A novel cell-cell junction system: the *cortex adhaerens* mosaic of lens fiber cells

Beate K. Straub[1], Judit Boda[1], Caecilia Kuhn[1], Martina Schnoelzer[2], Ulrike Korf[2], Tore Kempf[2], Herbert Spring[3], Mechthild Hatzfeld[4] and Werner W. Franke[1,*]

[1]Division of Cell Biology, German Cancer Research Center, Im Neuenheimer Feld 280, 69120 Heidelberg, Germany
[2]Protein Analysis Facility, German Cancer Research Center, Im Neuenheimer Feld 280, 69120 Heidelberg, Germany
[3]Structural Analysis Group, German Cancer Research Center, Im Neuenheimer Feld 280, 69120 Heidelberg, Germany
[4]Institute for Physiological Chemistry, Medical Faculty, University of Halle, Magdeburger Strasse 18, 06097 Halle/Saale, Germany
*Author for correspondence (e-mail: w.franke@dkfz.de)

Accepted 4 August
Journal of Cell Science 116, 4985-4995 © 2003 The Company of Biologists Ltd
doi:10.1242/jcs.00815

Summary

The anucleate prismoid fiber cells of the eye lens are densely packed to form a tissue in which the plasma membranes and their associated cytoplasmic coat form a single giant cell-cell adhesive complex, the *cortex adhaerens*. Using biochemical and immunoprecipitation methods in various species (cow, pig, rat), in combination with immunolocalization microscopy, we have identified two different major kinds of cortical complex. In one, the transmembrane glycoproteins N-cadherin and cadherin-11 [which also occur in heterotypic ('mixed') complexes] are associated with α- and β-catenin, plakoglobin (proportions variable among species), p120^{ctn} and vinculin. The other complex contains ezrin, periplakin, periaxin and desmoyokin (and so is called the EPPD complex), usually together with moesin, spectrin(s) and plectin. In sections through lens fiber tissue, the short sides of the lens fiber hexagons appear to be enriched in the cadherin-based complexes, whereas the EPPD complexes also occur on the long sides. Moreover, high resolution double-label fluorescence microscopy has revealed, on the short sides, a finer, almost regular mosaicism of blocks comprising the cadherin-based, catenin-containing complexes, alternating with patches formed by the EPPD complexes. The latter, a new type of junctional plaque ensemble of proteins hitherto known only from certain other cell types, must be added to the list of major lens cortex proteins. We here discuss its possible functional importance for the maintenance of lens structure and functions, notably clear and sharp vision.

Key words: Lens fibers, Adherens Junctions, *Cortex adhaerens*, Cadherins, Catenins, Ezrin, Moesin, Periaxin, Periplakin, Desmoyokin

Introduction

The functions of a specific tissue generally depend on its architecture, which is usually based on dual-function cell-cell adhesion elements. These morphologically distinct plasma membrane domains serve on the one hand as position-specific cell-cell attachment structures and, on the other, provide cytoplasmic anchorage plates ('plaques') for cytoskeletal filament bundles. Here, the 'adhering junctions' (e.g. Farquhar and Palade, 1963; Staehelin, 1974), which share some molecular principles because they comprise clusters of glycoproteins of the larger cadherin multigene family (Koch et al., 1990; Takeichi, 1988; Takeichi, 1991) and possess the common arm-repeat plaque protein plakoglobin (Cowin et al., 1986), are usually divided into two major categories with specific, mutually exclusive constituents. (1) The 'adherens junctions', pleiomorphic as *zonulae adhaerentes*, *fasciae adhaerentes* or *puncta adhaerentia*, are formed by 'classic' cadherins, which on their cytoplasmic side are complexed with a set of plaque proteins dominated by α- and β-catenins, and anchor actin microfilaments (Nagafuchi and Takeichi, 1989; Ozawa et al., 1989). (2) By contrast, the desmosomes (*maculae adhaerentes*) are assemblies of two special cadherin subgroups (the desmogleins and the desmocollins), with a plaque characterized by desmoplakin and members of the plakophilin subfamily of *arm*-repeat proteins, which anchors bundles of intermediate-sized filaments (IFs) (cf. Franke et al., 1981; Schmidt et al., 1994; Schmidt et al., 1999; Hatzfeld, 1999; Green and Gaudry, 2000).

It has, however, become increasingly clear over the past decade that there are diverse adhering junctions that cannot be subsumed under these two major categories but represent special structures sui generis. Examples include the *complexus adhaerentes* described in special vascular endothelia, notably the retothelial cells of lymph node sinus (Schmelz and Franke, 1993; Valiron et al., 1996), the M-/N-cadherin containing *contactus adhaerentes* connecting the cells of the granule layer of the cerebellum (Rose et al., 1995; Hollnagel et al., 2002), the *area composita* in the intercalated disks connecting cardiomyocytes (e.g. C. M. Borrmann, Molekulare Charakterisierung der Adhärens-Zellverbindungen des Herzens: Identifizierung einer neuen Art, der Area composita, PhD Thesis, University of Heidelberg, Germany, 2000) and the heterotypic adhering junctions connecting the photoreceptor and Mueller glia cells of the retina, which characteristically contain the arm-protein neurojungin (Paffenholz et al., 1999).

The eye lens contains a central mass of densely packed anucleate fiber cells surrounded by a layer of cells with epithelioid features, often referred to as 'epithelium'; this layer

is in turn surrounded by a capsule of extracellular matrix material (for reviews, see Maisel et al., 1981; Rafferty, 1985). The physical laws of the vision process require transparency and homogeneity of the lens body. Therefore, the tight package of the lens fibers, with frequent and often regularly spaced interdigitations, as well as the high concentration and homogenous distribution of cytoplasmic proteins, including loosely arranged cytoskeletal filaments, are crucial for lens function (Ramaekers et al., 1980; Benedetti et al., 1981; Maisel et al., 1981; Ramaekers and Bloemendal, 1981). It is thus not surprising that diverse disturbances of the composition and distribution of lens proteins all lead to cataract formation (e.g. Capetanaki et al., 1989; Duncan et al., 2000; He and Li, 2000; Jakobs et al., 2000; Krutovskikh and Yamasaki, 2000).

Previously, we have noted that the cell-cell interactive structure of the lens fibers represents a relatively thin but extended cortex around the entire cell, obviously a cell-type-specific junctional complex (Schmidt et al., 1994). This cortex comprises the plasma membrane proper and a subjacent plaque-equivalent layer that only rarely shows distinct substructures (cf. Franke et al., 1987; Lo et al., 1997; Lo et al., 2000) but is generally associated with actin microfilaments, actin-binding proteins (ABPs) and adhering junction proteins including plakoglobin (Franke et al., 1987), α- and β-catenin (Bassnett et al., 1999; Duncan et al., 2000; Bagchi et al., 2002), α-actinin and vinculin (Geiger et al., 1985; Beebe et al., 2001). N-Cadherin has been reported to be the most prominent, if not the only, cadherin present (e.g. Hatta and Takeichi, 1986; Atreya et al., 1989; Citi et al., 1994; Lo et al., 2000; Bagchi et al., 2002). More recently, however, we have noticed that this cortex is highly complex and heterogenous, and comprises a set of proteins hitherto not shown – or even expected – in the lens.

Materials and Methods

Tissues and cultured cells

Bovine and porcine eyes were obtained freshly from a local slaughterhouse, murine (mouse and rat) eyes from animals of the animal house of the German Cancer Research Center. For immunohistochemistry, eyes were enucleated and lenses routinely snap-frozen in isopentane cooled with liquid nitrogen to a temperature of about –130°C. For biochemical experiments, lenses were separated into the outermost 'epithelioid' cell layer attached to the capsule, the cortex, and the 'nucleus' and the dissected tissue portions were used either directly or frozen in liquid nitrogen and kept at –80°C until needed. Cultured epithelial cells of the human lines HaCaT, PLC (ATCC CRL-8024) and CaCo-2 (ATCC HTB-37), the canine line MDCK (ATCC CCL-34), the bovine line MDBK (ATCC CCL-22) and calf lens cells were grown as described (Ramaekers et al., 1980; Peitsch et al., 1999).

Antibodies and reagents

Antibodies used included mouse monoclonal antibodies (mAbs) against: ezrin (3C12), N-cadherin, vinculin (hvin-1), actin and tropomyosin from Sigma (St Louis, MO, USA); fodrin/spectrin (MAB 1822) from Chemicon (Hofheim/Taunus, Germany); drebrin from MoBiTec (Göttingen, Germany); E-, N-, P- and R-cadherin, cadherin-5, moesin, α- and β-catenin, and p120^{ctn} from Transduction Laboratories (Lexington, KY, USA); protein p0071 (mAb 6D-1-10) (Hatzfeld et al., 2003); and cadherin-11 from Zymed (South San Francisco, CA, USA). In addition, we used a series of mAbs from Progen Biotechnik (Heidelberg, Germany): desmoplakins (DP I/II, 2.15, 2.17 and 2.20) (Cowin et al., 1985), desmogleins Dsg1-Dsg3 (e.g. Dsg 1&2: 3.10.), desmocollins Dsc1-Dsc3 (e.g. mAbs U100 and U114), plakoglobin [Pg 5.10 (Cowin et al., 1986); 11E4], vimentin (3B4 and V9) (Hermann et al., 1989), plakophilins PKP1-PKP3 (Mertens et al., 1996; Schmidt et al., 1999) and an antibody to neurojungin (J 19.97) that in lens tissue also reacts with phakinin (cf. Paffenholz et al., 1999). Rabbit antibodies routinely used were against α-catenin, β-catenin, pan-cadherin, tropomyosin, α-actinin, l/s-afadin, l-afadin (Sigma) (for a review, see Takai and Nakanishi, 2003), protein ZO-1, connexin Cx 43, ponsin, claudin-1, occludin, cadherin-11 (from Zymed), non-muscle myosin heavy chain (Biotrend, Cologne, Germany), merlin/NF-2 from Santa Cruz Biotechnology (Heidelberg, Germany), CD44 (generous gift from M. Zöller, German Cancer Research Center) and protein p0071 (Hatzfeld et al., 2003). Specific guinea-pig antisera against plectin were also used (P2, from H. Herrmann, German Cancer Research Center) (cf. Schröder et al., 1999).

Monoclonal antibodies against desmoyokin (Dy 2.4. and Dy 47.27.5) obtained in this laboratory were systematically compared with desmoyokin rabbit antisera kindly provided by T. Hashimoto (Department of Dermatology, Keio University of Medicine, Tokyo, Japan) (cf. Hashimoto et al., 1993). To generate further antibodies specific for human desmoyokin, the synthetic peptides D1 (amino acids 2038-2056: PDVKIPKFKKPKFGFGPKS; 'AHNAK fragment', accession number A45259), D2 (amino acids 2792-2812: PKGKGGVTGSPEASISGSKGD) and D4 (amino acids 298-320: PNLEGTLTGPRLGSPSGKTGT; all peptides used were from Peptide Specialty Laboratories, Heidelberg, Germany) were coupled to KLH and used to immunize guinea pigs after dissolution in Freund's complete adjuvant (Sigma). After three booster injections using Freund's incomplete adjuvant, the animals were anesthetized and blood was collected by heart puncture.

Periplakin antibodies used included murine mAbs [AE11 (Ma and Sun, 1986); clone IIb (Simon and Green, 1984); generous gifts from T. T. Sun (Department of Dermatology, New York University Medical Center, NY) and M. Simon (School of Dental Medicine, SUNY, Stony Brooks, NY, USA)] as well as guinea pig antibodies generated against three synthetic peptides, P1 (amino acids 7-24: KRNKGKYSPTVQTRSISN; accession number AAC 17738), P2 (amino acids 336-356: LRKVDSDLNQKYGPDFKDRYQ) and P3 (amino acids 815-835: ENGRSSHVSKRARLQSPATKV) as described above for desmoyokin.

Immunohistochemistry

Lens cryosections of ~5 μm thickness were air dried for several hours and fixed for 5 minutes in methanol, followed by 5 minutes in acetone both at –20°C. Because of the fragile character of lens, the incubation protocols were optimized for relatively brief periods of incubation time, and the specimens were usually incubated with the primary antibodies diluted in PBS for 30 minutes, followed by two repeated washes in PBS for 5 minutes or less each, incubation with the secondary antibodies for 30 minutes, and two subsequent 5-minute washes in PBS. The sections were then rinsed in distilled water, fixed for 5 minutes in ethanol and mounted in Fluoromount G (Biozol Diagnostica, Eching, Germany). To enhance the accessibility of certain large proteins such as desmoyokin and periplakin to immunoglobulins, the specimens were initially exposed to PBS containing either 0.1% Triton X-100 or 0.1% saponin and then washed twice in PBS for 5 minutes each. Immunofluorescence of cell cultures and of other tissues was performed as described (Peitsch et al., 1999).

Epifluorescence was observed and documented with a Zeiss Axiophot photomicroscope, confocal laser-scanning immunofluorescence microscopy was done with a Zeiss LSM 510 (Zeiss, Jena, Germany).

Fractionation of tissues and cell cultures

Cortex preparations from several lenses were homogenized with either a Dounce or a Potter-Elvehjem homogenizer (Braun, Melsungen, Germany) at very high volume-to-tissue mass ratios in low salt buffer (50 mM Tris-HCl, pH 7.4, 5 mM $MgCl_2$, 1 mM DTT) containing the protease inhibitor phenylmethylsulfonylfluoride (PMSF) at 1 mM or Pefablock SC (Roche Diagnostics, Mannheim, Germany). Pellets obtained after centrifugation at 4°C at 18,000 ***g*** (in a Beckman centrifuge Optima XL-70, München, Germany) for 30 minutes contained the 'water-insoluble' particle fraction (WIF), primarily the cytoskeleton and membranous structures, whereas the supernatant contained the water-soluble particle fraction (WSF) including the crystallins (Alcala et al., 1975). This procedure was repeated twice with the WIF to minimize residual crystallins. Tissues other than lens and cell cultures were directly homogenized in SDS sample buffer as described (Peitsch et al., 1999).

Gel electrophoresis and immunoblotting SDS-PAGE, and two-dimensional gel electrophoresis involving either non-equilibrium pH-gradient electrophoresis (NEPHGE) or isoelectric focusing (IEF) were performed as described (Achtstätter et al., 1986). For SDS-PAGE, samples were suspended in electrophoresis buffer (250 mM Tris-HCl, pH 6.8, 20% SDS, 25% glycerol, 125 mM DTT), often with the addition of benzonase (1:1000; Merck, Darmstadt, Germany). For NEPHGE or IEF, protein samples were precipitated with methanol and chloroform, and solubilized in lysis buffer containing 9.5 M urea, 2.0% NP-40, 2.0% ampholine and 20 mM DTT.

Immunoblotting was performed using PVDF membranes (Millipore, Bedford, MA, USA). After blocking with 10% non-fat dry milk in Tris-buffered saline containing 0.1% Tween (TBST) for at least 1 hour, blots were incubated with the primary antibodies in PBS for 1 hour, followed by three washes in TBST for 30 minutes each. Horseradish peroxidase (HRP)-conjugated antibodies to rabbit, mouse or guinea pig IgG (diluted 1:10000 in TBST) were applied for 30 minutes, followed by a 30 minute wash in TBST and enhanced chemiluminescence (ECL; Amersham Biosciences, Freiburg, Germany).

Immunoprecipitations

Pelleted fractions were suspended in immunoprecipitation buffer (20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 5 mM EDTA or 0.5 mM $CaCl_2$, 1% Triton X-100, 1 mM DTT, 1 mM PMSF or Pefablock SC) and centrifuged for 15 minutes at 14,900 ***g*** (in an Eppendorf centrifuge 5414, Hamburg, Germany) and 4°C. The supernatant obtained was then precleared with protein-A- or protein-G-coupled Sepharose for several hours, and the supernatant obtained after centrifugation was reacted overnight with protein A and/or protein G beads coated with the specific antibody in 50 mM Tris-HCl, pH 7.5. The pellet obtained was solubilized in 20-40 μl sample buffer, and the immunoprecipitate was separated using SDS-PAGE. Protein gels stained with colloidal Coomassie Blue (Novex, Frankfurt, Germany) were used to analyse unknown bands by peptide mass fingerprinting [matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS)] and amino acid sequence analysis (see below).

Tryptic digestion

Protein bands were excised from the gel and cut into 1×1 mm pieces that were washed twice with deionized water, 50% acetonitrile/water (1:1) and acetonitrile. Proteins were digested overnight with sequencing-grade modified trypsin (Promega) in 40 mM ammonium bicarbonate at 37°C. The reaction was stopped by freezing.

MALDI-MS

MALDI mass spectra were recorded in positive ion reflector mode with delayed extraction on a Reflex II time-of-flight instrument (Bruker-Daltonik, Bremen, Germany) equipped with a SCOUT multiprobe inlet and a 337-nm nitrogen laser. The ion-acceleration voltage was set to 20.0 kV, the reflector voltage to 21.5 kV and the first extraction plate to 15.4 kV. Mass spectra were obtained by averaging 50-200 individual laser shots. Calibration of the spectra was performed internally by a two-point linear fit using the autolysis products of trypsin at mass:charge ratios of 842.50 and 2211.10.

For the mass spectrometric analysis of tryptic digests, MALDI samples were prepared on thin film spots (Jensen et al., 1996). Briefly, 0.3 μl aliquots of a nitrocellulose-containing saturated solution of α-cyano-4-hydroxycinnamic acid (Sigma-Aldrich) in acetone were deposited onto individual spots on the target. Subsequently, 0.8 μl 10% formic acid and 0.4 μl of the digest sample were loaded on top of the thin film spots and allowed to dry slowly at ambient temperature. To remove salts from the digestion buffer, the spots were washed with 1% formic acid and with water.

Post-source-decay analysis

Post-source-decay (PSD) analysis was performed in positive ion reflector mode with delayed extraction by setting an ion gate width of 40 Da around the ion of interest. Data were acquired in 14 segments by decreasing the reflector voltage in a stepwise fashion. For each segment, 200 individual laser shots were accumulated. The fragment ion spectrum was obtained by pasting together all segments to a single spectrum using the FAST software (Bruker). Fragment ion calibration was performed externally with the fragment masses of the adrenocorticotropic hormone (ACTH) 18-39 clip.

Sample preparation for PSD analysis was achieved by cocrystallization of matrix with samples concentrated using Zip Tip C18 (Millipore, Schwalbach, Germany). Briefly, the peptides in the supernatant of the in-gel digestion were absorbed to a prewashed (50% acetonitrile/water) and equilibrated (0.1% trifluoroacetic acid/water) Zip Tip C18 by repetitive pipetting steps. Following washing of the Zip Tip C18 by equilibration buffer, the peptides were eluted from the Zip Tip with 1 μl of matrix (α-cyano-4-hydroxycinnamic acid saturated in 50% acetonitrile/water).

Database search

Singly charged monoisotopic peptide masses were used for database searching. Searches were performed against the NCBInr database using the ProFound search algorithm (http://129.85.19.192/prowl-cgi/ProFound.exe) and the Protein prospector software developed at the University of California, San Francisco (http://prospector.ucsf.edu/), with an IEP range of 0-14 and the oxidation of methionine as a possible modification. Up to one missed tryptic cleavage was considered, and the mass tolerance for the monoisotopic peptide masses was set to ±100 ppm or ±0.1 Da.

Searches with fragment masses from PSD experiments were performed against the NCBInr database using the MS-Tag search algorithm provided by Protein prospector. Parent mass tolerance was set to ±100 ppm and fragment ion tolerance was set to ±1500 ppm.

Amino acid sequence analysis

For high-performance liquid chromatography (HPLC) separation the tryptic digest was extracted twice with 0.1% trifluoroacetic acid (TFA) in 60% acetonitrile. After concentration on a SpeedVac, the extracted tryptic peptides were separated on a HPLC system equipped with a 140B solvent delivery system (Applied Biosystems, Weiterstadt, Germany), Acurate splitter (LC Packings, Idstein, Germany), UV absorbance detector 759A (Applied Biosystems), U-Z capillary flow cell (LC Packings) and a Probot fraction collector (BAI, Lautertal, Germany) using a reversed-phase column (Hypersil C18 BDS, mean particle diameter 3 μm, 0.3×150 mm) and a linear gradient from 12% acetonitrile in 0.1%TFA to 64% acetonitrile in 0.08% TFA in 90 minutes, with a flow rate of 4 μl $minute^{-1}$ at room temperature.

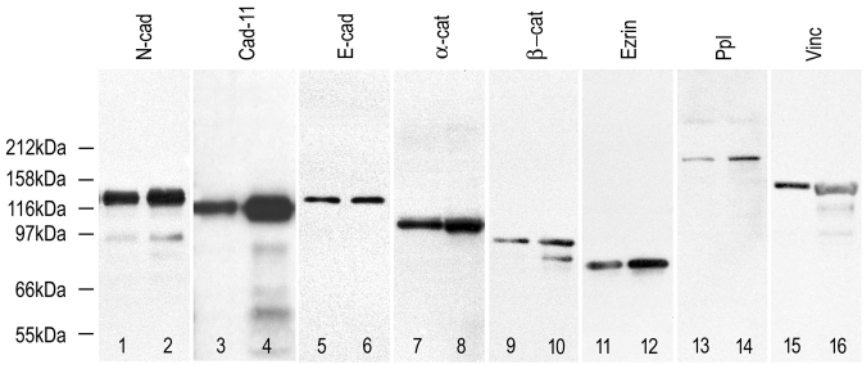

Fig. 1. SDS-PAGE and immunoblot analysis of junctional proteins of the cytoskeletal fractions from bovine lens fiber cells, including cortical regions. Proteins of water-insoluble fractions were separated by 8% PAGE and probed with antibodies to the following proteins: lanes 1 and 2, N-cadherin (N-cad); lanes 3 and 4, cadherin-11 (Cad-11); lanes 5 and 6, E-cadherin (E-cad); lanes 7 and 8, α-catenin (α-cat); lanes 9 and 10, β-catenin (β-cat); lanes 11 and 12, ezrin (81 kDa); lanes 13 and 14, periplakin (~190 kDa) (Ppl); lanes 15 and 16, vinculin (Vinc). Two lanes are shown for each probe, the left with a lower protein load and the right with a higher protein load. Molecular weights of reference proteins examined in parallel are given on the left margin.

Peptide elution was monitored at 214 nm and individual fractions from the HPLC separation were re-analysed by MALDI-MS. Sequence analysis of selected peptide-containing fractions was performed on a Procise Protein Sequencer 494 cLC using standard programs supplied by Applied Biosystems.

Results

In previous immunohistochemical studies of mammalian lenses and in biochemical analyses of cytoskeleton fractions obtained therefrom, we had noted that the border structures of the anucleate lens fiber cells were compositionally and structurally different from other kinds of junctions and collectively referred to this large complex as the *cortex adhaerens* (Franke et al., 1987; Schmidt et al., 1994). Moreover, because we found unexpectedly many proteins in lens cortical structures, we decided to examine the composition of the *cortex adhaerens* more systematically.

Biochemical analysis

When total proteins from mammalian lens fiber tissue, without the capsule and the outermost ‘epithelioid’ cell layer, were extracted with relatively large amounts of ‘low salt buffer’, a residual (‘cytoskeletal’) fraction was obtained that revealed, on SDS-PAGE or two-dimensional gel electrophoresis, a remarkably complex protein pattern. In addition to the IF proteins vimentin, phakinin and filensin (e.g. Lieska et al., 1980; Ramaekers et al., 1980; Merdes et al., 1991; Merdes et al., 1993; Perng et al., 1999) (U. Haus, Zur molekularbiologischen Charakterisierung von Cytoskelett-Proteinen der Rinderlinse. Diploma Thesis, University of Cologne, Germany, 1990), some typical junctional proteins were consistently seen in all three species studied. These included the transmembrane glycoproteins N-cadherin, cadherin-11 and – in much lower amounts – E-cadherin (Fig. 1, lanes 1-6), which seemed to be restricted to the outer layers, as well as the plaque proteins α- and β-catenin (Fig. 1, lanes 7-10), p120^{ctn} (see below) and, in amounts markedly differing in different species, plakoglobin. Although the finding of an N-cadherin-based ensemble of adhering junction proteins essentially confirmed earlier reports (e.g. Geiger et al., 1985; Cowin et al., 1986; Hatta and Takeichi, 1986; Franke et al., 1987; Atreya et al., 1989; Bassnett et al., 1999; Ferreira-Cornwell et al., 2000; Leong et al., 2000; Bagchi et al., 2002), the recognition that the *cortices adhaerentes* of lens fibers contained the type-II cadherin-11 in similar large amounts was novel. On the one hand, it was compatible with the general widespread occurrence of cadherin-11 in diverse kinds of mesenchymal and other mesodermally derived cells but, on the other hand, it was surprising because it had not been detected in previous studies of lens tissue (e.g. Hoffmann and Balling, 1995; Simonneau et al., 1995; Hadeball et al., 1998; Simonneau and Thiery, 1998). The other cadherins examined (see Materials and Methods) were not detected. Among the cytoskeletal proteins, we regularly detected vinculin (Fig. 1, lanes 15 and 16), α-actinin, actin, tropomyosin, myosin, spectrin, ankyrin and plectin (not shown), all of which had previously been reported to occur in lens-fiber cortices (e.g. Kibbelaar et al., 1979; Repasky et al., 1982; Allen et al., 1987; Franke et al., 1987; Weitzer and Wiche, 1987; Lee et al., 2000).

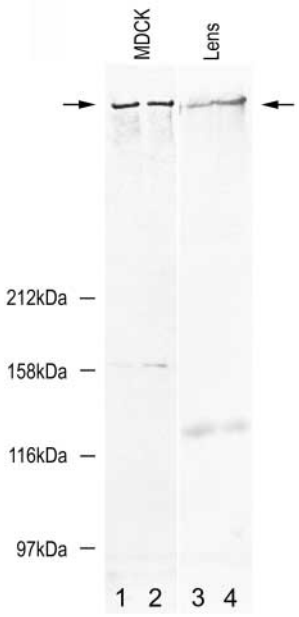

Fig. 2. SDS-PAGE and immunoblot detection of the very large (>600 kDa) protein desmoyokin. On SDS-PAGE-separation (5.5% gel) of total proteins, desmoyokin (arrows) is detected in cultured kidney epithelial cells of line MDCK (lanes 1 and 2) as a positive control and in bovine lens fiber cells (lanes 3 and 4). Molecular weights of reference proteins examined in parallel are indicated on the left.

Much to our surprise, however, we also noted among the major lens junction proteins a series of plaque components such as ezrin (Fig. 1, lanes 11 and 12), periplakin (Fig. 1, lanes

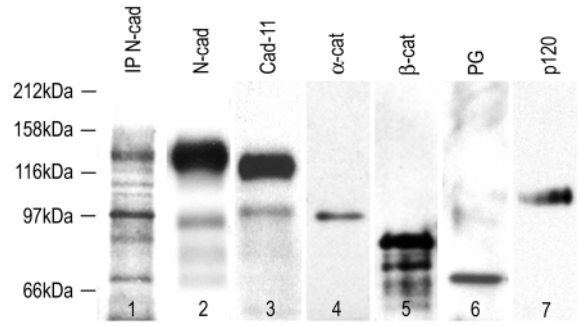

Fig. 3. Identification of the N-cadherin-associated protein complexes in lens fiber plasma membranes, demonstrated by immunoprecipitation, SDS-PAGE (8%) and immunoblotting. (Lane 1) Coomassie-Blue-stained major polypeptides of the immunoprecipitate obtained from water-insoluble, 1%-Triton X-100-soluble fractions from Sepharose-bound antibodies against N-cadherin. (Lanes 2-7) Proteins of immunoprecipitates subjected to SDS-PAGE were probed by immunoblotting with antibodies against N-cadherin (lane 2; N-cad), cadherin-11 (lane 3; Cad-11), α-catenin (lane 4; α-cat), β-catenin (lane 5; β-cat), plakoglobin (lane 6; PG) and p120^{ctn} (lane 7; p120). Antibodies against E-cadherin showed no immunoreactivity; similarly controls using Sepharose-bound uncoated or coated with antibodies against desmoplakins or desmocollins were negative. Molecular weight markers are indicated on the left.

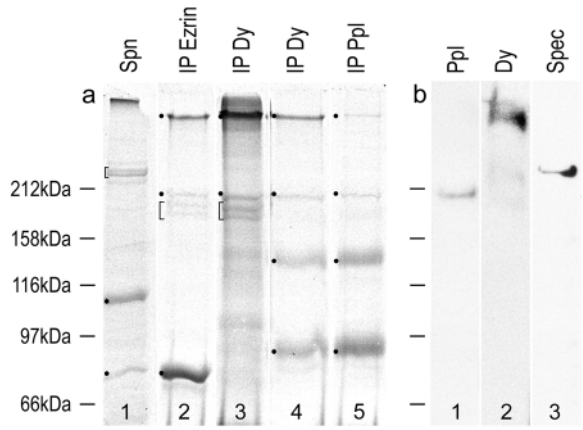

Fig. 4. Molecular complexes identified in junctional plaques present in cytoskeletal fractions from lens fibers, as identified by immunoprecipitations followed by SDS-PAGE (6%), immunoblotting and MALDI-MS. (a) Coomassie-Blue-stained proteins present in immunoprecipitates from water-insoluble, 1%-Triton-X-100-soluble fractions from bovine lens fiber cells. (Lane 1) Major proteins of the supernatant used for immunoprecipitation are indicated by symbols on the left margin (from top): [spectrin/fodrin, • filensin, and • ezrin (81 kDa). (Lane 2) Immunoprecipitate obtained with the monoclonal ezrin antibody 3C12, showing from top: • desmoyokin (>600 kDa), • periplakin (~190 kDa), [periaxin (two bands of ~170 kDa and ~150 kDa) and • ezrin. (Lane 3) Immunoprecipitation with rabbit antibodies against desmoyokin (IP Dy), showing (from top): • desmoyokin, • periplakin and [periaxin. (Lane 4) Immunoprecipitate obtained with guinea pig antibodies against desmoyokin (IP Dy), showing (from top): • desmoyokin, • periplakin, together with • and • guinea pig immunoglobulin chains. (Lane 5) Immunoprecipitate obtained with guinea pig antibodies against periplakin (IP Ppl): • desmoyokin and • periplakin, together with • and • guinea pig immunoglobulin chains. (b) Immunoprecipitate obtained with ezrin antibody [as in (a), lane 2] was subjected to SDS-PAGE and probed with antibodies against periplakin (lane 1; Ppl), desmoyokin (lane 2; Dy), spectrin/fodrin (lane 3; Spec), showing the presence of these proteins in the immunoprecipitated ezrin complexes. Controls of proteins bound to blank Sepharose and to Sepharose coated with antibodies to desmoplakins or desmocollins were negative. Molecular weight markers are indicated on the left.

13 and 14), periaxin (see also below) and desmoyokin (Figs 2, 4), which so far had only been reported from other kinds of cells [ezrin (Bretscher, 1983; Bretscher et al., 1997); periplakin (Simon and Green, 1984); periaxin (Gillespie et al., 1994); desmoyokin (cf. Hieda et al., 1989; Shtivelman et al., 1992; Hashimoto et al., 1993)]. In addition, we found considerable amounts of moesin in our immunoblots of total lens fiber proteins, but no significant signals for merlin [see below, however, for reports of the occurrence of merlin in outer (i.e. epithelioid) cells of lenses] (see Claudio et al., 1995; Claudio et al., 1997; Huynh et al., 1996).

To identify the protein complexes containing these lens cortex proteins, we performed immunoprecipitations, subjected the pelleted proteins to SDS-PAGE, excised the bands under question and analysed them by MALDI-MS, PSD and amino acid sequencing. The results allowed us to determine the complement of proteins associated with the specific antigen. For example, N-cadherin immunoprecipitates consistently contained not only the associated plaque proteins α- and β-catenin, plakoglobin and p120^{ctn}, but also remarkable amounts of cadherin-11 (Fig. 3). Conversely, considerable proportions of N-cadherin were identified in the immunoprecipitates obtained with antibodies to cadherin-11. In lens tissue material also containing outer cortical cell layers, junctional plaque proteins were detected in combinations with both N- and E-cadherin as well as with cadherin-11 (data not shown). These results also showed for the first time the existence of such heterotypic cadherin complexes with common plaque proteins but did not yet allow to distinguish between lateral heterocomplexes in the same membrane from transcellular heterotypic complexes of cadherins (e.g. Volk et al., 1987); that is, from the 'heterocadherins' in the sense used by Duguay et al. (Duguay et al., 2003) [for the controversial literature on cadherin organization see Shapiro et al., and others (Shapiro et al., 1995; Leckband and Sivasankar, 2000; Boggon et al., 2002; Ahrens et al., 2003)]. By contrast, ezrin immunoprecipitates contained desmoyokin, periplakin and periaxin (Table 1; Fig. 4a), and, when further probed with specific antibodies, positive reactions were also seen for spectrin (Fig. 4b), plectin and moesin (data not shown). These results left no doubt that desmoyokin was a major protein of lens fiber cells, where it mostly occurred in ezrin complexes, apparently together with periplakin and periaxin (Fig. 4a), suggesting the existence of a special category of large EPPD plaque complexes. For reasons not yet clarified, however, we detected little if any ezrin and moesin in the reciprocal immunoprecipitates of desmoyokin and periplakin (data not shown).

In similar biochemical experiments, we failed to detect in lens fibers significant amounts of desmosome-specific proteins such as desmoplakins, desmogleins, desmocollins and plakophilins, or of the plaque proteins neurojungin, merlin,

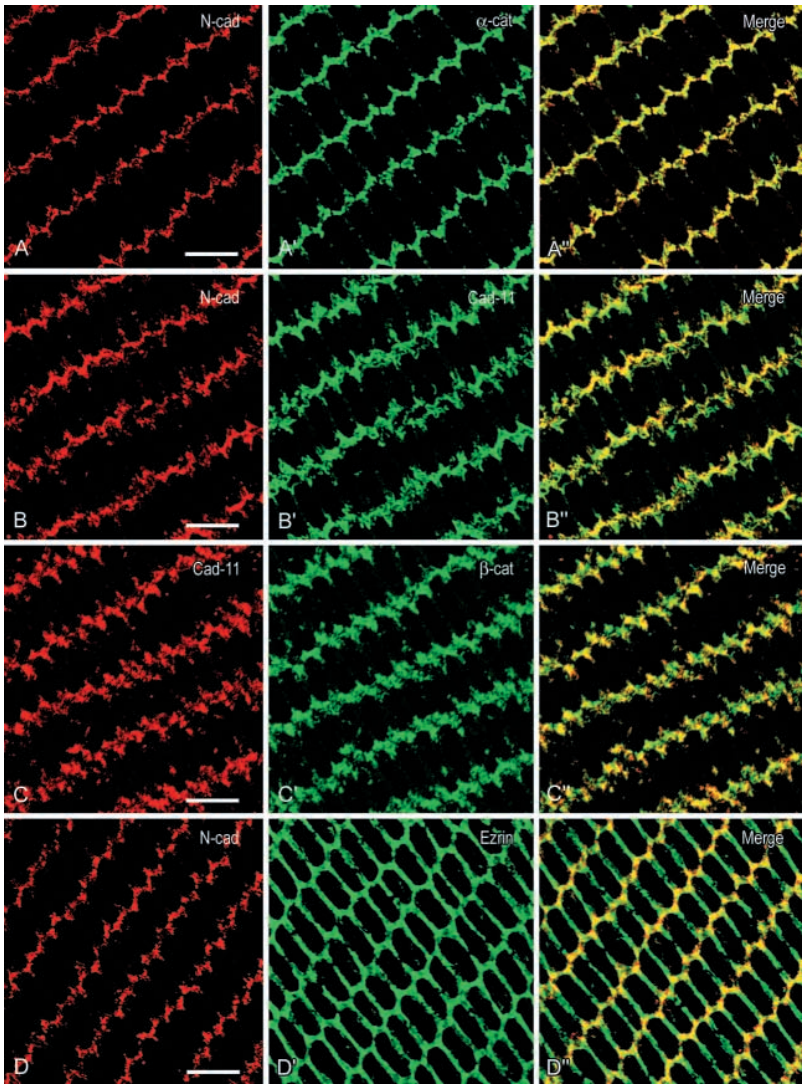

Fig. 5. Immunolocalization of adherens junction proteins in cryosections through near-cortical regions of bovine lens fiber cells. Immunofluorescence microscopy showing the hexagonal appearance of the fiber cells, with lateral long sides and apical short sides, after double immunolabeling with antibodies against N-cadherin (A,B,D, red; N-cad) in combination with either α-catenin (A′, green; α-cat) or cadherin-11 (B′, green; Cad-11), with antibodies against cadherin-11 in combination with β-catenin (C′, green; β-cat), or with a combination of antibodies to N-cadherin and ezrin (D′, green) observed by confocal laser scanning microscopy. Notice the far-reaching colocalizations of N-cadherin and α-catenin (A″, merged picture), cadherin-11 (B″, merge) and – shown here indirectly – with β-catenin (C″, merge), whereas N-cadherin localized close to ezrin on the short sides, often indicative of partial colocalization (D-D″; for higher resolution see, however, Fig. 8). Notice also the weak, if not absent, reaction of N-cadherin on the long sides. Scale bars, 10 μm.

Table 1. Peptides identified in proteins of ezrin and desmoyokin immunoprecipitates

Polypeptide (kDa)	Amino acid sequence	Identification
>600	FSMPGFK†	Desmoyokin
	ISMPDVDLNLK†	
	GEGPEVDVNLPK†	
~190	VVLQQDPQQAR*	Periplakin
	LTPAQYDR*	
	YEEEPGLR*	
~170 and ~150	PEGPRVAVGTGEAGFR* (1)	Periaxin
	IPEVELVTPGAQETEK* (2)	
	VPEMAVPEVR* (3)	
	FTAPQVELVGPR* (4)	

Immunoprecipitation was performed with antibodies against ezrin, using the fractions of water-insoluble and Triton X-100 soluble proteins from bovine lens tissue. Proteins were separated by SDS-PAGE, and bands were stained with colloidal Coomassie Blue, excised and analysed. The same four major proteins of >600, ~190, ~170 and ~150 kDa were also found to co-immunoprecipitate with desmoyokin. Amino acid sequences were identified by MALDI-MS and PSD (*), in some cases followed by amino acid sequence analysis (†). Peptides 1 and 2 show high homology to rat periaxin, whereas peptides 3 and 4 are homologous to KIAA 1620, a human brain protein most likely corresponding to human periaxin.

afadin, ponsin and drebrin (results not shown). The negative results obtained for the transmembrane glycoprotein CD44 were surprising because this protein has been reported in previous studies of lens material by other authors (Nishi et al., 1997; Saika et al., 1998), but we have not yet excluded all the diverse CD44 variants.

Immunohistochemistry

Neither in our electron micrographs nor by immunofluorescence microscopy did we find, in sections through lens fibers, any indication of the presence of tight junctions, including negative reactions for occludin and several claudins (cf. Langbein et al., 2002), and of desmosomes (see also Franke et al., 1987). By contrast, various sizes of gap junctions were regularly found by electron microscopy and with antibodies to both connexins and protein ZO-1, especially in the central region of the long cell sides, confirming previous reports (Giepmans and Molenaar, 1998; Toyofuku et al., 1998; Nielsen et al., 2001). The cytoplasm displayed the notorious intense positivity for IF proteins such as vimentin (cf. Ramaekers et al., 1980) and phakinin (e.g. Fig. 6B) (cf. Merdes et al., 1991; Merdes et al., 1993), whereas actin and the ABPs examined appeared to be generally enriched in the cortical zone (cf. Kibbelaar et al., 1979; Lo, 1988; Lo et al., 1997).

Using immunohistochemistry, N-cadherin and cadherin-11 were the only cadherins that consistently reacted at the contacts of lens fiber cells, very intensely at the short apical sides and rather weakly, sometimes hardly visible at all, along the long lateral sides (Fig. 5A-C). By contrast, immunostaining for E-cadherin was weakly present in the outer cortical layers but diminished centripetally in a steep gradient (not shown). Particularly at the short sides, both N-cadherin and cadherin-11 colocalized with both α-catenin and the major *arm*-repeat proteins β-catenin (Fig. 5A-C″), plakoglobin and p120^{ctn} (results not shown), as well as with actin and vinculin (cf. Volk and Geiger, 1984; Geiger et al., 1985; Franke et al., 1987).

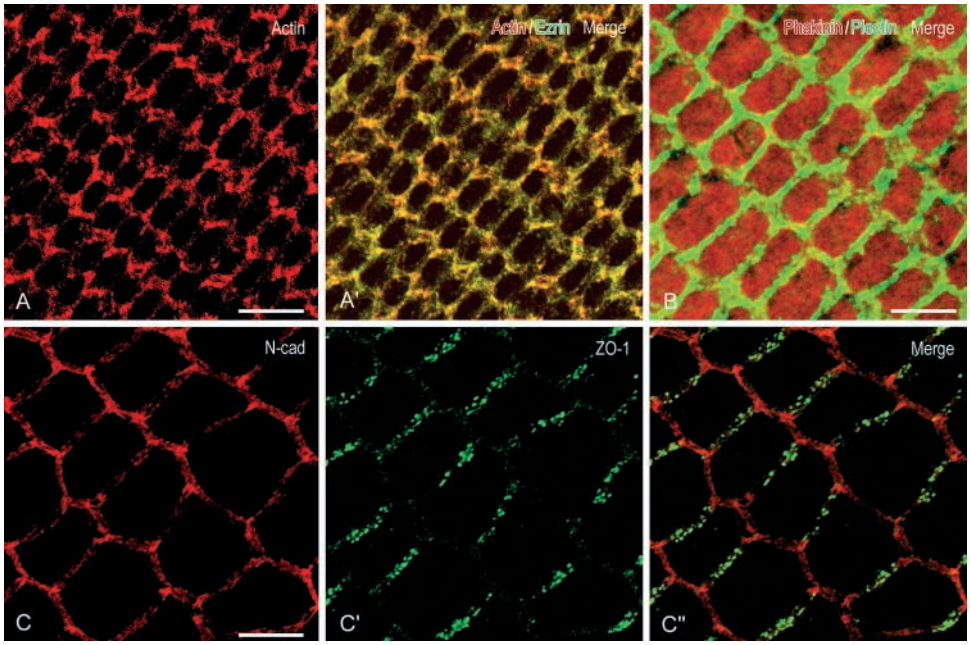

Fig. 6. Double-label immunofluorescence microscopy of bovine lens fiber cells stained for different cytoskeletal and junctional proteins. Actin (A, red) shows far-reaching colocalization with ezrin (green, notice the yellow reaction in A′, merged picture). Almost the entire cell cortex is also positive for plectin (B, green), shown in contrast to the glycoprotein phakinin (B, red). By contrast, in the plane shown in C-C″, antibodies to N-cadherin (C, red; N-cad) intensely decorate the narrow sides, but seem to be excluded from large parts of the lateral membranes, specifically the punctate reaction sites corresponding to gap junctions, which in turn are positive for protein ZO-1 (C″, merged picture). Scale bar, 10 μm.

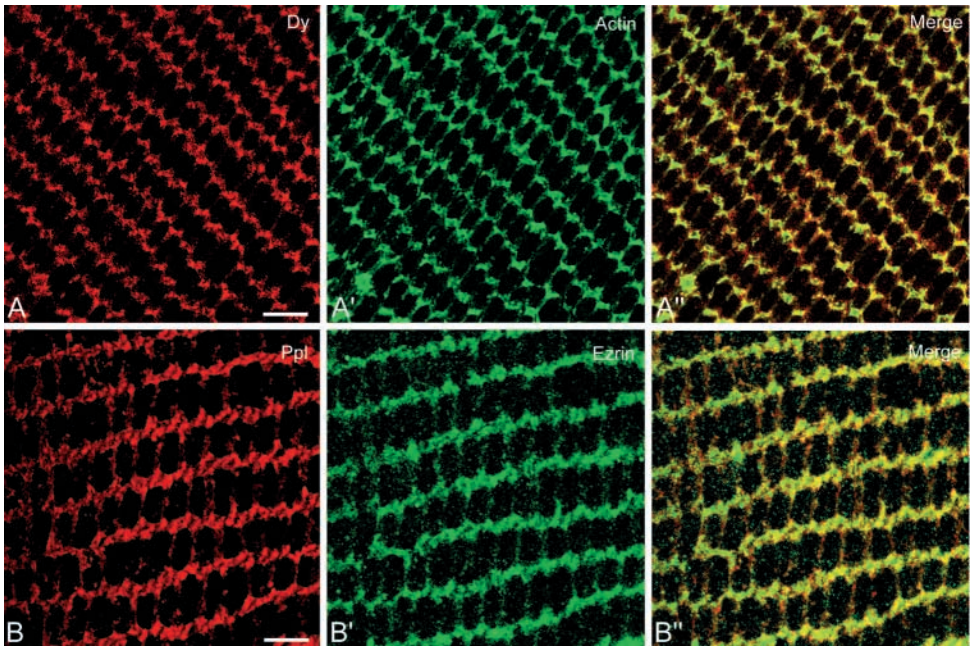

Fig. 7. Double-label immunofluorescence microscopy of desmoyokin (AHNAK protein), periplakin, ezrin and actin in cortical fiber cells of bovine lens. Desmoyokin (A, red, Dy) occurs along the entire membrane but is enriched on the short sides, showing far-reaching colocalization with actin (A′, green; A″ merged picture). Similarly, periplakin (B, red) shows extensive colocalization with ezrin (B′, green; B″, merged picture). Scale bar, 10 μm.

Specifically in bovine lens, the plakoglobin reaction was relatively strong in the outermost cell layers, but was practically undetectable in the lens interior [for other species, see Franke et al. (Franke et al., 1987)]. Again, the immunoreaction of all these proteins was very intense at the short sides and weak on the long sides.

This immunohistochemical reaction of typical proteins of the *cortex adhaerens* on the short sides was somewhat different from the pattern of other junction-associated proteins, in particular several ABPs such as ezrin (Fig. 5D-D″, Fig. 6A,A′), moesin (not shown) and plectin (Fig. 6B), all of which showed immunostaining on both the short and the long sides, although mostly again more intensely on the short sides. By stark contrast, gap junction proteins such as connexin Cx 43 and protein ZO-1 appeared in clusters of punctate reaction sites in the central region of the long sides (Fig. 6C-C″). Clearly, however, the newly discovered large lens proteins desmoyokin (Fig. 7A-A″) and periplakin (Fig. 7B-B″) showed, like ezrin and moesin, intense immunostaining on the short sides, whereas their reactions on the long sides were in most regions weak and appeared to be interrupted in places. Moreover, at higher resolution, the cortical proteins enriched on the short sides differed in their distribution patterns and displayed a mutually exclusive patchwork (Fig. 8) – although the typical junctional plaque proteins (such as α- and β-catenin) colocalized with N-cadherin (Fig. 8A) and cadherin-11 (Fig.

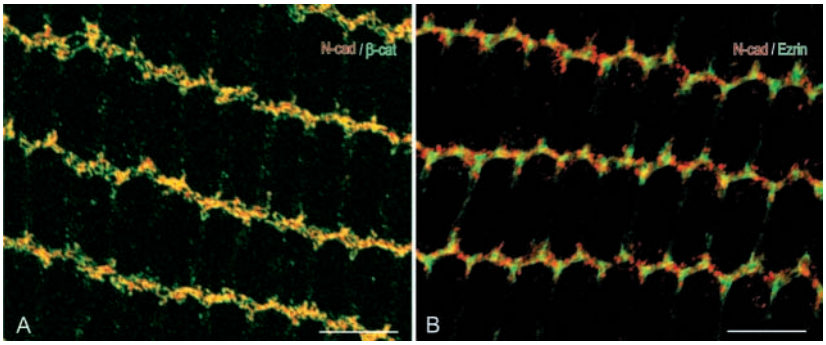

Fig. 8. Higher magnification of double-label immunofluorescence microscopy (merged images) showing N-cadherin (red) in combination with β-catenin (A, green; β-cat) and ezrin (B, green) on the short sides of bovine lens fiber cells. Notice the practically complete colocalization of N-cadherin with catenin (yellow), different from the only partial colocalization with ezrin, which for the most part seems to occur in patches (green) alternating with N-cadherin-positive (red) patches. Scale bar, 10 μm.

5C-C″), ezrin, moesin, periplakin and desmoyokin were concentrated in interspersed regions that did not react with N-cadherin (Fig. 8B) or any other cadherin (not shown), although there were also regions of overlap indicative of colocalization (Fig. 8B; see also Fig. 5D).

The immunofluorescence reaction pattern of merlin [another cortical protein related to ezrin and moesin (for a review, see Bretscher et al., 2002)] was surprising in two ways. It was generally weak, often negative, in the more cortical regions of the lens fiber mass and stronger in the deeper regions, opposite to what has been reported for mouse and chicken lens (e.g. Claudio et al., 1995; Claudio et al., 1997; Huynh et al., 1996); however, where positive, it often appeared with nearly equal intensity on both the long and the short sides.

Immunolocalization reactions for neurojungin, afadins, ponsin, drebrin and protein CD44 were negative.

Discussion

The extended structure which so tightly connects the plasma membranes of the anucleate fiber cells of the lens represents one large, mostly homogeneous-looking cortex suggestive of a continuous adhering junction complex. This giant *cortex adhaerens* is interrupted only sparsely by locally densified adherens plaques (e.g. Rafferty, 1985; Franke et al., 1987; Lo et al., 1997; Lo et al., 2000) and by gap junctions containing a specific set of connexins to which certain cytoplasmic plaque proteins such as ZO-1 are attached. Although this *cortex adhaerens* appears structurally rather uniform under the electron microscope (e.g. Ramaekers et al., 1980; Rafferty, 1985; Franke et al., 1987; Lo, 1988; Lo et al., 2000), our detailed studies have shown marked biochemical complexity and mosaicism.

Taken together, the present results show that the *cortex adhaerens* hexagons, notably the short sides, are characterized by a typical adhaerens junction ensemble, dominated by N-cadherin and cadherin-11 as major transmembrane glycoproteins and a plaque comprising not only α- and β-catenin, but also plakoglobin, p120^{ctn} and vinculin. The results of our N-cadherin/cadherin-11 cross-immunoprecipitation experiments have also directly demonstrated intimate complexes of different type-I and type-II cadherins with the plaque proteins mentioned, and experiments are under way to decide whether these are ipso- or heterocellular cadherin complexes. The immunocytochemical results further suggest that the long-side cortex of the fiber cells, at least in the species examined, contains much less of the adherens junction components but is relatively rich in ABPs characteristic of other microfilament-anchorage complexes, including band 4 proteins (Aster et al., 1984; Allen et al., 1987), spectrin (Nelson et al., 1983; Green and Maisel, 1984; Thomas, 2001) and plectin (Weitzer and Wiche, 1987).

Our surprising finding of a totally novel group of actin filament anchorage proteins in the lens now adds another ensemble of cytoskeletal proteins to the *cortex adhaerens* that have hitherto been reported only from diverse other cells. These include ezrin and moesin [cortical ABPs of various epithelial and certain non-epithelial cells (e.g. Bretscher, 1983; Bretscher et al., 1997; Yonemura et al., 1998; Bretscher et al., 2002)], periplakin [a desmoplakin-related protein so far found only in epidermis and other stratified epithelia (Ruhrberg et al., 1997; DiColandrea et al., 2000; Karashima and Watt, 2002) [for periplakin gene transcripts in certain other cells, see also Aho et al. (Aho et al., 1998)] and desmoyokin [a large protein with a hotly debated location (e.g. Hieda et al., 1989; Shtivelman et al., 1992; Hashimoto et al., 1993; Shtivelman and Bishop, 1993; Masanuga et al., 1995)]. In addition, in such complexes, we have detected periaxin, a protein originally identified in myelinating Schwann cells, where it is enriched at plasma membranes (e.g. Gillespie et al., 1994; Melendez-Vasquez et al., 2001). Our immunoprecipitation results have further shown that lens-fiber ezrin occurs in the same junctional plaque complexes as periplakin, periaxin and desmoyokin, often also in association with lens spectrin(s), which suggests but does not yet prove that all these proteins can co-assemble into a giant cortical EPPD complex.

The constitutive occurrence of desmoyokin and periaxin in the *cortex adhaerens* of lens fibers is especially noteworthy because both proteins have been reported as ‘dual location proteins’ that occur in certain plasma membrane regions as well as in the nucleoplasm of a broad variety of cell types (e.g. Shtivelman et al., 1992; Shtivelman and Bishop, 1993; Masanuga et al., 1995; Sherman and Brophy, 1999; Nie et al., 2000; Sussmann et al., 2001). The mere absence of nuclei in the lens fiber cells now also demonstrates that desmoyokin and periaxin are indeed major, stable components of adhering junctions that can occur in a long-lasting form in the absence of a nucleus. In this context, it is also worth mentioning that desmoyokin has recently been shown in the *area composita* plaques of cardiac intercalated disks (e.g. Hohaus et al., 2002).

At present, the transmembrane protein(s) to which EPPD complexes are attached are still elusive. Although we cannot formally exclude some contribution of cadherins to these cortical complexes, their relatively low concentrations along

the long sides of the fiber cell cortices (see Results) (Bassnett et al., 1999; Lo et al., 2000; Beebe et al., 2001) and their absence in our EPPD immunoprecipitates tend to suggest an involvement of other transmembrane proteins. Because the known ezrin-binding protein CD44 (Tsukita et al., 1994; Yonemura et al., 1998) has not been detected in our ezrin immunoprecipitates, we will have systematically to examine the series of known candidates of possible transmembrane partners in the EPPD complex (for a review, see Bretscher et al., 2002). It will also be interesting to investigate the possible existence of junctional plaque complexes of the EPPD category in other cell types.

Our high-resolution double-label immunofluorescence microscopy also revealed a mosaicism of the *cortex adhaerens*, in particular on the short sides of hexagons, where *puncta-adhaerentia*-type complexes comprising cadherins and catenins alternate with junctional structures containing EPPD complexes. Whether this regular patchwork pattern is a general characteristic of the junction system and how it is formed in the development of lens fibers from the 'epithelioid' cells of the lens surface, remain to be studied. Considering the frequency and sensitivity with which alterations of protein composition of lens fibers result in cataract formations, including changes of junctional components such as gap junction connexins (Martinez-Wittingham et al., 2003), we expect that gene abrogation experiments will probably help to elucidate the functional importance of the molecular complexity and pattern arrangement of the *cortex adhaerens*.

We thank C. Grund and S. Winter-Simanowski for excellent technical help, J. Osterholt for expert photographic work, and E. Gundel for typing the manuscript. The work has been supported in part by a grant of the Deutsche Forschungsgemeinschaft (DFG).

References

Achtstätter, T., Hatzfeld, M., Quinlan, R. A., Parmelee, D. C. and Franke, W. W. (1986). Separation of cytokeratin polypeptides by gel electrophoretic and chromatographic techniques and their identification by immunoblotting. *Methods Enzymol.* **134**, 355-371.

Aho, S., McLean, W. H. I., Li, K. and Uitto, J. (1998). cDNA cloning, mRNA expression, and chromosomal mapping of human and mouse periplakin genes. *Genomics* **48**, 242-247.

Ahrens, T., Lambert, M., Pertz, O., Sasaki, T., Schulthess, T., Mège, R.-M., Timpl, R. and Engel, J. (2003). Homoassociation of VE-cadherin follows a mechanism common to 'classical' cadherins. *J. Mol. Biol.* **325**, 733-742.

Alcala, J., Lieska, N. and Maisel, H. (1975). Protein composition of bovine lens cortical fiber cell membranes. *Exp. Eye Res.* **21**, 581-595.

Allen, D. P., Low, P. S., Dola, A. and Maisel, H. (1987). Band 3 and ankyrin homologues are present in eye lens: evidence for all major erythrocyte membrane components in same non-erythroid cell. *Biochem. Biophys. Res. Commun.* **149**, 266-275.

Aster, J. C., Brewer, G. J., Hanashi, M. and Maisel, H. (1984). Band 4.1-like proteins of the bovine lens. *Biochem. J.* **224**, 609-616.

Atreya, P. L., Barnes, J., Katar, M., Alcala, J. and Maisel, H. (1989). N-cadherin of the human lens. *Curr. Eye Res.* **8**, 947-956.

Bagchi, M., Katar, M., Lewis, J. and Maisel, H. (2002). Associated proteins of lens adherens junction. *J. Cell. Biochem.* **86**, 700-703.

Bassnett, S., Missey, H. and Vucemilo, I. (1999). Molecular architecture of the lens fiber cell basal membrane complex. *J. Cell Sci.* **112**, 2155-2165.

Beebe, D. C., Vasiliev, O., Guo, J., Shui, Y.-B. and Bassnett, S. (2001). Changes in adhesion complexes define stages in the differentiation of lens fiber cells. *Invest. Ophthalmol. Vis. Sci.* **42**, 727-734.

Benedetti, E. L., Dunia, I., Ramaekers, F. C. S. and Kibbelaar, M. A. (1981). Lenticular plasma membranes and cytoskeleton. In *The Ocular Lens: Structure, Function and Pathology* (ed. H. Maisel), pp. 137-188. New York: Marcel Dekker.

Boggon, T. J., Muray, J., Chappuis-Flament, S., Wong, E., Gumbiner, B. M. and Shapiro, L. (2002). C-cadherin ectodomain structure and implications for cell adhesion mechanisms. *Science* **296**, 1308-1313.

Bretscher, A. (1983). Purification of an 80,000 Dalton protein that is a component of the isolated microvillus cytoskeleton, and its localization in nonmuscle cells. *J. Cell Biol.* **97**, 425-432.

Bretscher, A., Reczek, D. and 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**, 3011-3018.

Bretscher, A., Edwards, K. and Fehon, R. G. (2002). ERM proteins and merlin: integrators at the cell cortex. *Nature* **3**, 586-599.

Capetanaki, Y., Smith, S. and Heath, J. P. (1989). Overexpression of the vimentin gene in transgenic mice inhibits normal lens cell differentiation. *J. Cell Biol.* **109**, 1653-1664.

Citi, S., Volberg, T., Bershadsky, A. D., Denisenko, N. and Geiger, B. (1994). Cytoskeletal involvement in the modulation of cell-cell junctions by the protein kinase inhibitor H-7. *J. Cell Sci.* **107**, 683-692.

Claudio, J. O., Lutchman, M. and Rouleau, G. A. (1995). Widespread but cell type-specific expression of the mouse neurofibromatosis type 2 gene. *Neuroreport* **6**, 1942-1946.

Claudio, J. O., Veneziale, R. W., Menko, A. S. and Rouleau, G. A. (1997). Expression of schwannomin in lens and Schwann cells. *Neuroreport* **27**, 2025-2030.

Cowin, P., Kapprell, H.-P. and Franke, W. W. (1985). The complement of desmosomal plaque proteins in different cell types. *J. Cell Biol.* **101**, 1442-1454.

Cowin, P., Kapprell, H.-P., Franke, W. W., Tamkun, J. and Hynes, R. O. (1986). Plakoglobin: a protein common to different kinds of intercellular adhering junctions. *Cell* **46**, 1063-1073.

DiColandrea, T., Karashima, T., Määttä, A. and Watt, F. M. (2000). Subcellular distribution of envoplakin and periplakin: insights into their role as precursors of the epidermal cornified envelope. *J. Cell Biol.* **151**, 573-585.

Duguay, D., Ramsey, A. F. and Steinberg, M. S. (2003). Cadherin-mediated cell adhesion and tissue segregation: qualitative and quantitative determinants. *Dev. Biol.* **253**, 309-323.

Duncan, M. K., Kozmik, Z., Cveklova, K., Piatigorsky, J. and Cvekl, A. (2000). Overexpression of PAX6(5a) in lens fiber cells results in cataract and upregulation of α5β1 integrin expression. *J. Cell Sci.* **113**, 3173-3185.

Farquhar, M. and Palade, G. E. (1963). Junctional complexes in various epithelia. *J. Cell Biol.* **17**, 375-412.

Ferreira-Cornwell, M. C., Veneziale, R. W., Grunwald, G. B. and Menko, A. S. (2000). N-Cadherin function is required for differentiation-dependent cytoskeletal reorganization in lens cells in vitro. *Exp. Cell Res.* **256**, 237-247.

Franke, W. W., Schmid, E., Grund, C., Müller, H., Engelbrecht, I., Moll, R., Stadler, J. and Jarasch, E.-D. (1981). Antibodies to high molecular weight polypeptides of desmosomes: specific localization of a class of junctional proteins in cells and tissues. *Differentiation* **20**, 217-241.

Franke, W. W., Kapprell, H.-P. and Cowin, P. (1987). Plakoglobin is a component of the filamentous subplasmalemmal coat of lens cells. *Eur. J. Cell Biol.* **43**, 301-315.

Geiger, B., Volk, T. and Volberg, T. (1985). Molecular heterogeneity of adherens junctions. *J. Cell Biol.* **101**, 1523-1531.

Giepmans, B. N. and Moolenaar, W. H. (1998). The gap junction protein connexin 43 interacts with the second PDZ domain of the zona occludens-1 protein. *Curr. Biol.* **140**, 1199-1209.

Gillespie, C. S., Sherman, D. L., Blair, G. E. and Brophy, P. J. (1994). Periaxin, a novel protein of myelinating Schwann cells with a possible role in axonal ensheathment. *Neuron* **12**, 497-508.

Green, J. and Maisel, H. (1984). Lens fodrin binds actin and calmodulin. *Curr. Eye Res.* **3**, 1433-1440.

Green, K. J. and Gaudry, C. A. (2000). Are desmosomes more than tethers for intermediate filaments? *Nat. Rev. Mol. Cell Biol.* **1**, 208-216.

Hadeball, B., Borchers, A. and Wedlich, D. (1998). *Xenopus* cadherin-11 (Xcadherin-11) expression requires the Wg/Wnt signal. *Mech. Dev.* **72**, 101-113.

Hashimoto, T., Amagai, M., Parry, D. A., Dixon, T. W., Tsukita, S., Tsukita, S., Miki, K., Sakai, K., Inokuchi, Y., Kudoh, J. et al. (1993).

Desmoyokin, a 630 kDa keratinocyte plasma membrane-associated protein, is a homologous to the protein encoded by human gene *AHNAK*. *J. Cell Sci.* **105**, 275-286.

Hatta, H. and Takeichi, M. (1986). Expression of N-cadherin adhesion molecules associated with early morphogenetic events in chick development. *Nature* **320**, 447-449.

Hatzfeld, M. (1999). The armadillo family of structural proteins. *Int. Rev. Cytol.* **186**, 179-224.

Hatzfeld, M., Green, K. J. and Sauter, H. (2003). Targeting of p0071 to desmosomes and adherens junctions is mediated by different protein domains. *J. Cell Sci.* **1**, 1219-1233.

He, W. and Li, S. (2000). Congenital cataracts: gene mapping. *Hum. Genet.* **106**, 1-13.

Herrmann, H., Fouquet, B. and Franke, W. W. (1989). Expression of intermediate filament proteins during development of *Xenopus laevis*. I. cDNA clones encoding different forms of vimentin. *Development* **105**, 279-298.

Hieda, Y., Tsukita, S. and Tsukita, S. (1989). A new high molecular mass protein showing unique localization in desmosomal plaque. *J. Cell Biol.* **109**, 1511-1518.

Hoffmann, I. and Balling, R. (1995). Cloning and expression analysis of a novel mesodermally expressed cadherin. *Dev. Biol.* **169**, 337-346.

Hohaus, A., Person, V., Behlke, J., Schaper, J., Morano, I. and Haase, H. (2002). The carboxyl-terminal region of AHNAK provides a link between cardiac L-type channels and the actin-based cytoskeleton. *FASEB J.* **16**, 1205-1216.

Hollnagel, A., Grund, C., Franke, W. W. and Arnold, H. H. (2002). The cell adhesion molecule M-cadherin is not essential for muscle development and regeneration. *Mol. Cell. Biol.* **22**, 4760-4770.

Huynh, D. P., Tran, T. M., Nechiporuk, T. and Pulst, S. M. (1996). Expression of neurofibromatosis 2 transcript and gene product during mouse fetal development. *Cell Growth Differ.* **7**, 1551-1561.

Jakobs, P. M., Hess, J. F., FitzGerald, P. G., Kramer, P., Weleber, R. G. and Litt, M. (2000). Autosomal-dominant congenital cataract associated with a deletion mutation in the human beaded filament structural protein gene *BFSP2*. *Am. J. Hum. Genet.* **66**, 1432-1436.

Jensen, O. N., Podtelejnikov, A. and Mann, M. (1996). Delayed extraction improves specificity in database searches by matrix-assisted laser desorption/ionization peptide maps. *Rapid Commun. Mass Spectrom.* **10**, 1371-1378.

Karashima, T. and Watt, F. M. (2002). Interaction of periplakin and envoplakin with intermediate filaments. *J. Cell Sci.* **115**, 556-563.

Kibbelaar, M. A., Selten-Versteegen, A.-M. E., Dunia, I., Benedetti, E. L. and Bloemendal, H. (1979). Actin in mammalian lens. *Eur. J. Biochem*. **95**, 543-549.

Koch, P. J., Walsh, M. J., Schmelz, M., Goldschmidt, M. D., Zimbelmann, R. and Franke, W. W. (1990). Identification of desmoglein, a constitutive desmosomal glycoprotein, as a member of the cadherin subfamily of cell adhesion molecules. *Eur. J. Cell Biol.* **53**, 1-12.

Krutovskikh, V. and Yamasaki, H. (2000). Connexin gene mutations in human genetic diseases. *Mut. Res.* **462**, 197-207.

Langbein, L., Grund, C., Kuhn, C., Praetzel, S., Kartenbeck, J., Brandner, J. M., Moll, I. and Franke, W. W. (2002). Tight junctions and compositionally related junctional structures in mammalian stratified epithelia and cell cultures derived therefrom. *Eur. J. Cell Biol.* **81**, 419-435.

Leckband, D. and Sivasankar, S. (2000). Mechanism of homophilic cadherin adhesion. *Curr. Opin. Cell Biol.* **12**, 587-592.

Lee, A., Fischer, R. S. and Fowler, V. M. (2000). Stabilization and remodeling of the membrane skeleton during lens fiber cell differentiation and maturation. *Dev. Dyn.* **217**, 257-270.

Leong, L., Menko, A. S. and Grunwald, G. B. (2000). Differential expression of N- and B-cadherin during lens development. *Invest. Ophthalmol. Vis. Sci.* **41**, 3503-3510.

Lieska, N., Chen, J., Maisel, H. and Romero-Herrera, A. E. (1980). Subunit characterization of lens intermediate filaments. *Biochem. Biophys. Acta* **626**, 136-153.

Lo, W. K. (1988). Adherens junctions in the ocular lens of various species. Ultrastructural analysis with an improved fixation. *Cell Tissue Res.* **254**, 31-40.

Lo, W. K., Shaw, A. P. and Wen, X. J. (1997). Actin filament bundles in cortical fiber cells of the rat lens. *Exp. Eye Res.* **65**, 691-701.

Lo, W. K., Shaw, A. P., Paulsen, D. F. and Mills, A. (2000). Spatiotemporal distribution of zonulae adherens and associated actin bundles in both epithelium and fiber cells during chicken lens development. *Exp. Eye Res.* **71**, 45-55.

Ma, A. S.-P. and Sun, T.-T. (1986). Differentiation-dependent changes in the solubility of a 195-kD protein in human epidermal keratinocytes. *J. Cell Biol.* **103**, 41-48.

Maisel, H., Hardling, C. V., Alcala, J. R., Kuszak, J. and Bradley, R. (1981). The morphology of the lens. In *Molecular and Cellular Biology of the Eye Lens* (ed. H. Bloemendal), pp. 49-84. New York: John Wiley and Sons.

Martinez-Wittingham, F. J., Sellitto, C., Li, L., Gong, X., Brink, P. R., Mathias, R. T. and White, T. W. (2003). Dominant cataracts result from incongruous mixing of wild-type lens connexins. *J. Cell Biol.* **161**, 969-978.

Masanuga, T., Shimizu, H., Ishiko, A., Fujiwara, T., Hashimoto, T. and Nishikawa, T. (1995). Desmoyokin/AHNAK protein localizes to the non-desmosomal keratinocye cell surface of human epidermis. *J. Invest. Dermatol.* **10**, 941-945.

Melendez-Vasquez, C. V., Rios, J. C., Zanazzi, G., Lambert, S., Bretscher, A. and Salzer, J. L. (2001). Nodes of Ranvier form in association with ezrin-radixin-moesin (ERM)-positive Schwann-cell processes. *Proc. Natl. Acad. Sci. USA* **98**, 1235-1240.

Merdes, A., Brunkener, M., Horstmann, H. and Georgatos, S. D. (1991). Filensin: a new vimentin-binding polymerization competent, and membrane-associated protein of the lens fiber cell. *J. Cell Biol.* **115**, 397-410.

Merdes, A., Gounari, F. and Georgatos, S. D. (1993). The 47-kD lens-specific protein phakinin is a tailless intermediate filament protein and an assembly partner of filensin. *J. Cell Biol.* **123**, 1507-1516.

Mertens, C., Kuhn, C. and Franke, W. W. (1996). Plakophilins 2a and 2b: constitutive proteins of dual location in the karyoplasm and the desmosomal plaque. *J. Cell Biol.* **135**, 1009-1025.

Nagafuchi, A. and Takeichi, M. (1989). Transmembrane control of cadherin-mediated cell adhesion: a 94kD protein functionally associated with a specific region of the cytoplasmic domain of E-cadherin. *Cell Regul.* **1**, 37-44.

Nelson, W. J., Granger, B. L. and Lazarides, E. (1983). Avian lens spectrin: subunit composition compared with erythrocyte and brain spectrin. *J. Cell Biol.* **97**, 1271-1276.

Nie, Z., Ning, W., Amagai, M. and Hashimoto, T. (2000). C-Terminus of desmoyokin/AHNAK protein is responsible for its translocation between the nucleus and cytoplasm. *J. Invest. Dermatol.* **114**, 1044-1049.

Nielsen, P. A., Baruch, A., Giepmans, B. N. and Kumar, N. M. (2001). Characterization of the association of connexins and ZO-1 in the lens. *Cell Commun. Adhes.* **8**, 213-217.

Nishi, O., Nishi, K., Akaishi, T. and Shirasawa, E. (1997). Detection of cell adhesion molecules in lens epithelial cells of human cataracts. *Invest. Ophthalmol. Vis. Sci.* **38**, 579-585.

Ozawa, M., Baribault, H. and Kemler, R. (1989). The cytoplasmic domain of the cell adhesion molecule uvomorulin associates with three independent proteins structurally related in different species. *EMBO J.* **8**, 1711-1717.

Paffenholz, R., Kuhn, C., Grund, C., Stehr, S. and Franke, W. W. (1999). The arm-repeat protein NPRAP (neurojungin) is a constituent of the plaques of the outer limiting zone in the retina, defining a novel type of adhering junction. *Exp. Cell Res.* **250**, 452-464.

Peitsch, W. K., Grund, C., Kuhn, C., Schnölzer, M., Spring, H., Schmelz, M. and Franke, W. W. (1999). Drebrin is a widespread actin-associating protein enriched at junctional plaques, defining a specific microfilament anchorage system in polar epithelial cells. *Eur. J. Cell Biol.* **78**, 767-778.

Perng, M. D., Cairns, L., van den Ijssel, P., Prescott, A., Hutcheson, A. M. and Quinlan, R. A. (1999). Intermediate filament interactions can be altered by HSP27 and alpha B-crystallin. *J. Cell Sci.* **112**, 2099-2112.

Rafferty, N. S. (1985). Lens morphology. In *The Ocular Lens: Structure, Function and Pathology* (ed. H. Maisel), pp. 1-60. New York: Marcel Dekker.

Ramaekers, F. C. S. and Bloemendal, H. (1981). Cytoskeletal and contractile structures in lens cell differentiation. In *The Ocular Lens: Structure, Function and Pathology* (ed. H. Maisel), pp. 85-136. New York: Marcel Dekker.

Ramaekers, F. C. S., Osborn, M., Schmid, E., Weber, K., Bloemendal, H. and Franke, W. W. (1980). Identification of the cytoskeletal proteins in lens-forming cells, a special epithelioid cell type. *Exp. Cell Res.* **127**, 309-327.

Repasky, E. A., Granger, B. L. and Lazarides, E. (1982). Widespread occurrence of avian spectrin in nonerythroid cells. *Cell* **29**, 821-833.

Rose, O., Grund, C., Reinhardt, S., Starzinski-Powitz, A. and Franke, W.

W. (1995). Contactus adherens, a special type of plaque-bearing adhering junction containing M-cadherin, in the granule cell layer of the cerebellar glomerulus. *Proc. Natl. Acad. Sci. USA* **92**, 6022-6026.

Ruhrberg, C., Hajibagheri, M. A. N., Parry, D. A. D. and Watt, F. M. (1997). Periplakin, a novel component of cornified envelopes and desmosomes that belongs to the plakin family and forms complexes with envoplakin. *J. Cell Biol.* **139**, 1835-1849.

Saika, S., Kawashima, Y., Miyamoto, T., Okada, Y., Tanaka, S., Yamanaka, O., Ohnishi, Y., Ooshima, A. and Yamanaka, A. (1998). Immunolocalization of hyaluronan and CD44 in quiescent and proliferating human lens cells. *J. Cataract Refract. Surg.* **24**, 1266-1270.

Schmelz, M. and Franke, W. W. (1993). Complexus adhaerentes, a new group of desmoplakin-containing junctions in endothelial cells: the syndesmos connecting retothelial cells of lymph nodes. *Eur. J. Cell Biol*. **61**, 274-289.

Schmidt, A., Heid, H. W., Schäfer, S., Nuber, U. A., Zimbelmann, R. and Franke, W. W. (1994). Desmosomal and cytoskeletal architecture in epithelial differentiation: cell-type specific plaque components and intermediate filament anchorage. *Eur. J. Cell Biol*. **65**, 229-245.

Schmidt, A., Langbein, L., Prätzel, S., Rode, M., Rackwitz, H.-R. and Franke, W. W. (1999). Plakophilin-3 – a novel cell-type specific desmosomal plaque protein. *Differentiation* **64**, 291-306.

Schröder, R., Warlo, I., Herrmann, H., van der Ven, P. F. M., Klasen, C., Blümcke, I., Mundegar, R. R., Fürst, D. O., Goebel, H. H. and Magin, T. M. (1999). Immunogold EM reveals a close association of plectin and the desmin cytoskeleton in human skeletal muscle. *Eur. J. Cell Biol.* **78**, 288-295.

Shapiro, L., Fannon, A. M., Kwong, P. D., Thompson, A., Lehmann, M. S., Grübel, G., Legrand, J.-F., Als-Nielsen, J., Colman, D. R. and Hendrickson, W. A. (1995). Structural basis of cell-cell adhesion by cadherins. *Nature* **374**, 327-337.

Sherman, D. L. and Brophy, P. J. (1999). A tripartite nuclear localization signal in the PDZ-domain protein L-periaxin. *J. Biol. Chem.* **275**, 4537-4540.

Shtivelman, E. and Bishop, J. M. (1993). The human gene *AHNAK* encodes a large phosphoprotein located primarily in the nucleus. *J. Cell Biol*. **120**, 625-630.

Shtivelman, E., Cohen, F. E. and Bishop, J. M. (1992). A human gene (*AHNAK*) encoding an unusually large protein with a 1.2-μm polyionic rod structure. *Proc. Natl. Acad. Sci. USA* **89**, 5472-5476.

Simon, M. and Green, H. (1984). Participation of membrane-associated proteins in the formation of the cross-linked envelope of the keratinocyte. *Cell* **36**, 827-834.

Simonneau, L. and Thiery, J. P. (1998). The mesenchymal cadherin-11 is expressed in restricted sites during the ontogeny of the rat brain in modes suggesting novel functions. *Cell Adhes. Commun.* **6**, 431-450.

Simonneau, L., Kitagawa, M., Suzuki, S. and Thiery, J. P. (1995). Cadherin-11 expression marks the mesenchymal phenotype: towards new functions for cadherins? *Cell Adhes. Commun.* **3**, 115-130.

Staehelin, L. A. (1974). Structure and function of intercellular junctions. *Int. Rev. Cytol.* **39**, 191-283.

Sussmann, J., Stokoe, D., Ossina, N. and Shtivelman, E. (2001). Protein kinase B phosphorylates AHNAK and regulates its subcellular localization. *J. Cell Biol.* **154**, 1019-1030.

Takai, Y. and Nakanishi, H. (2003). Nectin and afadin: novel organizers of intercellular junctions. *J. Cell Sci.* **116**, 17-27.

Takeichi, M. (1988). The cadherins: cell-cell adhesion molecules controlling animal morphogenesis. *Development* **102**, 639-655.

Takeichi, M. (1991). Cadherin adhesion receptors as a morphogenetic regulator. *Science* **251**, 1451-1455.

Thomas, G. H. (2001). Spectrin: the ghost in the machine. *BioEssays* **81**, 152-160.

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

Tsukita, S., Oishi, K., Sato, N., Sagara, J., Kawai, A. and Tsukita, S. (1994). ERM family members as molecular linkers between the cell surface glycoprotein CD44 and actin-based cytoskeletons. *J. Cell Biol.* **126**, 391-401.

Valiron, O., Chevrier, V., Usson, Y., Breviario, F., Job, D. and Dejana, E. (1996). Desmoplakin expression and organization at human umbilical vein endothelial cell-to-cell junctions. *J. Cell Sci.* **109**, 2141-2149.

Volk, T. and Geiger, B. (1984). A 135-kD membrane protein of intercellular adherens junctions. *EMBO J.* **3**, 2249-2260.

Volk, T., Cohen, O. and Geiger, B. (1987). Formation of heterotypic adherens-type junctions between L-CAM-containing liver cells and A-CAM-containing lens cells. *Cell* **5**, 987-994.

Weitzer, G. and Wiche, G. (1987). Plectin from bovine lenses. Chemical properties, structural analysis and initial identification of interaction partners. *Eur. J. Biochem.* **169**, 41-52.

Yonemura, S., Hirao, M., Doi, Y., Takanahashi, N., Kondo, T., Tsukita, S. and Tsukita, S. (1998). Ezrin/Radixin/Moesin (ERM) proteins bind to a positively charged amino acid cluster in the juxta-membrane cytoplasmic domain of CD44, CD43 and ICAM-2. *J. Cell Biol.* **140**, 885-895.