & Birchmeier W (2002) Hakai, a c-Cbl-like protein, ubiquitinates and induces endocytosis of the E-cadherin complex. Nat Cell Biol 4: 222-231.

Fukami K, Endo T, Imamura M & Takenawa T (1994) alpha-Actinin and vinculin are PIP2-binding proteins involved in signaling by tyrosine kinase. J Biol Chem 269: 1518-1522.

Fukata Y, Oshiro N & Kaibuchi K (1999) Activation of moesin and adducin by Rho-kinase downstream of Rho. Biophys Chem 82: 139-147.

Furuse M, Hirase T, Itoh M, Nagafuchi A, Yonemura S, Tsukita S & Tsukita S (1993) Occludin: a novel integral membrane protein localizing at tight junctions. J Cell Biol 123: 1777-1788.

Furuse M, Itoh M, Hirase T, Nagafuchi A, Yonemura S, Tsukita S & Tsukita S (1994) Direct association of occludin with ZO-1 and its possible involvement in the localization of occludin at tight junctions. J Cell Biol 127: 1617-1626.

Gallo R, Provenzano C, Carbone R, Di Fiore PP, Castellani L, Falcone G & Alema S (1997) Regulation of the tyrosine kinase substrate Eps8 expression by growth factors, v-Src and terminal differentiation. Oncogene 15: 1929-1936.

Gao L, Joberty G & Macara IG (2002) Assembly of epithelial tight junctions is negatively regulated by Par6. Curr Biol 12: 221-225.

Gardner K & Bennett V (1987) Modulation of spectrin-actin assembly by erythrocyte adducin. Nature 328: 359-362.

Garrod DR (1993) Desmosomes and hemidesmosomes. Curr Opin Cell Biol 5: 30-40.

Geiger B (1979) A 130K protein from chicken gizzard: its localization at the termini of microfilament bundles in cultured chicken cells. Cell 18: 193-205.

Geiger B & Ginsberg D (1991) The cytoplasmic domain of adherens-type junctions. Cell Motil Cytoskeleton 20: 1-6.

Getsios S, Chen GT, Huang DT & MacCalman CD (1998) Regulated expression of cadherin-11 in human extravillous cytotrophoblasts undergoing aggregation and fusion in response to transforming growth factor beta 1. J Reprod Fertil 114: 357-363.

Ghadimi BM, Behrens J, Hoffmann I, Haensch W, Birchmeier W & Schlag PM (1999) Immunohistological analysis of E-cadherin, alpha-, beta- and gamma- catenin expression in colorectal cancer: implications for cell adhesion and signaling. Eur J Cancer 35: 60-65.

Giepmans BN & Moolenaar WH (1998) The gap junction protein connexin43 interacts with the second PDZ domain of the zona occludens-1 protein. Curr Biol 8: 931-934.

Gilmore AP & Burridge K (1996) Regulation of vinculin binding to talin and actin by phosphatidyl-inositol-4-5-bisphosphate. Nature 381: 531-535.

Glukhova MA, Frid MG & Koteliansky VE (1990) Developmental changes in expression of contractile and cytoskeletal proteins in human aortic smooth muscle. J Biol Chem 265: 13042-13046.

Goldmann WH (2000) Kinetic determination of focal adhesion protein formation. Biochem Biophys Res Commun 271: 553-557.

Goodenough DA, Goliger JA & Paul DL (1996) Connexins, connexons, and intercellular communication. Annu Rev Biochem 65: 475-502.

Gopalakrishnan S, Raman N, Atkinson SJ & Marrs JA (1998) Rho GTPase signaling regulates tight junction assembly and protects tight junctions during ATP depletion. Am J Physiol 275: C798-C809.

Gottardi CJ, Arpin M, Fanning AS & Louvard D (1996) The junction-associated protein, zonula occludens-1, localizes to the nucleus before the maturation and during the remodeling of cell-cell contacts. Proc Natl Acad Sci U S A 93: 10779-10784.

Graff JM, Young TN, Johnson JD & Blackshear PJ (1989) Phosphorylation-regulated calmodulin binding to a prominent cellular substrate for protein kinase C. J Biol Chem 264: 21818-21823.

Green KJ, Guy SG, Cserhalmi-Friedman PB, McLean WH, Christiano AM & Wagner RM (1999) Analysis of the desmoplakin gene reveals striking conservation with other members of the plakin family of cytolinkers. Exp Dermatol 8: 462-470.

Griffiths G & Simons K (1986) The trans Golgi network: sorting at the exit site of the Golgi complex. Science 234: 438-443.

Groesch ME & Otto JJ (1990) Purification and characterization of an 85 kDa talin-binding fragment of vinculin. Cell Motil Cytoskeleton 15: 41-50.

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.

Gumbiner BM (1992) Epithelial morphogenesis. Cell 69: 385-387.

Gumbiner BM (1996) Cell adhesion: the molecular basis of tissue architecture and morphogenesis. Cell 84: 345-357.

Guo L, Degenstein L, Dowling J, Yu QC, Wollmann R, Perman B & Fuchs E (1995) Gene targeting of BPAG1: abnormalities in mechanical strength and cell migration in stratified epithelia and neurologic degeneration. Cell 81: 233-243.

Habets GG, Scholtes EH, Zuydgeest D, van der Kammen RA, Stam JC, Berns A & Collard JG (1994) Identification of an invasion-inducing gene, Tiam-1, that encodes a protein with homology to GDP-GTP exchangers for Rho-like proteins. Cell 77: 537-549.

Hall A (1998) Rho GTPases and the actin cytoskeleton. Science 279: 509-514.

Hamad NM, Elconin JH, Karnoub AE, Bai W, Rich JN, Abraham RT, Der CJ & Counter CM (2002) Distinct requirements for Ras oncogenesis in human versus mouse cells. Genes Dev 16: 2045-2057.

Hamaguchi M, Yamagata S, Thant AA, Xiao H, Iwata H, Mazaki T & Hanafusa H (1995) Augmentation of metalloproteinase (gelatinase) activity secreted from Rous sarcoma virus-infected cells correlates with transforming activity of src. Oncogene 10: 1037-1043.

Hartwig JH (1994) Actin-binding proteins 1: spectrin superfamily. Protein Profile 1: 706-778.

Hartwig JH & DeSisto M (1991) The cytoskeleton of the resting human blood platelet: structure of the membrane skeleton and its attachment to actin filaments. J Cell Biol 112: 407-425.

Hartwig JH, Thelen M, Rosen A, Janmey PA, Nairn AC & Aderem A (1992) MARCKS is an actin filament crosslinking protein regulated by protein kinase C and calcium-calmodulin. Nature 356: 618-622.

Hesse M, Franz T, Tamai Y, Taketo MM & Magin TM (2000) Targeted deletion of keratins 18 and 19 leads to trophoblast fragility and early embryonic lethality. EMBO J 19: 5060-5070.

Higgs HN, Pollard TD (2001) Regulation of actin filament network formation through ARP2/3 complex: activation by a diverse array of proteins. Annu Rev Biochem 70: 649-676.

Hinck L, Näthke IS, Papkoff J & Nelson WJ (1994) Dynamics of cadherin/catenin complex formation: novel protein interactions and pathways of complex assembly. J Cell Biol 125: 1327-1340.

Hirase T, Kawashima S, Wong EY, Ueyama T, Rikitake Y, Tsukita S, Yokoyama M & Staddon JM (2001) Regulation of tight junction permeability and occludin phosphorylation by Rhoa-p160ROCK-dependent and –independent mechanisms. J Biol Chem 276: 10423-10431.

Hirayama E, Inoue N, Kamata M, Ama M & Kim J (2000) Dynamic distribution of an antigen involved in the differentiation of avian myoblasts: II. Possible association of beta1 integrin with myofibril organization. Cell Motil Cytoskeleton 45: 27-41.

Hooley R, Yu CY, Symons M & Barber DL (1996) G alpha 13 stimulates Na+-H+ exchange through distint Cdc42-dependent and RhoA-dependent pathways. J Biol Chem 271: 6152-6158.

Hopkinson SB & Jones JC (2000) The N terminus of the transmembrane protein BP180 interacts with the N-terminal domain of BP230, thereby mediating keratin cytoskeleton anchorange to the cell surface at the site of the hemidesmosome. Mol Biol Cell 11: 277-286.

Hordijk PL, ten Klooster JP, van der Kammen RA, Michiels F, Oomen LC & Collard JG (1997) Inhibition of invasion of epithelial cells by Tiam1-Rac signaling. Science 278: 1464-1466.

Hotchin NA, Cover TL & Akhtar N (2000) Cell vacuolation induced by the VacA cytotoxin of Helicobacter pylori is regulated by the Rac1 GTPase. J Biol Chem 275: 14009-14012.

Hotchin NA & Hall A (1995) The assembly of integrin adhesion complexes requires both extracellular matrix and intracellular rho/rac GTPases. J Cell Biol 131: 1857-1865.

Howarth AG, Hughes MR & Stevenson BR (1992) Detection of the tight junction-associated protein ZO-1 in astrocytes and other nonepithelial cell types. Am J Physiol 262: C461-C469.

Hu RJ, Moorthy S & Bennett V (1995) Expression of functional domains of beta G-spectrin disrupts epithelial morphology in cultured cells. J Cell Biol 128: 1069-1080.

Huber O, Krohn M & Kemler R (1997) A specific domain in alpha-catenin mediates binding to beta-catenin or plakoglobin. J Cell Sci 110 (Pt 15): 1759-1765.

Hunter T & Sefton BM (1980) Transforming gene product of Rous sarcoma virus phosphorylates tyrosine. Proc Natl Acad Sci U S A 77: 1311-1315.

Huotari V, Sormunen R, Lehto VP & Eskelinen S (1995) The polarity of the membrane skeleton in retinal pigment epithelial cells of developing chicken embryos and in primary culture. Differentiation 58: 205-215.

Huotari V, Vääräniemi J, Lehto VP & Eskelinen S (1996) Regulation of the disassembly/assembly of the membrane skeleton in Madin-Darby canine kidney cells. J Cell Physiol 167: 121-130.

Hüttelmaier S, Illenberger S, Grosheva I, Rüdiger M, Singer RH & Jockusch BM (2001) Raver1, a dual compartment protein, is a ligand for PTB/hnRNPI and microfilament attachment proteins. J Cell Biol 155: 775-786.

Hüttelmaier S, Mayboroda O, Harbeck B, Jarchau T, Jockusch BM & Rüdiger M (1998) The interaction of the cell-contact proteins VASP and vinculin is regulated by phosphatidylinositol-4,5-bisphosphate. Curr Biol 8: 479-488.

Ikeda W, Nakanishi H, Miyoshi J, Mandai K, Ishizaki H, Tanaka M, Togawa A, Takahashi K, Nishioka H, Yoshida H, Mizoguchi A, Nishikawa S & Takai Y (1999) Afadin: A key molecule essential for structural organization of cell- cell junctions of polarized epithelia during embryogenesis. J Cell Biol 146: 1117-1132.

Ilver D, Arnqvist A, Ögren J, Frick IM, Kersulyte D, Incecik ET, Berg DE, Covacci A, Engstrand L & Bóren T (1998) Helicobacter pylori adhesin binding fucosylated histo-blood group antigens revealed by retagging. Science 279: 373-377.

Ishikawa H, Bischoff R & Holtzer H (1968) Mitosis and intermediate-sized filaments in developing skeletal muscle. J Cell Biol 38: 538-555.

Ismaili A, Philpott DJ, Dytoc MT, Soni R, Ratnam S & Sherman PM (1995) Alpha-actinin accumulation in epithelial cells infected with attaching and effacing gastrointestinal pathogens. J Infect Dis 172: 1393-1396.

Itoh M, Furuse M, Morita K, Kubota K, Saitou M & Tsukita S (1999) Direct binding of three tight junction-associated MAGUKs, ZO-1, ZO-2, and ZO-3, with the COOH termini of claudins. J Cell Biol 147: 1351-1363.

Itoh M, Nagafuchi A, Moroi S & Tsukita S (1997) Involvement of ZO-1 in cadherin-based cell adhesion through its direct binding to alpha catenin and actin filaments. J Cell Biol 138: 181-192.

Itoh M, Nagafuchi A, Yonemura S, Kitani-Yasuda T, Tsukita S & Tsukita S (1993) The 220-kD protein colocalizing with cadherins in non-epithelial cells is identical to ZO-1, a tight junction-associated protein in epithelial cells: cDNA cloning and immunoelectron microscopy. J Cell Biol 121: 491-502.

Itoh M, Yonemura S, Nagafuchi A, Tsukita S & Tsukita S (1991) A 220-kD undercoat-constitutive protein: its specific localization at cadherin-based cell-cell adhesion sites. J Cell Biol 115: 1449-1462.

Iwamoto H, Czajkowsky DM, Cover TL, Szabo G & Shao Z (1999) VacA from Helicobacter pylori: a hexameric chloride channel. FEBS Lett 450: 101-104.

Izumi Y, Hirose T, Tamai Y, Hirai S, Nagashima Y, Fujimoto T, Tabuse Y, Kemphues KJ & 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.

Jaffe AB & Hall A (2002) Rho GTPases in transformation and metastasis. Adv Cancer Res 84: 57-80.

Jaken S (1996) Protein kinase C isozymes and substrates. Curr Opin Cell Biol 8: 168-173.

Jaken S, Leach K & Klauck T (1989) Association of type 3 protein kinase C with focal contacts in rat embryo fibroblasts. J Cell Biol 109: 697-704.

Jin J, Anthopoulos N, Wetsch B, Binari RC, Isaac DD, Andrew DJ, Woodgett JR & Manoukian AS (2001) Regulation of Drosophila tracheal system development by protein kinase B. Dev Cell 1: 817-827.

Joberty G, Petersen C, Gao L & Macara IG (2000) The cell-polarity protein Par6 links Par3 and atypical protein kinase C to Cdc42. Nat Cell Biol 2: 531-539.

Jockusch BM, Bubeck P, Giehl K, Kroemker M, Moschner J, Rothkegel M, Rüdiger M, Schluter K, Stanke G & Winkler J (1995) The molecular architecture of focal adhesions. Annu Rev Cell Dev Biol 11: 379-416.

Jockusch BM & Rüdiger M (1996) Crosstalk between cell adhesion molecules: vinculin as a paradigm for regulation by conformation. Trends Cell Biol 6: 311-315.

Johansson A, Driessens M & Aspenström P (2000) The mammalian homologue of the Caenorhabditis elegans polarity protein PAR-6 is a binding partner for the Rho GTPases Cdc42 and Rac1. J Cell Sci 113 (Pt 18): 3267-3275.

Johnson KR, Lewis JE, Li D, Wahl J, Soler AP, Knudsen KA & Wheelock MJ (1993) P- and E-cadherin are in separate complexes in cells expressing both cadherins. Exp Cell Res 207: 252-260.

Johnson RP & Craig SW (1994) An intramolecular association between the head and tail domains of vinculin modulates talin binding. J Biol Chem 269: 12611-12619.

Johnson RP & Craig SW (1995a) F-actin binding site masked by the intramolecular association of vinculin head and tail domains. Nature 373: 261-264.

Johnson RP & Craig SW (1995b) The carboxy-terminal tail domain of vinculin contains a cryptic binding site for acidic phospholipids. Biochem Biophys Res Commun 210: 159-164.

Johnson RP, Niggli V, Durrer P & Craig SW (1998) A conserved motif in the tail domain of vinculin mediates association with and insertion into acidic phospholipid bilayers. Biochemistry 37: 10211-10222.

Joneson T, McDonough M, Bar-Sagi D & Van Aelst L (1996) RAC regulation of actin polymerization and proliferation by a pathway distinct from Jun kinase. Science 274: 1374-1376.

Jou TS, Schneeberger EE & Nelson WJ (1998) Structural and functional regulation of tight junctions by RhoA and Rac1 small GTPases. J Cell Biol 142: 101-115.

Just I, Hofmann F, Genth H & Gerhard R (2001) Bacterial protein toxins inhibiting low-molecular-mass GTP-binding proteins. Int J Med Microbiol 291: 243-250.

Just I, Selzer J, Hofmann F, Green GA & Aktories K (1996) Inactivation of Ras by Clostridium sordellii lethal toxin-catalyzed glucosylation. J Biol Chem 271: 10149-10153.

Kaiser HW, O'Keefe E & Bennett V (1989) Adducin: Ca++-dependent association with sites of cell-cell contact. J Cell Biol 109: 557-569.

Kanner SB, Reynolds AB & Parsons JT (1991) Tyrosine phosphorylation of a 120-kilodalton pp60src substrate upon epidermal growth factor and platelet-derived growth factor receptor stimulation and in polyomavirus middle-T-antigen-transformed cells. Mol Cell Biol 11: 713-720.

Kaplan KB, Bibbins KB, Swedlow JR, Arnaud M, Morgan DO & Varmus HE (1994) Association of the amino-terminal half of c-Src with focal adhesions alters their properties and is regulated by phosphorylation of tyrosine 527. EMBO J 13: 4745-4756.

Kartenbeck J, Schmelz M, Franke WW & Geiger B (1991) Endocytosis of junctional cadherins in bovine kidney epithelial (MDBK) cells cultured in low Ca2+ ion medium. J Cell Biol 113: 881-892.

Katso R, Okkenhaug K, Ahmadi K, White S, Timms J & Waterfield MD (2001) Cellular function of phosphoinositide 3-kinases: implications for development, homeostasis, and cancer. Annu Rev Cell Dev Biol 17: 615-675.

Kaufmann U, Kirsch J, Irintchev A, Wernig A & Starzinski-Powitz A (1999) The M-cadherin catenin complex interacts with microtubules in skeletal muscle cells: implications for the fusion of myoblasts. J Cell Sci 112 (Pt 1): 55-67.

Keates S, Sougioultzis S, Keates AC, Zhao D, Peek RM, Jr., Shaw LM & Kelly CP (2001) cag+ Helicobacter pylori induce transactivation of the epidermal growth factor receptor in AGS gastric epithelial cells. J Biol Chem 276: 48127-48134.

Khwaja A, Rodriguez-Viciana P, Wennstrom S, Warne PH & Downward J (1997) Matrix adhesion and Ras transformation both activate a phosphoinositide 3-OH kinase and protein kinase B/Akt cellular survival pathway. EMBO J 16: 2783-2793.

Kikyo M, Matozaki T, Kodama A, Kawabe H, Nakanishi H & Takai Y (2000) Cell-cell adhesion-mediated tyrosine phosphorylation of nectin-2delta, an immunoglobulin-like cell adhesion molecule at adherens junctions. Oncogene 19: 4022-4028.

Kim SK (2000) Cell polarity: new PARtners for Cdc42 and Rac. Nat Cell Biol 2: E143-E145.

Kimura K, Fukata Y, Matsuoka Y, Bennett V, Matsuura Y, Okawa K, Iwamatsu A & Kaibuchi K (1998) Regulation of the association of adducin with actin filaments by Rho- associated kinase (Rho-kinase) and myosin phosphatase. J Biol Chem 273: 5542-5548.

Kimura K, Ito M, Amano M, Chihara K, Fukata Y, Nakafuku M, Yamamori B, Feng J, Nakano T, Okawa K, Iwamatsu A & Kaibuchi K (1996) Regulation of myosin phosphatase by Rho and Rho-associated kinase (Rho- kinase). Science 273: 245-248.

Kinch MS, Clark GJ, Der CJ & Burridge K (1995) Tyrosine phosphorylation regulates the adhesions of ras-transformed breast epithelia. J Cell Biol 130: 461-471.

King WG, Mattaliano MD, Chan TO, Tsichlis PN & Brugge JS (1997) Phosphatidylinositol 3-kinase is required for integrin-stimulated AKT and Raf-1 mitogen-activated protein kinase pathway activation. Mol Cell Biol 17: 4406-4418.

Kioka N, Sakata S, Kawauchi T, Amachi T, Akiyama SK, Okazaki K, Yaen C, Yamada KM & Aota S (1999) Vinexin: a novel vinculin-binding protein with multiple SH3 domains enhances actin cytoskeletal organization. J Cell Biol 144: 59-69.

Knudsen KA, Frankowski C, Johnson KR & Wheelock MJ (1998) A role for cadherins in cellular signaling and differentiation. J Cell Biochem Suppl 30-31: 168-176.

Knudsen KA, Soler AP, Johnson KR & Wheelock MJ (1995) Interaction of alpha-actinin with the cadherin/catenin cell-cell adhesion complex via alpha-catenin. J Cell Biol 130: 67-77.

Korman NJ, Eyre RW, Klaus-Kovtun V & Stanley JR (1989) Demonstration of an adhering-junction molecule (plakoglobin) in the autoantigens of pemphigus foliaceus and pemphigus vulgaris. N Engl J Med 321: 631-635.

Kozma R, Ahmed S, Best A & Lim L (1995) The Ras-related protein Cdc42Hs and bradykinin promote formation of peripheral actin microspikes and filopodia in Swiss 3T3 fibroblasts. Mol Cell Biol 15: 1942-1952.

Kroemker M, Rüdiger AH, Jockusch BM & Rüdiger M (1994) Intramolecular interactions in vinculin control alpha-actinin binding to the vinculin head. FEBS Lett 355: 259-262.

Kroner C, Eybrechts K & Akkerman JW (2000) Dual regulation of platelet protein kinase B. J Biol Chem 275: 27790-27798.

Kumar NM & Gilula NB (1996) The gap junction communication channel. Cell 84: 381-388.

Kurki P, Linder E, Virtanen I & Stenman S (1977) Human smooth muscle autoantibodies reacting with intermediate (100 A) filaments. Nature 268: 240-241.

Kuroda S, Fukata M, Fujii K, Nakamura T, Izawa I & Kaibuchi K (1997) Regulation of cell-cell adhesion of MDCK cells by Cdc42 and Rac1 small GTPases. Biochem Biophys Res Commun 240: 430-435.

Kuroda S, Fukata M, Kobayashi K, Nakafuku M, Nomura N, Iwamatsu A & Kaibuchi K (1996) Identification of IQGAP as a putative target for the small GTPases, Cdc42 and Rac1. J Biol Chem 271: 23363-23367.

Kuroda S, Fukata M, Nakagawa M, Fujii K, Nakamura T, Ookubo T, Izawa I, Nagase T, Nomura N, Tani H, Shoji I, Matsuura Y, Yonehara S & Kaibuchi K (1998) Role of IQGAP1, a target of the small GTPases Cdc42 and Rac1, in regulation of E-cadherin- mediated cell-cell adhesion. Science 281: 832-835.

Kurschat P & Mauch C (2000) Mechanisms of metastasis. Clin Exp Dermatol 25: 482-489.

Kusunoki T, Nishida S, Murata K & Tomura T (2002) Correlation between morphology of colonies formed by human thyroid tumor cells in collagen gel culture and invasive ability. Thyroid 12: 281-286.

Kuzuya M & Kinsella JL (1994) Reorganization of endothelial cord-like structures on basement membrane complex (Matrigel): involvement of transforming growth factor beta 1. J Cell Physiol 161: 267-276.

Lacaz-Vieira F (2000) Tight junction dynamics: oscillations and the role of protein kinase C. J Membr Biol 178: 151-161.

Laffargue M, Raynal P, Yart A, Peres C, Wetzker R, Roche S, Payrastre B & Chap H (1999) An epidermal growth factor receptor/Gab1 signaling pathway is required for activation of phosphoinositide 3-kinase by lysophosphatidic acid. J Biol Chem 274: 32835-32841.

Lamarche N & Hall A (1994) GAPs for rho-related GTPases. Trends Genet 10: 436-440.

Lamarche N, Tapon N, Stowers L, Burbelo PD, Aspenström P, Bridges T, Chant J & Hall A (1996) Rac and Cdc42 induce actin polymerization and G1 cell cycle progression independently of p65PAK and the JNK/SAPK MAP kinase cascade. Cell 87: 519-529.

Lehto VP, Virtanen I & Kurki P (1978) Intermediate filaments anchor the nuclei in nuclear monolayers of cultured human fibroblasts. Nature 272: 175-177.

Leiser J & Molitoris BA (1993) Disease processes in epithelia: the role of the actin cytoskeleton and altered surface membrane polarity. Biochim Biophys Acta 1225: 1-13.

Lerm M, Selzer J, Hoffmeyer A, Rapp UR, Aktories K & Schmidt G (1999) Deamidation of Cdc42 and Rac by Escherichia coli cytotoxic necrotizing factor 1: activation of c-Jun N-terminal kinase in HeLa cells. Infect Immun 67: 496-503.

Leung T, Manser E, Tan L & Lim L (1995) A novel serine/threonine kinase binding the Ras-related RhoA GTPase which translocates the kinase to peripheral membranes. J Biol Chem 270: 29051-29054.

Levinson AD, Oppermann H, Levintow L, Varmus HE & Bishop JM (1978) Evidence that the transforming gene of avian sarcoma virus encodes a protein kinase associated with a phosphoprotein. Cell 15: 561-572.

Li D & Mrsny RJ (2000) Oncogenic Raf-1 disrupts epithelial tight junctions via downregulation of occludin. J Cell Biol 148: 791-800.

Li S, Wang Q, Chakladar A, Bronson RT & Bernards A (2000) Gastric hyperplasia in mice lacking the putative Cdc42 effector IQGAP1. Mol Cell Biol 20: 697-701.

Li X, Matsuoka Y & Bennett V (1998) Adducin preferentially recruits spectrin to the fast growing ends of actin filaments in a complex requiring the MARCKS-related domain and a newly defined oligomerization domain. J Biol Chem 273: 19329-19338.

Li X, Talts U, Talts JF, Arman E, Ekblom P & Lonai P (2001) Akt/PKB regulates laminin and collagen IV isotypes of the basement membrane. Proc Natl Acad Sci U S A 98: 14416-14421.

Lin D, Edwards AS, Fawcett JP, Mbamalu G, Scott JD & Pawson T (2000) A mammalian PAR-3-PAR-6 complex implicated in Cdc42/Rac1 and aPKC signalling and cell polarity. Nat Cell Biol 2: 540-547.

Lin R, Warn-Cramer BJ, Kurata WE & Lau AF (2001) v-Src phosphorylation of connexin 43 on Tyr247 and Tyr265 disrupts gap junctional communication. J Cell Biol 154: 815-828.

Liu SC, Derick LH & Palek J (1987) Visualization of the hexagonal lattice in the erythrocyte membrane skeleton. J Cell Biol 104: 527-536.

Lodish H, Berk A, Zipursky SL, Matsudaira P, Baltimore D & Darnell J (2000) Molecular Cell Biology. WH Freeman and Company, New York, p 1071.

Lombardo CR, Weed SA, Kennedy SP, Forget BG & Morrow JS (1994) Beta II-spectrin (fodrin) and beta I epsilon 2-spectrin (muscle) contain NH2- and COOH-terminal membrane association domains (MAD1 and MAD2). J Biol Chem 269: 29212-29219.

Lozano J, Berra E, Municio MM, Diaz-Meco MT, Dominguez I, Sanz L & Moscat J (1994) Protein kinase C zeta isoform is critical for kappa B-dependent promoter activation by sphingomyelinase. J Biol Chem 269: 19200-19202.

Machesky LM & Way M (1998) Actin branches out. Nature 394: 125-126.

Mackay DJ, Esch F, Furthmayr H & Hall A (1997) Rho- and rac-dependent assembly of focal adhesion complexes and actin filaments in permeabilized fibroblasts: an essential role for ezrin/radixin/moesin proteins. J Cell Biol 138: 927-938.

Mackay DJ & Hall A (1998) Rho GTPases. J Biol Chem 273: 20685-20688.

Magendantz M, Henry MD, Lander A & Solomon F (1995) Interdomain interactions of radixin in vitro. J Biol Chem 270: 25324-25327.

Malliri A, van der Kammen RA, Clark K, van d, V, Michiels F & Collard JG (2002) Mice deficient in the Rac activator Tiam1 are resistant to Ras-induced skin tumours. Nature 417: 867-871.

Mandai K, Nakanishi H, Satoh A, Obaishi H, Wada M, Nishioka H, Itoh M, Mizoguchi A, Aoki T, Fujimoto T, Matsuda Y, Tsukita S & Takai Y (1997) Afadin: A novel actin filament-binding protein with one PDZ domain localized at cadherin-based cell-to-cell adherens junction. J Cell Biol 139: 517-528.

Mandai K, Nakanishi H, Satoh A, Takahashi K, Satoh K, Nishioka H, Mizoguchi A & Takai Y (1999) Ponsin/SH3P12: an l-afadin- and vinculin-binding protein localized at cell-cell and cell-matrix adherens junctions. J Cell Biol 144: 1001-1017.

Mangeat P, Roy C & Martin M (1999) ERM proteins in cell adhesion and membrane dynamics. Trends Cell Biol 9: 187-192.

Marchetti M, Arico B, Burroni D, Figura N, Rappuoli R & Ghiara P (1995) Development of a mouse model of Helicobacter pylori infection that mimics human disease. Science 267: 1655-1658.

Mareel MM, Behrens J, Birchmeier W, De Bruyne GK, Vleminckx K, Hoogewijs A, Fiers WC & Van Roy FM (1991) Down-regulation of E-cadherin expression in Madin Darby canine kidney (MDCK) cells inside tumors of nude mice. Int J Cancer 47: 922-928.

Mariner DJ, Anastasiadis P, Keilhack H, Bohmer FD, Wang J & Reynolds AB (2001) Identification of Src phosphorylation sites in the catenin p120ctn. J Biol Chem 276: 28006-28013.

Masaki T & Takaiti O (1969) Some properties of chicken alpha-actinin. J Biochem (Tokyo) 66: 637-643.

Mathur M, Goodwin L & Cowin P (1994) Interactions of the cytoplasmic domain of the desmosomal cadherin Dsg1 with plakoglobin. J Biol Chem 269: 14075-14080.

Matlin KS & Caplan MJ (1992) Epithelial cell structure and polarity. The kidney: physiology and pathophysiology. Raven Press, New York, p 475-501.

Matsui T, Amano M, Yamamoto T, Chihara K, Nakafuku M, Ito M, Nakano T, Okawa K, Iwamatsu A & Kaibuchi K (1996) Rho-associated kinase, a novel serine/threonine kinase, as a putative target for small GTP binding protein Rho. EMBO J 15: 2208-2216.

Matsui T, Maeda M, Doi Y, Yonemura S, Amano M, Kaibuchi K, Tsukita S & Tsukita S (1998) Rho-kinase phosphorylates COOH-terminal threonines of ezrin/radixin/moesin (ERM) proteins and regulates their head-to-tail association. J Cell Biol 140: 647-657.

Matsuoka Y, Hughes CA & Bennett V (1996) Adducin regulation. Definition of the calmodulin-binding domain and sites of phosphorylation by protein kinases A and C. J Biol Chem 271: 25157-25166.

Matter K, Bucher K & Hauri HP (1990) 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.

Mays RW, Beck KA & Nelson WJ (1994) Organization and function of the cytoskeleton in polarized epithelial cells: a component of the protein sorting machinery. Curr Opin Cell Biol 6: 16-24.

Mbalaviele G, Chen H, Boyce BF, Mundy GR & Yoneda T (1995) The role of cadherin in the generation of multinucleated osteoclasts from mononuclear precursors in murine marrow. J Clin Invest 95: 2757-2765.

McCarthy KM, Skare IB, Stankewich MC, Furuse M, Tsukita S, Rogers RA, Lynch RD & Schneeberger EE (1996) Occludin is a functional component of the tight junction. J Cell Sci 109 (Pt 9): 2287-2298.

McLean GW, Fincham VJ & Frame MC (2000) v-Src induces tyrosine phosphorylation of focal adhesion kinase independently of tyrosine 397 and formation of a complex with Src. J Biol Chem 275: 23333-23339.

Michiels F, Habets GG, Stam JC, van der Kammen RA & Collard JG (1995) A role for Rac in Tiam1-induced membrane ruffling and invasion. Nature 375: 338-340.

Miller GJ & Ball EH (2001) Conformational change in the vinculin C-terminal depends on a critical histidine residue (His-906). J Biol Chem 276: 28829-28834.

Mogensen MM (1999) Microtubule release and capture in epithelial cells. Biol Cell 91: 331-341.

Montesano R, Soriano JV, Hosseini G, Pepper MS & Schramek H (1999) Constitutively active mitogen-activated protein kinase kinase MEK1 disrupts morphogenesis and induces an invasive phenotype in Madin-Darby canine kidney epithelial cells. Cell Growth Differ 10: 317-332.

Moorby C & Patel M (2001) Dual functions for connexins: Cx43 regulates growth independently of gap junction formation. Exp Cell Res 271: 238-248.

Mooseker MS (1985) Organization, chemistry, and assembly of the cytoskeletal apparatus of the intestinal brush border. Annu Rev Cell Biol 1: 209-241.

Müller G, Ayoub M, Storz P, Rennecke J, Fabbro D & Pfizenmaier K (1995) PKC zeta is a molecular switch in signal transduction of TNF-alpha, bifunctionally regulated by ceramide and arachidonic acid. EMBO J 14: 1961-1969.

Nakagawa M, Fukata M, Yamaga M, Itoh N & Kaibuchi K (2001) Recruitment and activation of Rac1 by the formation of E-cadherin- mediated cell-cell adhesion sites. J Cell Sci 114: 1829-1838.

Nakanishi H, Brewer KA & Exton JH (1993) Activation of the zeta isozyme of protein kinase C by phosphatidylinositol 3,4,5-trisphosphate. J Biol Chem 268: 13-16.

Nakano K, Takaishi K, Kodama A, Mammoto A, Shiozaki H, Monden M & Takai Y (1999) Distinct actions and cooperative roles of ROCK and mDia in Rho small G protein-induced reorganization of the actin cytoskeleton in Madin-Darby canine kidney cells. Mol Biol Cell 10: 2481-2491.

Narumiya S (1996) The small GTPase Rho: cellular functions and signal transduction. J Biochem (Tokyo) 120: 215-228.

Nelson WJ (1991) Cytoskeleton functions in membrane traffic in polarized epithelial cells. Semin Cell Biol 2: 375-385.

Nelson WJ, Shore EM, Wang AZ & Hammerton RW (1990) Identification of a membrane-cytoskeletal complex containing the cell adhesion molecule uvomorulin (E-cadherin), ankyrin, and fodrin in Madin- Darby canine kidney epithelial cells. J Cell Biol 110: 349-357.

Nelson WJ & Veshnock PJ (1987) Ankyrin binding to (Na+ + K+)ATPase and implications for the organization of membrane domains in polarized cells. Nature 328: 533-536.

Newton AC (1993) Interaction of proteins with lipid headgroups: lessons from protein kinase C. Annu Rev Biophys Biomol Struct 22: 1-25.

Nieman MT, Prudoff RS, Johnson KR & Wheelock MJ (1999) N-cadherin promotes motility in human breast cancer cells regardless of their E-cadherin expression. J Cell Biol 147: 631-644.

Niemann C, Brinkmann V, Spitzer E, Hartmann G, Sachs M, Naundorf H & Birchmeier W (1998) Reconstitution of mammary gland development in vitro: requirement of c- met and c-erbB2 signaling for branching and alveolar morphogenesis. J Cell Biol 143: 533-545.

Nishizuka Y (1989) The Albert Lasker Medical Awards. The family of protein kinase C for signal transduction. JAMA 262: 1826-1833.

Nishizuka Y (1995) Protein kinase C and lipid signaling for sustained cellular responses. FASEB J 9: 484-496.

Nishizuka Y & Nakamura S (1995) Lipid mediators and protein kinase C for intracellular signalling. Clin Exp Pharmacol Physiol 22 Suppl 1: S202-S203.

Nobes CD & Hall A (1995) Rho, rac, and cdc42 GTPases regulate the assembly of multimolecular focal complexes associated with actin stress fibers, lamellipodia, and filopodia. Cell 81: 53-62.

Nobes CD, Hawkins P, Stephens L & Hall A (1995) Activation of the small GTP-binding proteins rho and rac by growth factor receptors. J Cell Sci 108 (Pt 1): 225-233.

Noe V, Chastre E, Bruyneel E, Gespach C & Mareel M (1999) Extracellular regulation of cancer invasion: the E-cadherin-catenin and other pathways. Biochem Soc Symp 65: 43-62.

Noren NK, Liu BP, Burridge K & Kreft B (2000) p120 catenin regulates the actin cytoskeleton via Rho family GTPases. J Cell Biol 150: 567-580.

Nuckolls GH, Turner CE & Burridge K (1990) Functional studies of the domains of talin. J Cell Biol 110: 1635-1644.

O'Brien LE, Jou TS, Pollack AL, Zhang Q, Hansen SH, Yurchenco P & Mostov KE (2001) Rac1 orientates epithelial apical polarity through effects on basolateral laminin assembly. Nat Cell Biol 3: 831-838.

Ochieng J, Basolo F, Albini A, Melchiori A, Watanabe H, Elliott J, Raz A, Parodi S & Russo J (1991) Increased invasive, chemotactic and locomotive abilities of c-Ha-ras- transformed human breast epithelial cells. Invasion Metastasis 11: 38-47.

O'Halloran T, Molony L & Burridge K (1986) Purification and assay of vinculin, metavinculin, and talin. Methods Enzymol 134: 69-77.

Ohno S (2001) Intercellular junctions and cellular polarity: the PAR-aPKC complex, a conserved core cassette playing fundamental roles in cell polarity. Curr Opin Cell Biol 13: 641-648.

Ohtsuka H, Yajima H, Maruyama K & Kimura S (1997) The N-terminal Z repeat 5 of connectin/titin binds to the C-terminal region of alpha-actinin. Biochem Biophys Res Commun 235: 1-3.

Otto JJ (1983) Detection of vinculin-binding proteins with an 125I-vinculin gel overlay technique. J Cell Biol 97: 1283-1287.

Ozawa M, Ringwald M & Kemler R (1990) Uvomorulin-catenin complex formation is regulated by a specific domain in the cytoplasmic region of the cell adhesion molecule. Proc Natl Acad Sci U S A 87: 4246-4250.

Pai R, Wyle FA, Cover TL, Itani RM, Domek MJ & Tarnawski AS (1998) Helicobacter pylori culture supernatant interferes with epidermal growth factor-activated signal transduction in human gastric KATO III cells. Am J Pathol 152: 1617-1624.

Parra M, Gascard P, Walensky LD, Gimm JA, Blackshaw S, Chan N, Takakuwa Y, Berger T, Lee G, Chasis JA, Snyder SH, Mohandas N & Conboy JG (2000) Molecular and functional characterization of protein 4.1B, a novel member of the protein 4.1 family with high level, focal expression in brain. J Biol Chem 275: 3247-3255.

Parsons JT & Parsons SJ (1997) Src family protein tyrosine kinases: cooperating with growth factor and adhesion signaling pathways. Curr Opin Cell Biol 9: 187-192.

Paululat A, Holz A & Renkawitz-Pohl R (1999) Essential genes for myoblast fusion in Drosophila embryogenesis. Mech Dev 83: 17-26.

Pavalko FM, Schneider G, Burridge K & Lim SS (1995) Immunodetection of alpha-actinin in focal adhesions is limited by antibody inaccessibility. Exp Cell Res 217: 534-540.

Pawson T (1995) Protein modules and signalling networks. Nature 373: 573-580.

Pece S, Chiariello M, Murga C & Gutkind JS (1999) Activation of the protein kinase Akt/PKB by the formation of E-cadherin- mediated cell-cell junctions. Evidence for the association of phosphatidylinositol 3-kinase with the E-cadherin adhesion complex. J Biol Chem 274: 19347-19351.

Peifer M, McCrea PD, Green KJ, Wieschaus E & Gumbiner BM (1992) The vertebrate adhesive junction proteins beta-catenin and plakoglobin and the Drosophila segment polarity gene armadillo form a multigene family with similar properties. J Cell Biol 118: 681-691.

Perez-Moreno M, Avila A, Islas S, Sanchez S & Gonzalez-Mariscal L (1998) Vinculin but not alpha-actinin is a target of PKC phosphorylation during junctional assembly induced by calcium. J Cell Sci 111 (Pt 23): 3563-3571.

Perona R, Montaner S, Saniger L, Sanchez-Perez I, Bravo R & Lacal JC (1997) Activation of the nuclear factor-kappaB by Rho, CDC42, and Rac-1 proteins. Genes Dev 11: 463-475.

Petersen AM, Blom J, Andersen LP & Krogfelt KA (2000) Role of strain type, AGS cells and fetal calf serum in Helicobacter pylori adhesion and invasion assays. FEMS Immunol Med Microbiol 29: 59-67.

Podbilewicz B & White JG (1994) Cell fusions in the developing epithelial of C. elegans. Dev Biol 161: 408-424.

Pollard TD (1976) Cytoskeletal functions of cytoplasmic contractile proteins. J Supramol Struct 5: 317-334.

Popoff MR, Chaves-Olarte E, Lemichez E, Eichel-Streiber C, Thelestam M, Chardin P, Cussac D, Antonny B, Chavrier P, Flatau G, Giry M, de Gunzburg J & Boquet P (1996) Ras, Rap, and Rac small GTP-binding proteins are targets for Clostridium sordellii lethal toxin glucosylation. J Biol Chem 271: 10217-10224.

Pradhan D, Lombardo CR, Roe S, Rimm DL & Morrow JS (2001) alpha -Catenin binds directly to spectrin and facilitates spectrin- membrane assembly in vivo. J Biol Chem 276: 4175-4181.

Price-Schiavi SA, Carraway CA, Fregien N & Carraway KL (1998) Post-transcriptional regulation of a milk membrane protein, the sialomucin complex (Ascites sialoglycoprotein (ASGP)-1/ ASGP-2, rat muc4), by transforming growth factor beta. J Biol Chem 273: 35228-35237.

Puius YA, Mahoney NM & Almo SC (1998) The modular structure of actin-regulatory proteins. Curr Opin Cell Biol 10: 23-34.

Purchio AF, Erikson E, Brugge JS & Erikson RL (1978) Identification of a polypeptide encoded by the avian sarcoma virus src gene. Proc Natl Acad Sci U S A 75: 1567-1571.

Qiu RG, Abo A & Steven MG (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.

Rahikkala M, Sormunen R & Eskelinen S (2001) Effects of src kinase and TGFbeta1 on the differentiation and morphogenesis of MDCK cells grown in three-dimensional collagen and Matrigel environments. J Pathol 195: 391-400.

Rasheed S, Norman GL & Heidecker G (1983) Nucleotide sequence of the Rasheed rat sarcoma virus oncogene: new mutations. Science 221: 155-157.

Ren XD, Bokoch GM, Traynor-Kaplan A, Jenkins GH, Anderson RA & Schwartz MA (1996) Physical association of the small GTPase Rho with a 68-kDa phosphatidylinositol 4-phosphate 5-kinase in Swiss 3T3 cells. Mol Biol Cell 7: 435-442.

Retta SF, Barry ST, Critchley DR, Defilippi P, Silengo L & Tarone G (1996) Focal adhesion and stress fiber formation is regulated by tyrosine phosphatase activity. Exp Cell Res 229: 307-317.

Reynolds AB, Daniel J, McCrea PD, Wheelock MJ, Wu J & Zhang Z (1994) Identification of a new catenin: the tyrosine kinase substrate p120cas associates with E-cadherin complexes. Mol Cell Biol 14: 8333-8342.

Reynolds AB, Herbert L, Cleveland JL, Berg ST & Gaut JR (1992) p120, a novel substrate of protein tyrosine kinase receptors and of p60v-src, is related to cadherin-binding factors beta-catenin, plakoglobin and armadillo. Oncogene 7: 2439-2445.

Ridley AJ (2001) Rho GTPases and cell migration. J Cell Sci 114: 2713-2722.

Ridley AJ, Comoglio PM & Hall A (1995) Regulation of scatter factor/hepatocyte growth factor responses by Ras, Rac, and Rho in MDCK cells. Mol Cell Biol 15: 1110-1122.

Ridley AJ & Hall A (1992) The small GTP-binding protein rho regulates the assembly of focal adhesions and actin stress fibers in response to growth factors. Cell 70: 389-399.

Ridley AJ, Paterson HF, Johnston CL, Diekmann D & Hall A (1992) The small GTP-binding protein rac regulates growth factor-induced membrane ruffling. Cell 70: 401-410.

Rieder G, Hatz RA, Moran AP, Walz A, Stolte M & Enders G (1997) Role of adherence in interleukin-8 induction in Helicobacter pylori-associated gastritis. Infect Immun 65: 3622-3630.

Rimm DL, Koslov ER, Kebriaei P, Cianci CD & Morrow JS (1995) Alpha 1(E)-catenin is an actin-binding and -bundling protein mediating the attachment of F-actin to the membrane adhesion complex. Proc Natl Acad Sci U S A 92: 8813-8817.

Rindler MJ, Ivanov IE & Sabatini DD (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 & Nelson WJ (1989) Morphogenesis of the polarized epithelial cell phenotype. Science 245: 718-725.

Rodriguez-Boulan E & Powell SK (1992) Polarity of epithelial and neuronal cells. Annu Rev Cell Biol 8: 395-427.

Rodriguez Fernandez JL, Geiger B, Salomon D & Ben Ze'ev A (1992) Overexpression of vinculin suppresses cell motility in BALB/c 3T3 cells. Cell Motil Cytoskeleton 22: 127-134.

Rodriguez Fernandez JL, Geiger B, Salomon D & Ben Ze'ev A (1993) Suppression of vinculin expression by antisense transfection confers changes in cell morphology, motility, and anchorage-dependent growth of 3T3 cells. J Cell Biol 122: 1285-1294.

Rodriguez-Viciana P, Warne PH, Khwaja A, Marte BM, Pappin D, Das P, Waterfield MD, Ridley A & Downward J (1997) Role of phosphoinositide 3-OH kinase in cell transformation and control of the actin cytoskeleton by Ras. Cell 89: 457-467.

Rüdiger M (1998) Vinculin and alpha-catenin: shared and unique functions in adherens junctions. Bioessays 20: 733-740.

Saez JC, Berthoud VM, Moreno AP & Spray DC (1993) Gap junctions. Multiplicity of controls in differentiated and undifferentiated cells and possible functional implications. Adv Second Messenger Phosphoprotein Res 27: 163-198.

Sahai E & Marshall CJ (2002) ROCK and Dia have opposing effects on adherens junctions downstream of Rho. Nat Cell Biol 4: 408-415.

Saito Y (1997) [Analysis of the cellular functions of the small GTP-binding protein rho p21 with Clostridium botulinum C3 exoenzyme]. Nippon Yakurigaku Zasshi 109: 13-17.

Sakamoto M, Takamura M, Ino Y, Miura A, Genda T & Hirohashi S (2001) Involvement of c-Src in carcinoma cell motility and metastasis. Jpn J Cancer Res 92: 941-946.

Samakovlis C, Manning G, Steneberg P, Hacohen N, Cantera R & Krasnow MA (1996) Genetic control of epithelial tube fusion during Drosophila tracheal development. Development 122: 3531-3536.

Sander EE, ten Klooster JP, van Delft S, van der Kammen RA & Collard JG (1999) Rac downregulates Rho activity: reciprocal balance between both GTPases determines cellular morphology and migratory behavior. J Cell Biol 147: 1009-1022.

Sander EE, van Delft S, ten Klooster JP, Reid T, van der Kammen RA, Michiels F & Collard JG (1998) Matrix-dependent Tiam1/Rac signaling in epithelial cells promotes either cell-cell adhesion or cell migration and is regulated by phosphatidylinositol 3-kinase. J Cell Biol 143: 1385-1398.

Santos OF & Nigam SK (1993) HGF-induced tubulogenesis and branching of epithelial cells is modulated by extracellular matrix and TGF-beta. Dev Biol 160: 293-302.

Sato N, Funayama N, Nagafuchi A, Yonemura S, Tsukita S & Tsukita S (1992) A gene family consisting of ezrin, radixin and moesin. Its specific localization at actin filament/plasma membrane association sites. J Cell Sci 103 (Pt 1): 131-143.

Sato N, Yonemura S, Obinata T, Tsukita S & Tsukita S (1991) Radixin, a barbed end-capping actin-modulating protein, is concentrated at the cleavage furrow during cytokinesis. J Cell Biol 113: 321-330.

Satoh-Horikawa K, Nakanishi H, Takahashi K, Miyahara M, Nishimura M, Tachibana K, Mizoguchi A & Takai Y (2000) Nectin-3, a new member of immunoglobulin-like cell adhesion molecules that shows homophilic and heterophilic cell-cell adhesion activities. J Biol Chem 275: 10291-10299.

Satoh T, Nakafuku M & Kaziro Y (1992) Function of Ras as a molecular switch in signal transduction. J Biol Chem 267: 24149-24152.

Schlessinger J & Geiger B (1983) The dynamic interrelationships of actin and vinculin in cultured cells. Cell Motil 3: 399-403.

Schmidt G, Sehr P, Wilm M, Selzer J, Mann M & Aktories K (1997) Gln 63 of Rho is deamidated by Escherichia coli cytotoxic necrotizing factor-1. Nature 387: 725-729.

Schmidt JM, Zhang J, Lee HS, Stromer MH & Robson RM (1999) Interaction of talin with actin: sensitive modulation of filament crosslinking activity. Arch Biochem Biophys 366: 139-150.

Schmidt JW, Piepenhagen PA & Nelson WJ (1993) Modulation of epithelial morphogenesis and cell fate by cell-to-cell signals and regulated cell adhesion. Semin Cell Biol 4: 161-173.

Schneeberger EE & Lynch RD (1992) Structure, function, and regulation of cellular tight junctions. Am J Physiol 262: L647-L661.

Scholey JM, Rogers GC & Sharp DJ (2001) Mitosis, microtubules, and the matrix. J Cell Biol 154: 261-266.

Schwarz MA, Owaribe K, Kartenbeck J & Franke WW (1990) Desmosomes and hemidesmosomes: constitutive molecular components. Annu Rev Cell Biol 6: 461-491.

Schwartz MA, Schaller MD & Ginsberg MH (1995) Integrins: emerging paradigms of signal transduction. Annu Rev Cell Dev Biol 11: 549-599.

Scita G, Tenca P, Frittoli E, Tocchetti A, Innocenti M, Giardina G & Di Fiore PP (2000) Signaling from Ras to Rac and beyond: not just a matter of GEFs. EMBO J 19: 2393-2398.

Sefton BM, Hunter T, Ball EH & Singer SJ (1981) Vinculin: a cytoskeletal target of the transforming protein of Rous sarcoma virus. Cell 24: 165-174.

Segal ED, Cha J, Lo J, Falkow S & Tompkins LS (1999) Altered states: involvement of phosphorylated CagA in the induction of host cellular growth changes by Helicobacter pylori. Proc Natl Acad Sci U S A 96: 14559-14564.

Segal ED, Falkow S & Tompkins LS (1996) Helicobacter pylori attachment to gastric cells induces cytoskeletal rearrangements and tyrosine phosphorylation of host cell proteins. Proc Natl Acad Sci U S A 93: 1259-1264.

Segal ED, Shon J & Tompkins LS (1992) Characterization of Helicobacter pylori urease mutants. Infect Immun 60: 1883-1889.

Seger R & Krebs EG (1995) The MAPK signaling cascade. FASEB J 9: 726-735.

Seibenhener ML, Roehm J, White WO, Neidigh KB, Vandenplas ML & Wooten MW (1999) Identification of Src as a novel atypical protein kinase C-interacting protein. Mol Cell Biol Res Commun 2: 28-31.

Seidensticker MJ & Behrens J (2000) Biochemical interactions in the wnt pathway. Biochim Biophys Acta 1495: 168-182.

Sells MA, Knaus UG, Bagrodia S, Ambrose DM, Bokoch GM & Chernoff J (1997) Human p21-activated kinase (Pak1) regulates actin organization in mammalian cells. Curr Biol 7: 202-210.

Sharma SA, Tummuru MK, Blaser MJ & Kerr LD (1998) Activation of IL-8 gene expression by Helicobacter pylori is regulated by transcription factor nuclear factor-kappa B in gastric epithelial cells. J Immunol 160: 2401-2407.

Sheth B, Fesenko I, Collins JE, Moran B, Wild AE, Anderson JM & Fleming TP (1997) Tight junction assembly during mouse blastocyst formation is regulated by late expression of ZO-1 alpha+ isoform. Development 124: 2027-2037.

Small JV (1988) The actin cytoskeleton. Electron Microsc Rev 1: 155-174.

Small JV, Kaverina I, Krylyshkina O & Rottner K (1999a) Cytoskeleton cross-talk during cell motility. FEBS Lett 452: 96-99.

Small JV, Rottner K & Kaverina I (1999b) Functional design in the actin cytoskeleton. Curr Opin Cell Biol 11: 54-60.

Smoot DT, Resau JH, Naab T, Desbordes BC, Gilliam T, Bull-Henry K, Curry SB, Nidiry J, Sewchand J, Mills-Robertson K, Frontin K, Abebe E, Dillon M, Chippendale GR, Phelps PC, Scott VF & Mobley HLT (1993) Adherence of Helicobacter pylori to cultured human gastric epithelial cells. Infect Immun 61: 350-355.

Soriano P, Montgomery C, Geske R & Bradley A (1991) Targeted disruption of the c-src proto-oncogene leads to osteopetrosis in mice. Cell 64: 693-702.

Sormunen RT, Leong AS, Vääräniemi JP, Fernando SS & Eskelinen SM (1999) Immunolocalization of the fodrin, E-cadherin, and beta-catenin adhesion complex in infiltrating ductal carcinoma of the breast-comparison with an in vitro model. J Pathol 187: 416-423.

Stein M, Bagnoli F, Halenbeck R, Rappuoli R, Fantl WJ & Covacci A (2002) c-Src/Lyn kinases activate Helicobacter pylori CagA through tyrosine phosphorylation of the EPIYA motifs. Mol Microbiol 43: 971-980.

Steinert PM & Liem RK (1990) Intermediate filament dynamics. Cell 60: 521-523.

Steinert PM & Roop DR (1988) Molecular and cellular biology of intermediate filaments. Annu Rev Biochem 57: 593-625.

Stevenson BR, Siliciano JD, Mooseker MS & Goodenough DA (1986) Identification of ZO-1: a high molecular weight polypeptide associated with the tight junction (zonula occludens) in a variety of epithelia. J Cell Biol 103: 755-766.

Stossel TP (1984) Contribution of actin to the structure of the cytoplasmic matrix. J Cell Biol 99: 15s-21s.

Strasser P, Gimona M, Herzog M, Geiger B & Small JV (1993) Variable and constant regions in the C-terminus of vinculin and metavinculin. Cloning and expression of fragments in E. coli. FEBS Lett 317: 189-194.

Sulston JE, Schierenberg E, White JG & Thomson JN (1983) The embryonic cell lineage of the nematode Caenorhabditis elegans. Dev Biol 100: 64-119.

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.

Szaszi K, Kurashima K, Kapus A, Paulsen A, Kaibuchi K, Grinstein S & Orlowski J (2000) RhoA and rho kinase regulate the epithelial Na+/H+ exchanger NHE3. Role of myosin light chain phosphorylation. J Biol Chem 275: 28599-28606.

Takahashi K, Nakanishi H, Miyahara M, Mandai K, Satoh K, Satoh A, Nishioka H, Aoki J, Nomoto A, Mizoguchi A & Takai Y (1999) Nectin/PRR: an immunoglobulin-like cell adhesion molecule recruited to cadherin-based adherens junctions through interaction with Afadin, a PDZ domain-containing protein. J Cell Biol 145: 539-549.

Takai Y, Sasaki T & Matozaki T (2001) Small GTP-binding proteins. Physiol Rev 81: 153-208.

Takaishi K, Sasaki T, Kotani H, Nishioka H & Takai Y (1997) Regulation of cell-cell adhesion by rac and rho small G proteins in MDCK cells. J Cell Biol 139: 1047-1059.

Takeda H, Nagafuchi A, Yonemura S, Tsukita S, Behrens J, Birchmeier W & Tsukita S (1995) V-src kinase shifts the cadherin-based cell adhesion from the strong to the weak state and beta catenin is not required for the shift. J Cell Biol 131: 1839-1847.

Takeda H & Tsukita S (1995) Effects of tyrosine phosphorylation on tight junctions in temperature-sensitive v-src-transfected MDCK cells. Cell Struct Funct 20: 387-393.

Takeichi M (1995) Morphogenetic roles of classic cadherins. Curr Opin Cell Biol 7: 619-627.

Takeuchi K, Sato N, Kasahara H, Funayama N, Nagafuchi A, Yonemura S, Tsukita S & Tsukita S (1994) Perturbation of cell adhesion and microvilli formation by antisense oligonucleotides to ERM family members. J Cell Biol 125: 1371-1384.

Taniguchi H & Manenti S (1993) Interaction of myristoylated alanine-rich protein kinase C substrate (MARCKS) with membrane phospholipids. J Biol Chem 268: 9960-9963.

Tapon N, Nagata K, Lamarche N & Hall A (1998) A new rac target POSH is an SH3-containing scaffold protein involved in the JNK and NF-kappaB signalling pathways. EMBO J 17: 1395-1404.

Temm-Grove CJ, Wert D, Thompson VF, Allen RE & Goll DE (1999) Microinjection of calpastatin inhibits fusion in myoblasts. Exp Cell Res 247: 293-303.

Thomas SM & Brugge JS (1997) Cellular functions regulated by Src family kinases. Annu Rev Cell Dev Biol 13: 513-609.

Thoreson MA, Anastasiadis PZ, Daniel JM, Ireton RC, Wheelock MJ, Johnson KR, Hummingbird DK & Reynolds AB (2000) Selective uncoupling of p120(ctn) from E-cadherin disrupts strong adhesion. J Cell Biol 148: 189-202.

Tolias KF, Cantley LC & Carpenter CL (1995) Rho family GTPases bind to phosphoinositide kinases. J Biol Chem 270: 17656-17659.

Tombola F, Carlesso C, Szabo I, de Bernard M, Reyrat JM, Telford JL, Rappuoli R, Montecucco C, Papini E & Zoratti M (1999) Helicobacter pylori vacuolating toxin forms anion-selective channels in planar lipid bilayers: possible implications for the mechanism of cellular vacuolation. Biophys J 76: 1401-1409.

Towbin H, Staehelin T & Gordon J (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci U S A 76: 4350-4354.

Toyofuku T, Yabuki M, Otsu K, Kuzuya T, Hori M & Tada M (1998) Direct association of the gap junction protein connexin-43 with ZO-1 in cardiac myocytes. J Biol Chem 273: 12725-12731.

Trave G, Pastore A, Hyvönen M & Saraste M (1995) The C-terminal domain of alpha-spectrin is structurally related to calmodulin. Eur J Biochem 227: 35-42.

Troyanovsky SM, Troyanovsky RB, Eshkind LG, Leube RE & Franke WW (1994) Identification of amino acid sequence motifs in desmocollin, a desmosomal glycoprotein, that are required for plakoglobin binding and plaque formation. Proc Natl Acad Sci U S A 91: 10790-10794.

Tsukamoto T & Nigam SK (1999) Role of tyrosine phosphorylation in the reassembly of occludin and other tight junction proteins. Am J Physiol 276: F737-F750.

Tsukita S, Furuse M & Itoh M (1999) Structural and signalling molecules come together at tight junctions. Curr Opin Cell Biol 11: 628-633.

Tsukita S, Furuse M & Itoh M (2001) Multifunctional strands in tight junctions. Nat Rev Mol Cell Biol 2: 285-293.

Tsukita S, Oishi K, Akiyama T, Yamanashi Y, Yamamoto T & Tsukita S (1991) Specific proto-oncogenic tyrosine kinases of src family are enriched in cell-to-cell adherens junctions where the level of tyrosine phosphorylation is elevated. J Cell Biol 113: 867-879.

Tsukita S, Tsukita S, Nagafuchi A & Yonemura S (1992) Molecular linkage between cadherins and actin filaments in cell-cell adherens junctions. Curr Opin Cell Biol 4: 834-839.

Tsukita S, Yonemura S & Tsukita S (1997a) ERM proteins: head-to-tail regulation of actin-plasma membrane interaction. Trends Biochem Sci 22: 53-58.

Tsukita S, Yonemura S & Tsukita S (1997b) ERM (ezrin/radixin/moesin) family: from cytoskeleton to signal transduction. Curr Opin Cell Biol 9: 70-75.

Turner CE, Glenney JR, Jr. & Burridge K (1990) Paxillin: a new vinculin-binding protein present in focal adhesions. J Cell Biol 111: 1059-1068.

Vaheri A, Carpen O, Heiska L, Helander TS, Jääskeläinen J, Majander-Nordenswan P, Sainio M, Timonen T & Turunen O (1997) The ezrin protein family: membrane-cytoskeleton interactions and disease associations. Curr Opin Cell Biol 9: 659-666.

Van Aelst L & D'Souza-Schorey C (1997) Rho GTPases and signaling networks. Genes Dev 11: 2295-2322.

Van Aelst L, Joneson T & Bar-Sagi D (1996) Identification of a novel Rac1-interacting protein involved in membrane ruffling. EMBO J 15: 3778-3786.

Vanhaesebroeck B, Stein RC & Waterfield MD (1996) The study of phosphoinositide 3-kinase function. Cancer Surv 27: 249-270.

Vojtek AB & Der CJ (1998) Increasing complexity of the Ras signaling pathway. J Biol Chem 273: 19925-19928.

Volberg T, Geiger B, Dror R & Zick Y (1991) Modulation of intercellular adherens-type junctions and tyrosine phosphorylation of their components in RSV-transformed cultured chick lens cells. Cell Regul 2: 105-120.

Volberg T, Geiger B, Kam Z, Pankov R, Simcha I, Sabanay H, Coll JL, Adamson E & Ben Ze'ev A (1995) Focal adhesion formation by F9 embryonal carcinoma cells after vinculin gene disruption. J Cell Sci 108 (Pt 6): 2253-2260.

Volberg T, Zick Y, Dror R, Sabanay I, Gilon C, Levitzki A & Geiger B (1992) The effect of tyrosine-specific protein phosphorylation on the assembly of adherens-type junctions. EMBO J 11: 1733-1742.

Vääräniemi J, Huotari V, Lehto VP & Eskelinen S (1994) The effects of PMA and TFP and alterations in intracellular pH and calcium concentration on the membrane associations of phospholipid- binding proteins fodrin, protein kinase C and annexin II in cultured MDCK cells. Biochim Biophys Acta 1189: 21-30.

Vääräniemi J, Huotari V, Lehto VP & Eskelinen S (1997) Effect of PMA on the integrity of the membrane skeleton and morphology of epithelial MDCK cells is dependent on the activity of amiloride- sensitive ion transporters and membrane potential. Eur J Cell Biol 74: 262-272.

Vääräniemi J, Palovuori R, Lehto VP & Eskelinen S (1999) Translocation of MARCKS and reorganization of the cytoskeleton by PMA correlates with the ion selectivity, the confluence, and transformation state of kidney epithelial cell lines. J Cell Physiol 181: 83-95.

Wang AZ, Ojakian GK & Nelson WJ (1990) Steps in the morphogenesis of a polarized epithelium. I. Uncoupling the roles of cell-cell and cell-substratum contact in establishing plasma membrane polarity in multicellular epithelial (MDCK) cysts. J Cell Sci 95 (Pt 1): 137-151.

Wang DS & Shaw G (1995) The association of the C-terminal region of beta I sigma II spectrin to brain membranes is mediated by a PH domain, does not require membrane proteins, and coincides with a inositol-1,4,5 triphosphate binding site. Biochem Biophys Res Commun 217: 608-615.

Warren SL & Nelson WJ (1987) Nonmitogenic morphoregulatory action of pp60v-src on multicellular epithelial structures. Mol Cell Biol 7: 1326-1337.

Watanabe N, Madaule P, Reid T, Ishizaki T, Watanabe G, Kakizuka A, Saito Y, Nakao K, Jockusch BM & Narumiya S (1997) p140mDia, a mammalian homolog of Drosophila diaphanous, is a target protein for Rho small GTPase and is a ligand for profilin. EMBO J 16: 3044-3056.

Watton SJ & Downward J (1999) Akt/PKB localisation and 3' phosphoinositide generation at sites of epithelial cell-matrix and cell-cell interaction. Curr Biol 9: 433-436.

Weekes J, Barry ST & Critchley DR (1996) Acidic phospholipids inhibit the intramolecular association between the N- and C-terminal regions of vinculin, exposing actin-binding and protein kinase C phosphorylation sites. Biochem J 314 (Pt 3): 827-832.

Weiss EE, Kroemker M, Rüdiger AH, Jockusch BM & Rüdiger M (1998) Vinculin is part of the cadherin-catenin junctional complex: complex formation between alpha-catenin and vinculin. J Cell Biol 141: 755-764.

Welch MD & Mullins RD (2002) Cellular control of actin nucleation. Annu Rev Cell Dev Biol 18: 247-288.

Wells A (1999) EGF receptor. Int J Biochem Cell Biol 31: 637-643.

Werth DK, Niedel JE & Pastan I (1983) Vinculin, a cytoskeletal substrate of protein kinase C. J Biol Chem 258: 11423-11426.

Wessler S, Hocker M, Fischer W, Wang TC, Rosewicz S, Haas R, Wiedenmann B, Meyer TF & Naumann M (2000) Helicobacter pylori activates the histidine decarboxylase promoter through a mitogen-activated protein kinase pathway independent of pathogenicity island-encoded virulence factors. J Biol Chem 275: 3629-3636.

Wheelock MJ & Jensen PJ (1992) Regulation of keratinocyte intercellular junction organization and epidermal morphogenesis by E-cadherin. J Cell Biol 117: 415-425.

Whitmarsh AJ, Yang SH, Su MS, Sharrocks AD & Davis RJ (1997) Role of p38 and JNK mitogen-activated protein kinases in the activation of ternary complex factors. Mol Cell Biol 17: 2360-2371.

Winkelmann JC & Forget BG (1993) Erythroid and nonerythroid spectrins. Blood 81: 3173-3185.

Wodarz A & Nusse R (1998) Mechanisms of Wnt signaling in development. Annu Rev Cell Dev Biol 14: 59-88.

Wolburg H, Wolburg-Buchholz K, Liebner S & Engelhardt B (2001) Claudin-1, claudin-2 and claudin-11 are present in tight junctions of choroid plexus epithelium of the mouse. Neurosci Lett 307: 77-80.

Woods DF & Bryant PJ (1993) Apical junctions and cell signalling in epithelia. J Cell Sci Suppl 17: 171-181.

Xu W, Baribault H & Adamson ED (1998) Vinculin knockout results in heart and brain defects during embryonic development. Development 125: 327-337.

Yahiro K, Niidome T, Kimura M, Hatakeyama T, Aoyagi H, Kurazono H, Imagawa K, Wada A, Moss J & Hirayama T (1999) Activation of Helicobacter pylori VacA toxin by alkaline or acid conditions increases its binding to a 250-kDa receptor protein-tyrosine phosphatase beta. J Biol Chem 274: 36693-36699.

Yamada KM & Geiger B (1997) Molecular interactions in cell adhesion complexes. Curr Opin Cell Biol 9: 76-85.

Yamada KM & Miyamoto S (1995) Integrin transmembrane signaling and cytoskeletal control. Curr Opin Cell Biol 7: 681-689.

Yamaguchi H & Wang HG (2001) The protein kinase PKB/Akt regulates cell survival and apoptosis by inhibiting Bax conformational change. Oncogene 20: 7779-7786.

Yamamoto T, Harada N, Kano K, Taya S, Canaani E, Matsuura Y, Mizoguchi A, Ide C & Kaibuchi K (1997) The Ras target AF-6 interacts with ZO-1 and serves as a peripheral component of tight junctions in epithelial cells. J Cell Biol 139: 785-795.

Yamaoka Y, Kodama T, Gutierrez O, Kim JG, Kashima K & Graham DY (1999) Relationship between Helicobacter pylori iceA, cagA, and vacA status and clinical outcome: studies in four different countries. J Clin Microbiol 37: 2274-2279.

Yap AS, Brieher WM & Gumbiner BM (1997) Molecular and functional analysis of cadherin-based adherens junctions. Annu Rev Cell Dev Biol 13: 119-146.

Yap AS, Niessen CM & Gumbiner BM (1998) The juxtamembrane region of the cadherin cytoplasmic tail supports lateral clustering, adhesive strengthening, and interaction with p120ctn. J Cell Biol 141: 779-789.

Yasuda T, Ohtsuka T, Inoue E, Yokoyama S, Sakisaka T, Kodama A, Takaishi K & Takai Y (2000) Importance of spatial activation of Cdc42 and rac small G proteins by frabin for microspike formation in MDCK cells. Genes Cells 5: 583-591.

Yeaman C, Grindstaff KK, Hansen MD & Nelson WJ (1999) Cell polarity: Versatile scaffolds keep things in place. Curr Biol 9: R515-R517.

Yonemura S, Itoh M, Nagafuchi A & Tsukita S (1995) Cell-to-cell adherens junction formation and actin filament organization: similarities and differences between non-polarized fibroblasts and polarized epithelial cells. J Cell Sci 108 (Pt 1): 127-142.

Young P, Ferguson C, Banuelos S & Gautel M (1998) Molecular structure of the sarcomeric Z-disk: two types of titin interactions lead to an asymmetrical sorting of alpha-actinin. EMBO J 17: 1614-1624.

Yu J, Fischman DA & Steck TL (1973) Selective solubilization of proteins and phospholipids from red blood cell membranes by nonionic detergents. J Supramol Struct 1: 233-248.

Zahraoui A, Joberty G, Arpin M, Fontaine JJ, 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.

Zamir E & Geiger B (2001) Molecular compexity and dynamics of cell-matrix adhesions. J Cell Sci 114: 3583-3590.

Zhang S, Han J, Sells MA, Chernoff J, Knaus UG, Ulevitch RJ & Bokoch GM (1995) Rho family GTPases regulate p38 mitogen-activated protein kinase through the downstream mediator Pak1. J Biol Chem 270: 23934-23936.

Zheng Y, Zangrilli D, Cerione RA & Eva A (1996) The pleckstrin homology domain mediates transformation by oncogenic dbl through specific intracellular targeting. J Biol Chem 271: 19017-19020.

Zhong C, Kinch MS & Burridge K (1997) Rho-stimulated contractility contributes to the fibroblastic phenotype of Ras-transformed epithelial cells. Mol Biol Cell 8: 2329-2344.

Zondag GC, Evers EE, ten Klooster JP, Janssen L, van der Kammen RA & Collard JG (2000) Oncogenic Ras downregulates Rac activity, which leads to increased Rho activity and epithelial-mesenchymal transition. J Cell Biol 149: 775-782.

Zschiesche W, Schonborn I, Behrens J, Herrenknecht K, Hartveit F, Lilleng P & Birchmeier W (1997) Expression of E-cadherin and catenins in invasive mammary carcinomas. Anticancer Res 17: 561-567.