Intracellular Routing of Wild-Type and Mutated Polymeric Immunoglobulin Receptor in Hippocampal Neurons in Culture

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Abstract. Certain epithelial cells synthesize the polymeric immunoglobulin receptor (pIgR) to transport immunoglobulins (Igs) A and M into external secretions. In polarized epithelia, newly synthesized receptor is first delivered to the basolateral plasma membrane and is then, after binding the Ig, transcytosed to the apical plasma membrane, where the receptor-ligand complex is released by proteolytic cleavage. In a previous work (Ikonen et al., 1993), we implied the existence of a dendro-axonal transcytotic pathway for the rabbit pIgR expressed in hippocampal neurons via the Semliki Forest Virus (SFV) expression system. By labeling surface-exposed pIgR in live neuronal cells, we now show (*a*) internalization of the receptor from the dendritic plasma membrane to the dendritic early endosomes, (*b*) redistribution of the receptor from the dendritic to the axonal domain, (*c*) inhibition of this movement by brefeldin A (BFA) and (*d*) stimulation by the activation of protein kinase C (PKC) via phorbol myristate acetate (PMA). In addition, we show that a mutant form of the receptor lacking the epithelial basolateral sorting signal is directly delivered to the axonal domain of hippocampal neurons. Although this mutant is internalized into early endosomes, no transcytosis to the dendrites could be observed. In epithelial Madin-Darby Canine Kidney (MDCK) cells, the mutant receptor could also be internalized into basolaterally derived early endosomes. These results suggest the existence of a dendro-axonal transcytotic pathway in neuronal cells which shares similarities with the basolateral to apical transcytosis in epithelial cells and constitute the basis for the future analysis of its physiological role.

I N polarized cells, such as epithelial cells and neurons, the plasma membrane is divided into two domains that differ morphologically, functionally and biochemically. In most epithelia, and possibly also in neurons, the asymmetric organization of the plasma membrane is generated by the sorting of membrane constituents into distinct vesicles in the TGN (for reviews see Rodriquez-Boulan and Powell, 1992; de Hoop and Dotti, 1993; Craig and Banker, 1994). Besides direct sorting, certain polarized cells generate membrane asymmetry by another vesicular pathway called transcytosis (Bartles et al., 1987; for reviews see Rodman et al., 1990; Nelson, 1992; Mostov et al., 1992). Transcytosis is also used to transport substances from one domain to the other. This includes the transepithelial transport of transferrin (Soda and Tavassoli, 1984; Friden et al., 1991), low density lipoprotein (Hashida et al., 1986), serum albumin (Ghitescu and Bendayan, 1992), insulin (King and Johnson, 1985; Pardridge, 1991), epidermal growth factor (Brändli et al., 1991; Gonnella et al., 1987), nerve growth factor (Siminoski et al., 1986), thyroglobulin (Herzog, 1983), and antibodies (Kraehenbuhl and

Neutra, 1992; Hunziker and Mellman, 1989; Sooranna and Contractor, 1991).

The best characterized transcytotic molecule is the polymeric immunoglobulin receptor (pIgR)[1]. The pIgR has a large extracellular ligand-binding domain, called the secretory component (SC), a single transmembrane domain and an intracellular cytoplasmic tail of 103 amino acids (Mostov et al., 1984). In the polarized kidney epithelial cell line MDCK newly synthesized pIgR is sorted in the TGN to the basolateral plasma membrane where it can bind its ligand, dimeric IgA (dIgA). The receptor-ligand complexes, as well as unoccupied receptors are efficiently internalized into the basolateral early endosomes. Here, both the occupied and the unoccupied receptors are sorted into transcytotic vesicles, although some receptors recycle to the basolateral plasma membrane (Song et al., 1994; Breitfeld et al., 1989). Transcytotic vesicles are delivered to apical endocytic structures and from there to the apical plasma membrane (Apodaca et al., 1994; Cardone et al., 1994; Barroso and Sztul, 1994). Either during its intracellular transport or upon insertion into the plasma membrane,

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1. *Abbreviations used in this paper:* BFA, brefeldin A; pIgR, polymeric immunoglobulin receptor; PKC, protein kinase C; SC, secretory component; SFV, Semliki Forest Virus.

the SC is proteolytically cleaved by a leupeptin-sensitive protease and released in complex with the bound ligand (Aroeti et al., 1992).

Several signals have been identified in the amino acid sequence of the receptor that regulate its complex intracellular routing. A sequence of 14 amino acids (from amino acid 655 to 668, bearing the sequence RHRRNV-DRVSICSY) in the cytoplasmic tail directs the receptor to the basolateral plasma membrane (Casanova et al., 1991). Deletion of this basolateral-sorting signal results in direct transport of the receptor to the apical plasma membrane. Two tyrosines present at positions 668 and 734 in the cytoplasmic tail mediate endocytosis, although mutating tyrosine 734 affects endocytosis more than mutations in tyrosine 668 (Okamoto et al., 1992). Transcytosis is regulated by various mechanisms. Although the unoccupied receptor is able to transcytose, transcytosis is stimulated by the binding of its natural ligand dIgA (Song et al., 1994). Phosphorylation of serine 664 and 726 enhances transcytosis (Okamoto et al., 1994; Casanova et al., 1990; Hirt et al., 1993). Although the identity of the kinases is unknown, the involvement of protein kinase A and C in transcytosis has been demonstrated (Cardone et al., 1994; Hansen and Casanova, 1994). Transcytosis is also controlled by heterotrimeric G proteins that have been shown to modulate many steps of the endocytic and exocytic traffic (Bomsel and Mostov, 1992, 1993; Barroso and Sztul, 1994; Hansen and Casanova, 1994). Finally it has been demonstrated that the drug brefeldin A (BFA) inhibits transcytosis, probably by interfering with the sorting of pIgR into transcytotic vesicles in the basolateral early endosomes (Hunziker et al., 1991, 1992).

In our previous work we showed the direct delivery of the wild-type pIgR to the dendrites of cultured hippocampal neurons and its subsequent appearance in the axons upon addition of dIgA (Ikonen et al., 1993). In the present paper we describe the direct sorting of the wild-type pIgR to the dendritic membrane, and by following the movement of the antibody-labeled receptor in living cells, we demonstrate its transcytotic movement to the axonal domain. This transcytotic movement is inhibited by BFA and stimulated by PMA which activates PKC. We also show that, in contrast to the wild-type receptor, a mutant form of the receptor lacking the epithelial basolateral targeting signal (Casanova et al., 1991) is delivered directly to the axons.

Materials and Methods

Chemicals

Phorbol 12-myristate 13-acetate (PMA), also called 12-*O*-tetradecanoylphorbol 13-acetate (TPA), was stored as a 0.1 mg/ml stock solution in ethanol at −20°C and was used at a final concentration of 2 μM. Brefeldin A was stored as a stock solution of 5 mg/ml in ethanol, and used at 10 μg/ml. Leupeptine was dissolved in water at 50 mg/ml, neutralized, and stored at −20°C until use at a concentration of 100 μg/ml. All these chemicals were purchased from Sigma (Sigma Chem. Co., St. Louis, MO).

Antibodies

The monoclonal antibody against the membrane and cytoplasmic domain of the rabbit pIgR (SC166) has been described (Kühn and Kraehenbuhl, 1983; Solari et al., 1985). A goat polyclonal antibody against the secretory component of the rabbit pIgR was obtained from Cooper Biomedical (Malvern, PA). The polyclonal antibody against MAP 2 was generously provided by Dr. Diez-Guerra, Centro de Biologia Molecular (CBM) (Madrid, Spain).

DNA Constructs and Production of Recombinant Semliki Forest Virus

The cloning of the pSFV1-pIgR wild-type and the preparation of recombinant SFV has been described (Ikonen et al., 1993; Olkkonen et al., 1993). To obtain the pIgR Δ655-668 mutant, the cDNA encoding wild-type pIgR (Mostov et al., 1984) was subcloned from plasmid pCB6 (Brown et al., 1989) into Bluescript KS, resulting in the plasmid KS-pIgR in which the 5′end of the pIgR gene faces the T3 promoter. This plasmid was amplified with the 5′ mutagenic primer (5′ GCC ATG GCC GTG GCC ATA GCC AGA GCC AGG ACA GAC ATT AGC ATG TCA GAC) and the 3′ primer (5′ GCT CCT CAA TCT CGA GGG TGC TC). The PCR product was digested with MscI and XbaI and ligated into the MscI and XbaI-cut Bluescript KS-pIgR. The MscI site is in the pIgR transmembrane domain, XbaI in the Bluescript polylinker; this cloning procedure essentially exchanged the wild-type cytoplasmic tail for the mutagenized cytoplasmic tail. The region amplified by PCR was sequenced. pIgR Δ655-668 was excised with SpeI and EcoRV, made blunt ended with the Klenow fragment of DNA polymerase and cloned into the dephosphorylated SmaI-cut plasmid pSFV1 (Liljeström and Garoff, 1991).

Detailed protocols on the preparation and application of recombinant SFVs in hippocampal neurons have been published (Liljeström and Garoff, 1991; Olkkonen et al., 1993; de Hoop et al., 1994).

Cell Culture and Infections

Primary cultures of rat hippocampal neurons were prepared as described (Goslin and Banker, 1991). Cells were grown in N2 medium (Bottenstein and Sato, 1979) supplemented with 1 ng/ml basic fibroblast growth factor (Boehringer Mannheim Biochemica, Mannheim, Germany). Unless stated otherwise, neurons used had been cultured for 9–14 d (stage 5 cells; Dotti et al., 1988). Hippocampal neurons were infected with recombinant SFV as described (de Hoop et al., 1994). After 1 h infection, cells were incubated for 3–5 h to allow SFV-driven protein synthesis. In some experiments cycloheximide (Sigma Chem. Co.) was added (20 μg/ml) during the last 2 h of expression.

MDCK cells expressing either wild-type (Hunziker et al., 1990; Mostov and Deitcher, 1986) or pIgR Δ655-668 (Casanova et al., 1991) under control of the CMV promoter were cultured at 37°C and 5% CO_2 in MEM with 5% FCS, 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 0.25 mg/ml geneticin (G418; Gibco BRL Life Technologies Inc., Grand Island, NY) for cells expressing the mutant Δ655-668 or 0.25 mg/ml hygromycin B (Calbiochem-Behring, La Jolla, CA) for cells expressing wild-type pIgR.

Immunofluorescence

Neurons on coverslips were fixed with 4% paraformaldehyde in PBS for 20 min at room temperature followed by a 15-min incubation in 50 mM NH_4Cl in PBS to quench excess paraformaldehyde. Cells were permeabilized via graded ethanol series. Nonspecific binding sites were blocked by incubation in 2% bovine serum albumin, 2% FCS and 0.2% cold-water fish-skin gelatin (Sigma Chem. Co.) in water for 30 min. The cells were then incubated with primary antibody for 1 h at room temperature and, after extensive washing with PBS, with species-specific secondary antibody (from Cappel, Durham, NC and from Dianova, Hamburg, Germany). The coverslips were mounted in Mowiol 4-88 (Calbiochem-Nova-biochem Corp., La Jolla, CA) containing 2.5% DABCO (Sigma Chem. Co.) as an antibleaching agent and microscopy was performed with an Axiophot microscope (Zeiss, Oberkochen, Germany).

Filter-grown MDCK cells were fixed with 4% paraformaldehyde and permeabilized with saponin (0.025% in PBS+ containing 0.7% cold-water fish-skin gelatin) as described (Huber et al., 1993a). Cells were mounted with spacers in 50% glycerol containing 2.5% DABCO and viewed with the European Molecular Biology Laboratory Confocal Microscope.

Identification and Quantitation of pIgR Labeled Processes

To precisely determine the axonal or dendritic distribution of the wild-

type or mutant receptor, low multiplicity of infection was used. In this way, very few cells per coverslip were infected and it was thus possible to unequivocably distinguish the origin, trajectory, and ending of the labeled processes. The identification of the processes as dendrites was done by double labeling with the dendritic marker MAP2 (Caceres et al., 1984). A process was identified as axon by its lack of MAP2 staining. The distribution was classified into the following pattern: soma and dendrites, soma and axons, and soma and both axons and dendrites. Several coverslips from different experiments were examined by two independent observers, the number of infected cells and process distribution averaged and the standard deviation calculated. Significance was determined by student's t test.

Internalization Experiments

The internalization experiments in neurons were performed as follows: FITC-coupled polyclonal antibodies against the secretory component of the rabbit pIgR were dissolved 1:100 in N_2 medium. Coverslips with attached neurons were put on top of small droplets of prewarmed antibody-containing medium during 20 min at 5% CO_2 and 36.5°C. Cells were then washed with cold HBSS containing 0.2% BSA, fixed with 4% paraformaldehyde in PBS and processed for immunofluorescence. To label the early endocytic compartment of neurons, Lucifer yellow–CH (dilithium salt, Sigma Chem. Co.) was added to the medium at 10 mg/ml. Neurons were incubated in Lucifer yellow–containing medium for 20 min, followed by a thorough wash with ice cold HBSS containing 0.2% BSA, fixed with 4% paraformaldehyde and processed for immunofluorescence.

To label the pIgRs present in the early endocytic compartment of filter-grown MDCK cells, the antibody against the secretory component of the rabbit pIgR (dissolved 1:40 in MEM containing 0.05% BSA and 10 mM Hepes-NaOH, pH 7.3; MEM-BSA) was added to the medium and the cells were incubated on ice for 45 min. Unbound antibody was removed by a quick wash in ice-cold MEM-BSA. Then, the basolateral side of the cells was exposed to 10 mg/ml lucifer yellow-CH (5 min at 37°C in MEM-BSA) to allow internalization of surface-bound antibodies and the fluid-phase ligand. Finally, the cells were thoroughly washed with ice-cold MEM-BSA, fixed with 4% paraformaldehyde and processed for immunofluorescence as described above.

Transcytosis Assay

Transcytosis of the pIgR in living cells was analyzed as follows: cells were infected for 1 h at 36.5°C, excess virus was removed and the cells were further incubated for 3 h in the presence of 100 μg/ml leupeptin to prevent the proteolytic removal of the secretory component (Apodaca et al., 1991). At the end of the 3-h expression period, the coverslips were incubated on ice for 30 min in small drops (± 100 μl) of air-MEM (MEM containing 4 mM NaHCO₃, 10 mM Hepes, pH 7.3, 2 mM glutamine, 0.6% glucose, 110 μg/ml pyruvic acid) with 5% FCS, 100 μg/ml leupeptin and 1:100 diluted FITC-conjugated anti-SC. Coverslips were subsequently washed in cold HBSS with 0.2% BSA and either fixed to examine the distribution of surface-bound antibody against the ectodomain (the secretory component) or transferred to prewarmed maintenance medium containing BFA (10 μg/ml) or PMA (2 μM) or medium without additions for 20 min to 2 h at 36.5°C. Finally, the cells were fixed, washed, and mounted to examine the distribution of the anti-SC antibody by fluorescence microscopy.

Western Blot Analysis

Hippocampal neurons were plated directly onto polylysine-coated 6-cm culture dishes (Nunc, Roskilde, Denmark) at a density of 300,000–400,000 cells per dish and cultured for three days. 5 h after infection with approximately equal amounts of recombinant virus, carrying either the wild-type or Δ655-668 pIgR cDNA, the neurons were lysed (de Hoop et al., 1994) and the cell extracts were analyzed by Western blotting using the anti-CT monoclonal antibody SC166 (diluted 1:100), followed by ECL detection as recommended by the supplier (Amersham International, Buckinghamshire, England).

Results

Expression of the Polymeric Immunoglobulin Receptor in Hippocampal Neurons in Culture

In a previous work we showed by indirect immunofluores-

cence that infection of polarized hippocampal neurons in culture with recombinant SFV resulted in the appearance of the pIgR in the dendrites and, to a lesser extent, also in axons. The axonal labeling was increased 2–4-fold upon addition of ligand (Ikonen et al., 1993). The aim of the present work was to analyze the mechanisms of this transcytotic route and the sorting of the Δ655-668 mutant, which lacks a basolateral targeting signal.

We first examined by Western blot the SFV-driven expression (Liljeström and Garoff, 1991; Olkkonen et al., 1993) of wild-type and Δ655-668 mutant pIgR in polarized hippocampal neurons in culture. In neurons expressing wild-type pIgR, a band of ~120 kD was visible (Fig. 1, lane 2) agreeing with the molecular weight of endogenous pIgR in liver (Sztul, 1992). In neurons expressing the Δ655-668 pIgR mutant (lane 3), a band with slightly higher electrophoretic mobility, due to the deletion, was observed. As expected, no expression was detected in control neurons and MDCK cells (lanes 1 and 4). The intensity of the 120-kD band differed slightly between neurons infected with truncated and wild-type pIgR. This is due to a difference in the efficiency of infection. The band of ~35 kD visible in MDCK cells expressing wild-type pIgR (arrow in lane 5) represents the cleaved cytoplasmic tail and membrane domain of the receptor (Kühn and Kraehenbuhl, 1983; Solari et al., 1985).

Intracellular Distribution of Newly Synthesized Wild-Type and Mutant pIgR

To analyze the routing of newly synthesized pIgR, fully polarized hippocampal neurons were infected with recombinant virus which express the pIgR and the distribution of the receptor was determined by immunofluorescence microscopy at 3 h postinfection. This was the earliest time point at which pIgR expression was detectable. The wild-type receptor (Fig. 2 B) was predominantly found in the somatodendritic region. Quantitative analysis revealed exclusive cell body and dendritic labeling in 85% of the cells (Fig. 3 A). In the remaining cells (15%), the labeling was present in the cell body and both dendrites and axons. We did not find cells with cell body and axonal staining in the
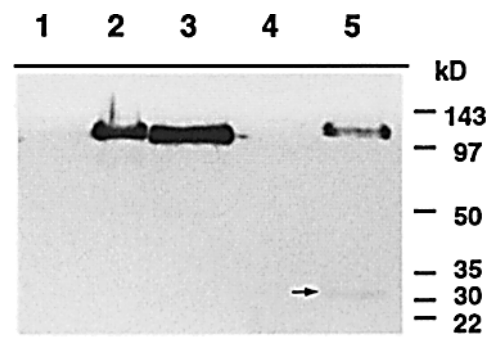
Figure 1. Western blot analysis of pIgR expression in infected hippocampal neurons. (Lane 1) Noninfected neurons; (lane 2) neurons infected with SFV–wild-type pIgR; (lane 3) neurons infected with SFV-mutant (Δ655-668) pIgR; (lane 4) control MDCK cells; (lane 5) stably transfected MDCK cells expressing wild-type pIgR. 40 μg protein was loaded in each lane. Positions of molecular weight markers are indicated on the right.
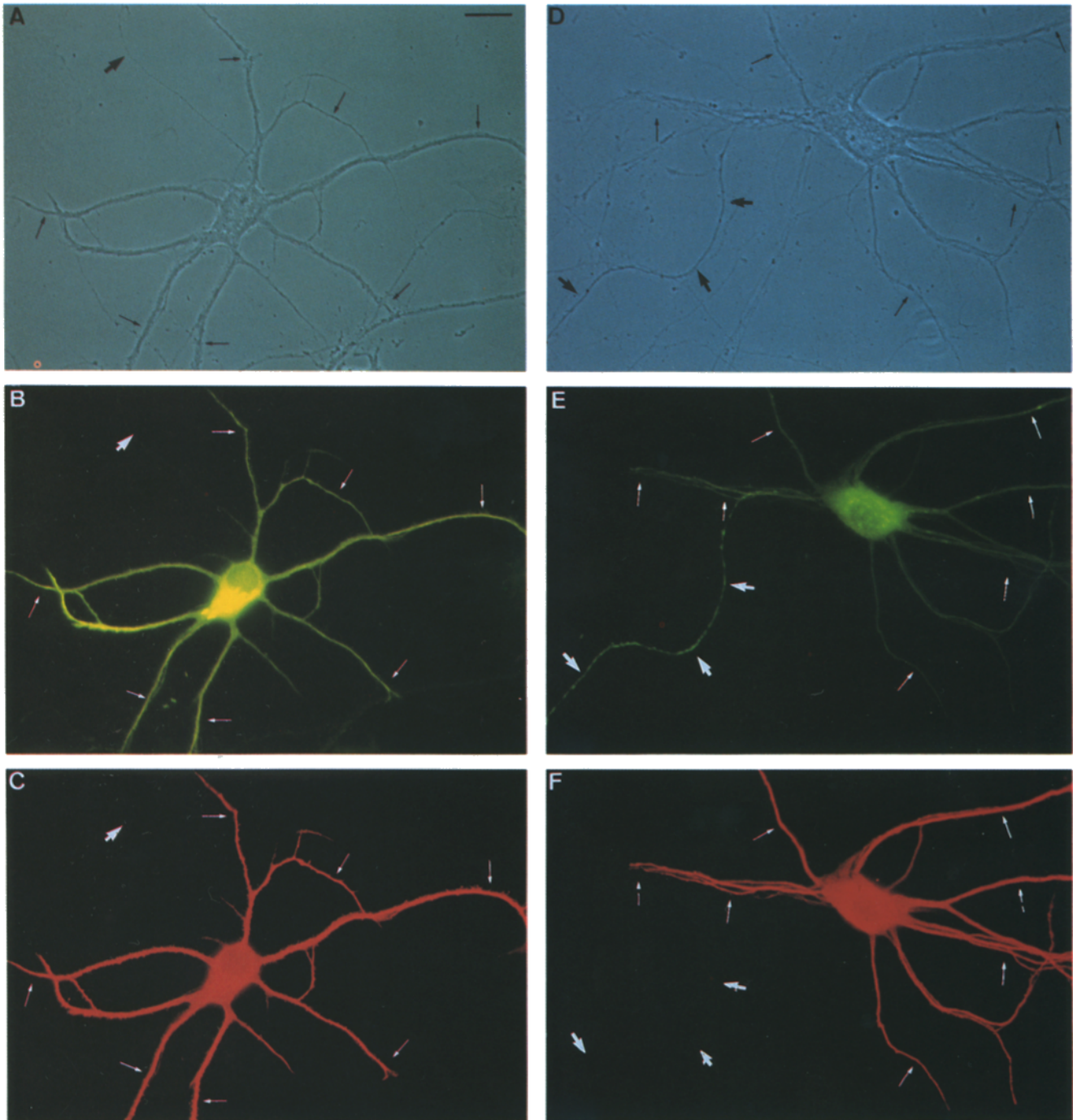
Figure 2. Intracellular distribution of wild-type and Δ655-688 pIgR in polarized neurons at early time after infection. Hippocampal neurons infected with SFV encoding the wild-type pIgR (*A–C*) or pIgR Δ655-688 (*D–F*) were fixed at 3 h postinfection and the intracellular distribution of the expressed receptor was determined by immunofluorescence using the anti-CT antibody. The wild-type receptor is present in the dendrites (*B, small arrows*), identified by the presence of MAP2 (*C*). The axon (*large arrows*) is completely devoid of labeling. The mutant receptor is present in the axon (MAP2-negative neurite, large arrows in *E*), while the dendrites (*small arrows*, identified by MAP2 staining, panel *F*) are devoid of labeling. Bar, 20 μm.

absence of dendritic labeling. This result indicates the direct delivery of the wild-type pIgR from the cell body to the dendritic domain.

In contrast to the cells expressing wild-type pIgR, the majority (64%) of the cells expressing the mutant pIgR displayed cell body and axonal labeling (Fig. 2, *D–F*, Fig. 3

A). Occasionally, labeling was also observed in the proximal, but not the distal, part of the dendrites. This labeling might correspond to receptor accumulated in the organelles of the secretory pathway which, in these cells, are found some distance into the dendrites (Krijnse-Locker, J., R. G. Parton, S. D. Fuller, G. Griffiths, and C. G. Dotti,

A

B
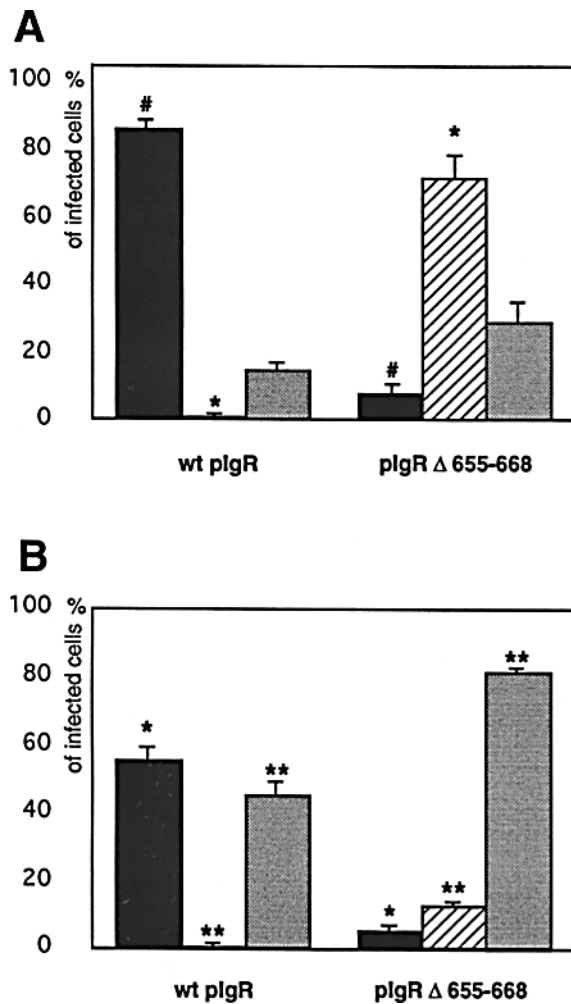
Figure 3. Quantitation of the distribution of wild-type and mutant pIgR in polarized neurons. The percentage of cells showing pIgR labeling in dendrites (*black bars*), axons (*striped bars*) or axons and dendrites (*gray bars*) was determined at 3 h postinfection (*A*) and at 5 h postinfection (*B*). The numbers are the averages of six different experiments, comprising 161 (wild type, 3 h post infection); 122 (mutant, 3 h postinfection), 201 (wild type, 5 h, postinfection), and 175 (mutant, 5 h postinfection) cells. Error bars represent SEM. The differences in distribution between wild-type and mutant pIgR at 3 h postinfection have the following significancies; #, $p < 0.025$; *, $p < 0.05$. At 5 h postinfection the differences in sorting between wild-type and mutant pIgR were less significant (*, $p < 0.05$; **, $p < 0.10$).

manuscript submitted for publication). In 29% of the cells both the axons and the dendrites were labeled. The fact that in most infected cells the axon was the only labeled process suggests the direct axonal sorting of mutant pIgR.

The Intracellular Distribution of Overexpressed pIgR Changes at Long Postinfection Times

In epithelial cells, the pIgR transcytoses from the basolateral plasma membrane to the apical surface even in the absence of ligand (Song et al., 1994). To determine whether or not the dendritically delivered pIgR would move to the

axonal domain in the absence of ligand, we analyzed its distribution at long (5 h) postinfection times. At this time 45% of the cells showed cell body and axonal and dendritic labeling (Fig. 3 *B*), a threefold increase in the number of cells with axonal labeling compared to that of 3 h postinfection (see above). Although not so dramatic, an increase (1.6-fold) in axonal labeling was still observed in the presence of cycloheximide during the last 2 h of the postinfection time (not shown), suggesting either a transcytotic redistribution of wild-type pIgR from the dendritic to the axonal domain or missorting induced by the high expression obtained by the efficient viral expression system.

At long postinfection times (5 h), the percentage of cells expressing mutant pIgR with exclusive axonal staining had decreased to one fifth, from 64% (3 h postinfection) to 13% (5 h postinfection) (Fig. 3 *B*). This decrease in prevalent axonal staining was to a large extent prevented when cycloheximide was added during the last two hours of the postinfection period (data not shown), indicating that the dendritic labeling of the mutant pIgR might be predominantly caused by missorting due to overexpression rather than redistribution.

Overexpressed pIgR Is Internalized into Early Endosomes

A prerequisite for the transcytosis of pIgR in epithelial cells is internalization in basolateral early endosomes where sorting into transcytotic vesicles takes place (Hunziker et al., 1992). Hence, we analyzed next whether overexpressed wild-type pIgR would undergo internalization into the dendritic early endosomes. Infected neurons were incubated with FITC-labeled anti-SC antibody during the last 20 min of the postinfection period. After excess antibody was removed, the cells were fixed, permeabilized, labeled with anti-CT antibodies, and analyzed by immunofluorescence microscopy. Fig. 4 shows the results of such an experiment. The anti-SC antibody was found in vesicular structures present in the cell body and the dendrites (Fig. 4 *C*) which also labeled with the fluid-phase marker Lucifer yellow (not shown). The internalized antibody represented only a fraction of the total pool of wild-type pIgR as revealed by the anti-CT staining (Fig. 4 *B*). No antibody internalization was observed in noninfected cells, showing that the labeling with the anti-SC antibody was not caused by nonspecific endocytosis.

The anti-SC antibody was also internalized in cells expressing the mutant pIgR. Fig. 4 *F* shows that the antibody accumulated intracellularly in vesicular structures. The total pool of the mutant receptor, as detected by the anti-CT antibody (Fig. 4 *E*), seemed to colocalize almost completely with the internalized antibodies. In addition, an almost overlapping staining was found between the total pool of mutant pIgR and Lucifer yellow, that had been internalized for 20 min (data not shown).

Considering that in MDCK cells more than 95% of the mutant pIgR is directly delivered to the apical surface (Casanova et al., 1991), the intense endosomal labeling, present also in the dendritic domain of the hippocampal neurons, was somewhat unexpected. Since the studies in MDCK cells accounted exclusively for the mutant protein that had reached the surface, it was still possible that in
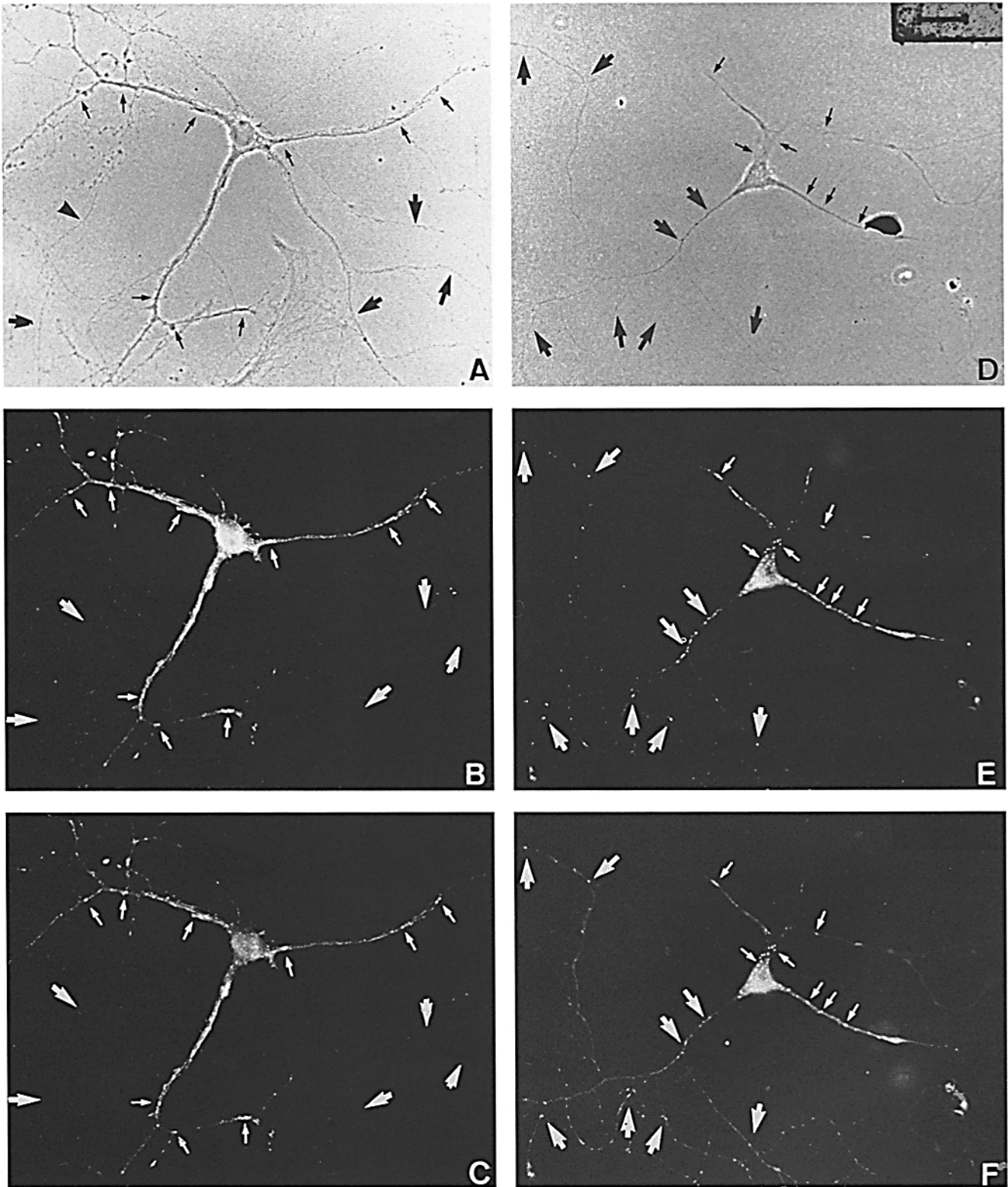
Figure 4. Internalization of pIgR in hippocampal neurons. Hippocampal neurons expressing either wild-type pIgR (*A–C*) or Δ655-668 pIgR (*D–F*) were incubated in the last 20 min of the postinfection period with FITC-conjugated anti-SC antibody, washed, and fixed. *B* and *E* show the total pool of pIgR as revealed by labeling with the anti-CT antibody, and *C* and *F,* the endocytosed anti-SC antibodies. In cells expressing the wild-type pIgR, the internalized antibody can be seen in the dendrites and in the cell body (*small arrows*), but not in any of the axons in the field (*large arrows*). The mutant pIgR is present in regularly shaped structures in both the axon (*large arrows*) as well as in the dendrites (*small arrows*). Bar represents 20 μm.

these cells the Δ655-668 pIgR was also present in the baso-lateral endosomes. To detect the pIgR present in basolateral endosomes, MDCK cells expressing Δ655-668 pIgR were incubated with anti-SC antibody and basolaterally added Lucifer yellow and processed for confocal immunofluorescence microscopy. We observed colocalization of Lucifer yellow (Fig. 5 B) with the internalized antibody (Fig. 5 A), indicating that in MDCK cells part of the mutant receptor is present in endosomal structures and also reaches the basolateral plasma membrane.

The Transcytotic Movement of Anti-SC Antibody

We examined next whether the wild-type pIgR in the dendritic early endosomes would be transcytosed to the axonal domain. For that purpose we bound fluorescently labeled anti-SC antibody at 4°C at 3 h postinfection and analyzed its distribution immediately thereafter or after allowing internalization at 36.5°C for 2 h (see Materials and Methods and Fig. 6 A). In this way, we only look at the pIgR that had reached the dendritic plasma membrane at this short postinfection time, and we thus avoid the interfering visualization of newly synthesized receptor. Directly after antibody binding, an intense uniform labeling was observed at the surface of the cell body and dendrites, but not at the axons (Fig. 7, A–C). After 2 h at 36.5°C, the staining pattern was vesicular and the axon was now intensively labeled (Fig. 7, D–F). These results show the change in the distribution of the receptor from the dendritic surface to the axonal domain. Using a similar experimental paradigm, we did not detect a change in distribution of Δ655-668 pIgR, from axonal to dendritic, suggesting lack of trancytosis of the mutant receptor. In addition, we were also unable to observe a change in the distribution of mutated pIgR upon the addition of the dIgA (data not

shown), which increases axonal labeling of the wild-type receptor (Ikonen et al., 1993).

Transcytosis Is Inhibited by BFA and Stimulated by PMA

The basolateral to apical transcytosis in MDCK cells is inhibited by the fungal metabolite BFA (Hunziker et al., 1991, 1992). To investigate whether transcytosis in hippocampal neurons is regulated by a similar mechanism, we analyzed the effect of BFA on the dendro-axonal transcytosis of pIgR. Infected neurons were incubated for 30 min at 4°C with FITC-labeled anti-SC at the end of the 3-h postinfection period, excess unbound antibody removed and the cells warmed at 36.5°C for 2 h in the presence or absence of 10 μg/ml BFA (see Fig. 6 B). The results of these experiments are shown in Fig. 8 A. In the absence of BFA more than 50% of the infected neurons showed axonal anti-SC labeling at the end of the experiment. In the presence of BFA, on the contrary, axonal labeling did not occur and most cells retained the anti-SC labeling in the dendrites. The small percentage of cells with axonal labeling after BFA treatment was comparable to the percentage of axonal labeling observed at 3 h postinfection, before transcytosis was allowed to occur. These results show, similarly to the transcytosis of pIgR in MDCK cells, the inhibitory effect of BFA in neuronal dendro-axonal transcytosis.

Recently it was shown that transcytosis of the pIgR in MDCK cells is stimulated by phorbol esters (Cardone et al., 1994). Using the assay described above, we tested if the phorbol ester PMA would increase axonal transcytosis of the pIgR. Anti-SC binding at 4°C was followed by a short incubation (20 min) at 36.5°C in the presence or absence of 2 μM PMA (see Fig. 6 C). At this short time after anti-SC binding, no significant axonal labeling was observed in the
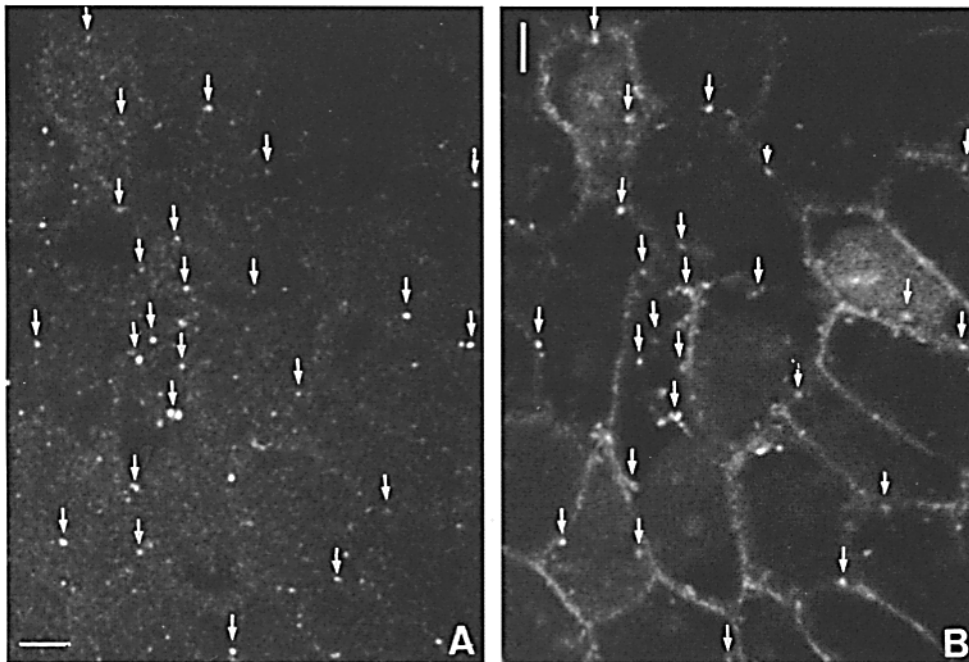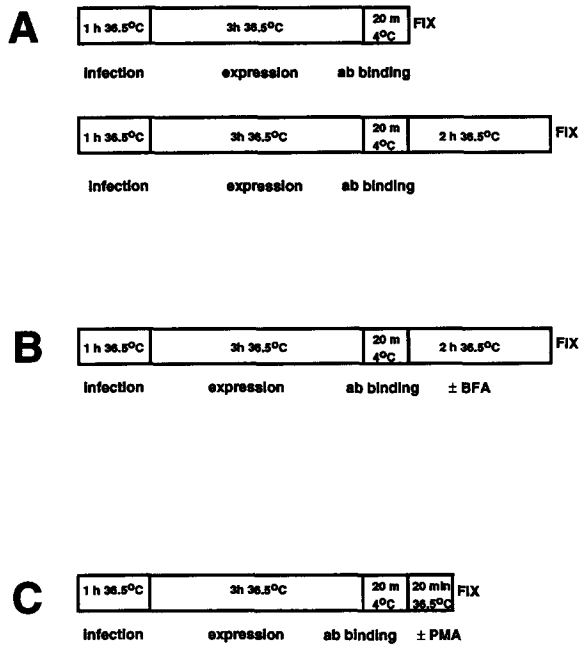

Figure 5. Δ655-668 pIgR is present in basolateral endosomes of MDCK cells. In MDCK cells expressing the mutant receptor the internalized FITC-conjugated anti-SC antibodies (A) colocalize with the fluid-phase marker Lucifer yellow endocytosed for 5 min from the basolateral side (B). The pictures represent a section from the basal part of the cell. Bar represents 2 μm.

Figure 6. Schematic representation of the transcytotic assay. Hippocampal neurons are infected with recombinant SFV and at 3 h postinfection incubated with FITC-labeled antibodies against the ectodomain of pIgR (ab binding). At this time, most of the wild-type receptor is observed in the somato-dendritic domain (see Fig. 3). At the end of the labeling excess antibody is removed and the cells are either fixed or further incubated at 36.5°C for 2 h to allow the dendro-axonal movement of the labeled pIgR (Fig. 6 *A*) and fixed at the end of this incubation period. Fixed cells are permeabilized to visualize the dendrites using antibodies against MAP2. To analyze the effect of BFA or PMA on transcytosis, these drugs were added during the incubation period after the labeling of the cells with FITC-bound antibodies. Incubations with BFA lasted 2 h (Fig. 6 *B*), incubation in the presence of PMA only 20 min (Fig. 6 *C*).

absence of PMA. However, PMA induced a rapid redistribution of the receptor from the dendritic to the axonal domain; the number of cells with axonal labeling increased about fourfold compared to control cells (Fig. 8 *B*). This dramatic increase shows that in neuronal cells, like in MDCK cells, PKC stimulates the axonal appearance of wild-type pIgR.

Intracellular Routing of Wild-Type and Δ655-668 pIgR in Immature Hippocampal Neurons

After 2–3 d in culture hippocampal neurons acquire morphological polarity; one axon and several short neurites ("stage 3 cells"; Dotti et al., 1988). We tested whether wild-type and the mutant Δ655-668 pIgR were sorted in a polarized fashion in these cells. Stage 3 neurons were infected with the recombinant viruses, fixed at 3 h postinfection and the distribution of the expressed receptors was analyzed by immunofluorescence microscopy (Fig. 9). In contrast to fully mature neurons ("stage 5 cells"), fixed at the same postinfection time, no selective delivery of pIgR receptors to any processes was observed in stage 3 cells.

Discussion

Direct Sorting of Wild-Type and Mutant pIgR in Hippocampal Neurons

In MDCK cells, membrane proteins are sorted in the *trans*-Golgi network into distinct vesicles destined for either the apical or the basolateral plasma membrane (Wandinger-Ness et al., 1990). In polarized neurons, membrane proteins are sorted to the axonal and to the dendritic surfaces (for a review see Craig and Banker, 1994). In most cases membrane proteins, targeted to the apical surface of MDCK cells, are delivered to the axonal surface of neurons, whereas those with a basolateral fate in MDCK cells are delivered to the dendritic surface of neurons (for reviews see Rodriguez-Boulan and Powell, 1992; de Hoop and Dotti, 1993; Craig and Banker, 1994). In MDCK cells, the wild-type pIgR is delivered to the basolateral membrane whereas the mutant pIgR is preferentially delivered to the apical membrane (Mostov, 1993; Casanova et al., 1991). Using the SFV-expression system in cultured rat hippocampal neurons, we observed a dendritic distribution of the wild-type receptor and axonal distribution of mutated receptor at 3 h postinfection, the earliest time at which any expression is detectable. This strengthens the case for homologous sorting mechanisms in epithelial and neuronal cells. Although we are analyzing the distribution of a heterologous protein in neurons, endogenous proteins expressed via the SFV system are appropriately sorted and distributed to the right compartments (Olkkonen et al., 1993; de Hoop et al., 1994; Simons et al., 1995). We therefore infer that the distribution of the pIgR at the earliest postinfection time reflects the domain to which direct sorting has taken place.

The Distribution of Wild-Type pIgR Changes at Longer Postinfection Times

With longer expression times, increasing amounts of the receptors were observed in the opposite domain; wild-type receptor appeared in the axons and the Δ655-668 mutant was detected in the dendrites. This change in distribution could be due to either transcytosis or missorting. We favor the idea that the late axonal appearance of the wild-type receptor is partly due to transcytosis. First, using an antibody binding and internalization assay, we noticed that wild-type receptors could migrate from dendrites to axons. Second, the labeling of axons was prevented by BFA and stimulated by PMA. Our previous results showing an increase of axonal pIgR upon addition of dIgA (Ikonen et al., 1993) further supports dendro-axonal transcytosis of pIgR. On the contrary, we consider the presence of the mutant receptor in the dendrites to be the consequence of missorting. Neither could we detect any change in intracellular distribution in the transcytosis assay nor had the addition of ligand any effect. The same mutant receptor expressed in MDCK cells does not transcytose (Casanova et al., 1991). In these cells, although most of the mutant protein is delivered apically, ~5% of the protein is detected on the basolateral surface (Casanova et al., 1991), also suggesting missorting. We found that in MDCK cells, part of the Δ665-668 receptor can internalize anti-SC antibody
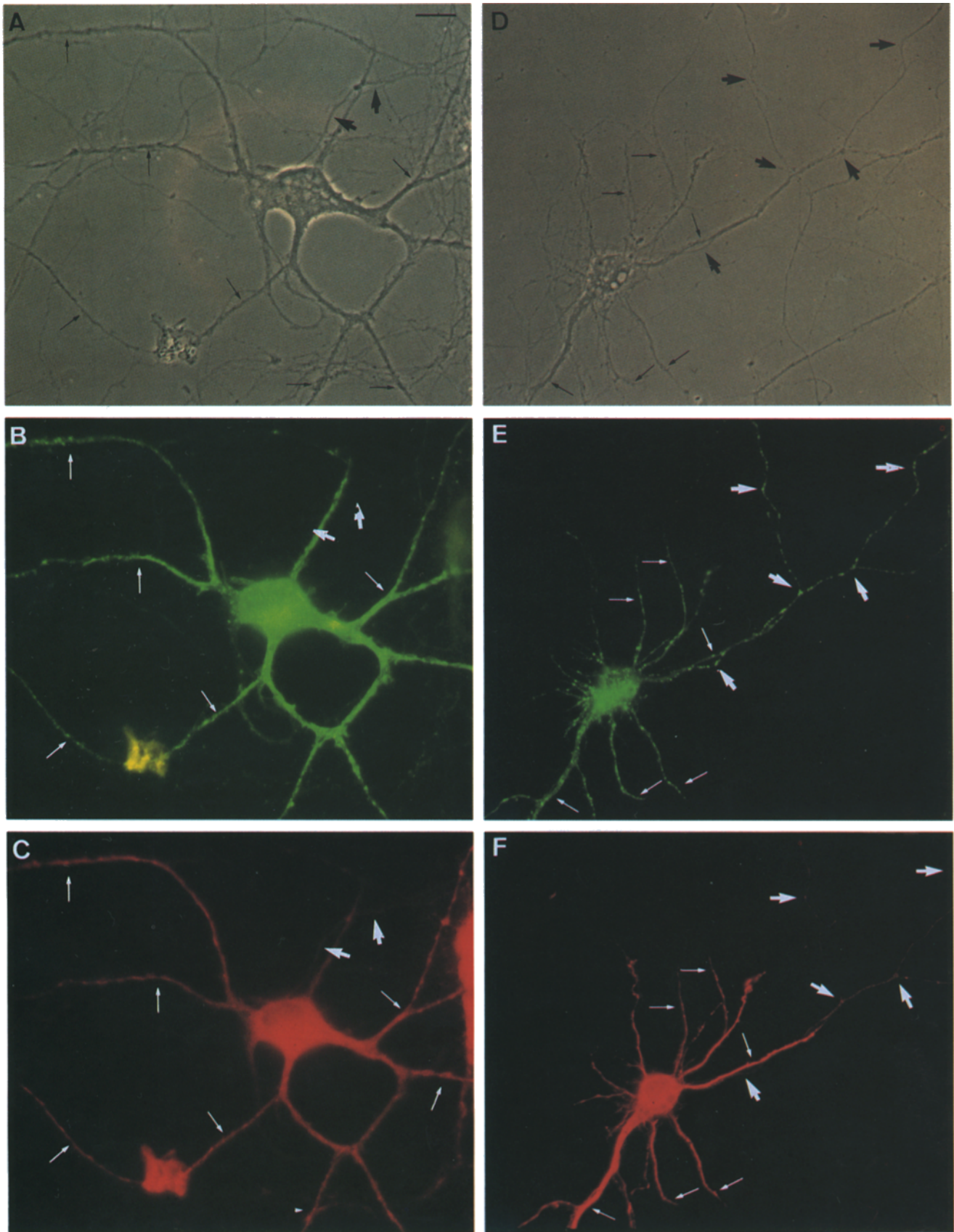
Figure 7. Transcytosis of the pIgR in neurons. Infected cells were incubated at 3 h postinfection with FITC-conjugated anti-SC for 30 min at 4°C, excess antibody was removed, and the cells were fixed (*A–C*) or further incubated for 2 h at 36.5°C, and then fixed (*D–F*). Directly after the binding of the FITC-labeled anti-SC, a surface labeling of the dendrites and cell body is revealed (*B; small arrows*). Permeabilization after fixation permitted the labeling of the dendrites with antibodies against MAP2 (*C* and *F*). The axons (MAP2-negative; see *C*), visible in the phase contrast photograph in *A*, are unlabeled (*large arrows*). Fixation of the cells 2 h after binding and detection of the bound antibody reveals strong labeling in the axon (*E; large arrows*) as well as in the dendrites. In contrast to *B*, the staining in *E* appears to be predominantly punctate. Bar represents 20 μm.

A

B
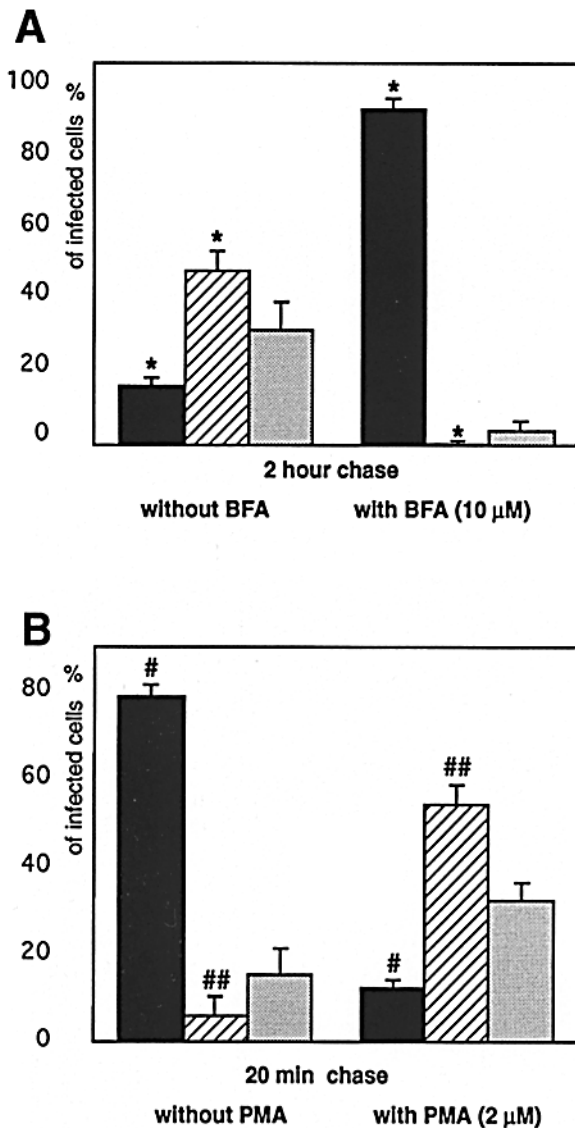
Figure 8. BFA inhibits axonal transcytosis whereas PMA increases it. Cells expressing wild-type pIgR were incubated at 3 h postinfection with FITC-labeled anti-SC for 30 min at 4°C, excess antibody washed away and the cells further incubated at 36.5°C either for 2 h with or without BFA (*A*), or for 20 min with or without PMA (*B*). (*Black bar*) Percentage of cells with labeling in the somatodendritic domain; (*striped bars*) percentage of cells with immunofluorescence in axonal domain; (*gray bars*) percentage of cells with immunofluorescence in all neurites. (*A*) In untreated neurons, 83% of the cells (*striped plus gray bars*) show axonal anti-SC labeling after 2 h incubation at 36.5°C. Incubation with BFA during these 2 h completely blocks axonal appearance. (*B*) After 20 min at 36.5°C only 21% of control cells (*striped plus gray bars*) have axonal labeling. The inclusion of PMA during that time increases axonal labeling to 88% of the cells. The numbers are the averages of two different experiments, comprising 56 (−BFA); 26 (+BFA); 39 (−PMA); 67 (+PMA) cells. Error bars represent SEM values. Significancies in the differences in distribution of the receptor in the absence or presence of the drug (PMA, BFA) are as follows: *, $p < 0.05$; #, $p < 0.025$; ##, $p < 0.10$.

from the basolateral surface to the basolateral early endosomes.

Neuronal Transcytosis of Wild-Type pIgR Is Sensitive to BFA and PMA

Transcytosis of the polymeric immunoglobulin receptor in MDCK cells appears to be sensitive to protein kinase C (stimulated via PMA) (Cardone et al., 1994) and brefeldin A (BFA) (Hunziker et al., 1992). We analyzed the effect of the compounds BFA and PMA to determine whether transcytosis of the pIgR in neurons was affected in a way similar to MDCK cells. With our transcytosis assay we found that BFA inhibited the axonal migration of receptor that had been fluorescently labeled at the dendritic surface. In the presence of PMA, transcytosis from the dendrites to the axon was accelerated. This increase in axonal labeling could be observed in times as short as 20 min. Thus, these experiments suggest that the transcytotic pathway in neurons resembles that of MDCK cells; in the sense that it is inhibited by BFA and stimulated by PMA. Treatment of hippocampal neurons with BFA induces endosomal tubulation without affecting the uptake of fluid-phase ligands (Cid-Arregui et al., 1995). Therefore, as in MDCK cells (Hunziker et al., 1991), BFA might block the dendro-axonal transcytosis of pIgR by specifically affecting the formation of transcytotic vesicles in the early endosomes. Whether BFA also affects the transport to the degradative compartment and/or the recycling to the plasma membrane in neurons is not known. In MDCK cells, however, BFA does not affect these pathways (Hunziker et al., 1991).

In MDCK cells PMA increases the movement of pIgR from the apical endosomes to the apical surface and accelerates the rate of transcytosis from the basolateral endosomes (Cardone et al., 1994). Although we did not determine the effect of PMA on the release of IgA from the axonal endosomes, our work clearly shows that in neurons PMA increases dendro-axonal transcytosis by accelerating the migration of pIgR from the dendritic early endosomes or from the dendritic plasma membrane to the axonal domain. It is believed that the effect of PMA on MDCK cells to stimulate transcytosis mimics the action of cholinergic agonsits to stimulate the release of IgA by nasal and intestinal epithelia in vivo (Raphael et al., 1988; Freier et al., 1987; Wilson et al., 1992). Since hippocampal neurons in culture also appear to respond to PMA by increasing the rate of transcytosis, we envision that in vivo neurons may regulate the traffic of endogenous transcytosed molecules in response to different hormones and neurotransmitters.

Sorting of the pIgR Is Developmentally Regulated

In stage 3 hippocampal neurons morphological polarization is already visible, the longer neurite will develop into the futural axon (Dotti et al., 1988). This polarity is however not yet fixed; amputation of the futural axon results in the acquisition of axonal properties of one of the other neurites and the acquisition of dendritic properties of the amputated axon (Dotti and Banker, 1987; Goslin and Banker, 1989). Expression of either wild-type or mutant pIgR in stage 3 hippocampal neurons, led to the nonpolarized distribution of the receptor. Accordingly, certain
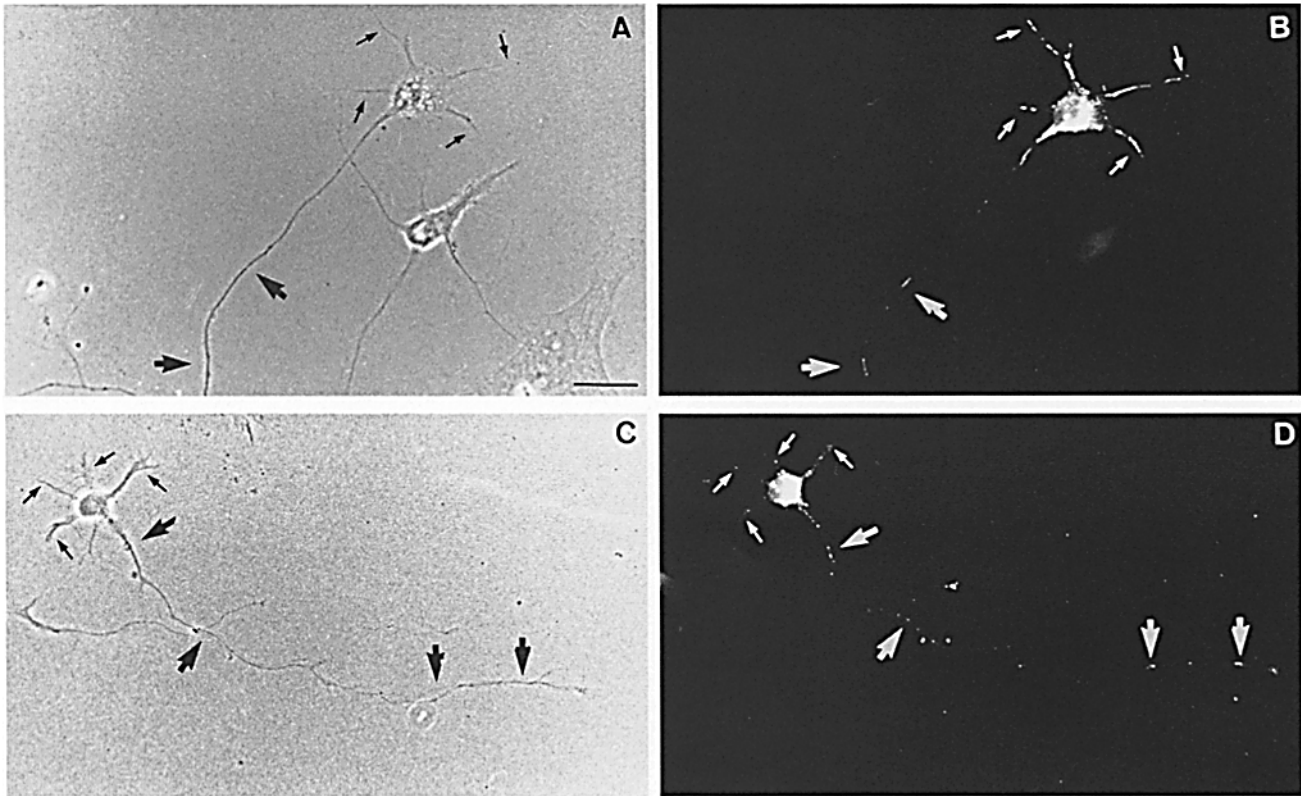
Figure 9. Immature neurons do not sort pIgR. Distribution of wild-type (*A* and *B*) and Δ655-668 (*C* and *D*) mutant pIgR receptor in young neurons in culture. Cells were fixed at 3 h postinfection and analyzed for the distribution of receptor using anti-CT antibody. Both the wild-type as well as the Δ655-668 pIgR could be observed in the small neurites that will form the dendrites (*small arrows*) as well as in the longer outgrowing neurite that will from the axon (*large arrows*). Bar, 20 μm.

endogenous proteins, with polarized distribution in fully differentiated stage 5 hippocampal neurons, are evenly distributed in the stage 3 cells, despite their morphological polarity (Killisch et al., 1991; Craig et al., 1993; van Lookeren Campagne et al., 1992; Huber et al., 1993b; see however, Goslin et al., 1990 and Fletcher et al., 1991). Similarly, viral glycoproteins expressed in differentiated neurons are sorted to the axon or the dendrites of fully mature (stage 5) neurons, whereas the same proteins are not sorted in morphologically polarized stage 3 neurons (Dotti and Simons, 1990). Altogether, these results suggest the asynchronous development of the machinery responsible for the establishment of morphological polarity and polarized protein delivery. The establishment of the machinery for molecular sorting is also a late occurring event in epithelial cells. Two different types of epithelial cells, MDCK and FRT, show lack of protein sorting at early developmental stages (Wollner et al., 1992; Zurzolo et al., 1992). The mechanism which regulates the stabilization of neuronal polarity and molecular sorting remain to be identified.

Implications of Neuronal Transcytosis

Bearing in mind the similarities between epithelial MDCK cells and neurons, it was logical to hypothesize that neurons could make use of a dendro-axonal transcytotic route, either as a way to generate membrane asymmetry or to transport macromolecules. However, little is known about

the existence of a transcellular vesicular transport pathway in neurons. Wheat germ agglutinin (WGA) has been shown to undergo dendritic to axonal transneuronal transport in retinal ganglion cells (LaVail and Margolis, 1987; Ruda and Coulter, 1982). However, it may not use the same transcytotic pathway as the pIgR. Unlike pIgR, after dendritic uptake WGA accumulates in the Golgi apparatus of most cells, including hippocampal neurons (Dotti and Banker, 1991). Therefore, the presence of WGA along vesicular profiles in axons of the visual system might represent exocytic rather than transcytotic vesicles. Horseradish peroxidase and fibroblast growth factor have also been shown to undergo anterograde transport in the optic nerve after uptake in the ganglion cells (Pickard and Silverman, 1981; Ferguson et al., 1990), possibly by a transcytotic mechanism similar to that of the pIgR. There are also indications for a transneuronal axo-dendritic transport (Schwab and Thoenen, 1978; Schwab et al., 1979; Evinger and Erichsen, 1986; Fabian, 1991; Simons et al., 1995).

Altogether our work reveals the existence of a transcytotic pathway in neurons and shows similarities of this pathway between epithelial and neuronal cells. To what extent transcytotic pathways are important for the establishment and maintenance of neuronal cell polarity or for transneuronal communication remains to be established. Transcytosis might be the pathway by which growth factors, like basic fibroblast growth factor, are transported during neuronal development (Ferguson et al., 1990), and

thus be an essential mode of transcellular communication. In support of this are the observations that an axonal to dendritic transcytotic process might be involved in the generation of motor-neuron diseases and other neurological malfunctions (Fabian, 1991; LaRocca and Wiley, 1988) and that the amyloid precursor protein (APP) is transcytosed from axons to dendrites (Simons et al., 1995). However, the true in vivo physiological role of neuronal transcytosis remains to be identified.

References

Apodaca, G., L.A. Katz, and K.E. Mostov. 1994. Receptor-mediated transcytosis of IgA in MDCK cells is via apical recycling endosomes. *J. Cell Biol.* 125: 67–86.

Apodaca, G., M. Bomsel, J. Arden, P. Breitfeld, K. Tang, and K. Mostov. 1991. The polymeric immunoglobulin receptor. A model protein to study transcytosis. *J. Clin. Invest.* 87:1877–1882.

Aroeti, B., J. Casanova, C. Okamoto, M. Cardone, A. Pollack, K. Tang, and K. Mostov. 1992. Polymeric immunoglobulin receptor. *Int. Rev. Cytol.* 137B: 157–169.

Barroso, M., and E. S. Sztul. 1994. Basolateral to apical transcytosis in polarized cells is indirect and involves BFA and trimeric G protein sensitive passage through the apical endosome. *J. Cell Biol.* 124:83–100.

Bartles, J. R., H. M. Feracci, B. Stieger, and A. L. Hubbard. 1987. Biogenesis of the rat hepatocyte plasma membrane in vivo: comparison of the pathways taken by apical and basolateral proteins using subcellular fractionation. *J. Cell Biol.* 105:1241–1252.

Bomsel, M., and K. E. Mostov. 1992. Role of heterotrimeric G proteins in membrane traffic. *Mol. Biol. Cell.* 3:1317–1328.

Bottenstein, J. E., and G. H. Sato. 1979. Growth of a rat neuroblastoma cell line in serum-free supplemented medium. *Proc. Natl. Acad. Sci. USA.* 76:514–517.

Brändli, A.W., E. D. Adamson, and K. Simons. 1991. Transcytosis of epidermal growth factor. *J. Biol. Chem.* 266:8560–8566.

Breitfeld, P., J. Harris, and K. E. Mostov. 1989. Postendocytic sorting of the ligand for the polymeric immunoglobulin receptor in Madin-Darby Canine Kidney cells. *J. Cell Biol.* 109:475–486.

Brown, D. A., B. Crise, and J. K. Rose. 1989. Mechanism of membrane anchoring affects polarized expression of two membrane proteins in MDCK cells. *Science (Wash. DC).* 245:1499–1501.

Caceres, A., G. A. Banker, O. Steward, L. Binder, and M. Payne. 1984. MAP2 is localized to the dendrites of hippocampal neurons which develop in culture. *Dev. Brain Res.* 13:314–318.

Cardone, M. H., B. L. Smith, W. Song, D. Mochly-Rosen, and K. Mostov. 1994. Phorbol myristate acetate-mediated stimulation of transcytosis and apical recycling in MDCK cells. *J. Cell Biol.* 124:717–727.

Casanova, J. E., P. P. Breitfeld, S. A. Ross, and K. E. Mostov. 1990. Phosphorylation of the polymeric immunoglobulin receptor required for its efficient transcytosis. *Science (Wash. DC).* 248:742–744.

Casanova, J. E., G. Apodaca, and K. E. Mostov. 1991. An autonomous signal for basolateral sorting in the cytoplasmic domain of the polymeric immunoglobulin receptor. *Cell.* 66:65–75.

Cid-Arregui, A., R. Parton, K. Simons, and C. Dotti. 1995. Nocodazole-dependent transport, and brefeldin A-sensitive processing and sorting of newly synthesized membrane proteins in cultured neurons. *J. Neurosci.* 15:4259–4269.

Craig, A. M., and G. Banker. 1994. Neuronal polarity. *Annu. Rev. Neurosci.* 17: 267–310.

Craig, A. M., C. D. Blackstone, R. L. Huganir, and G. Banker. 1993. The distribution of glutamate receptors in cultured rat hippocampal neurons: postsynaptic clustering of AMPA-selective subunits. *Neurons.* 10:1055–1068.

Dotti, C. G., and G. A. Banker. 1987. Experimentally induced alteration in the polarity of developing neurons. *Nature (Lond.).* 330:254–256.

Dotti, C. G., and K. Simons. 1990. Polarized sorting of viral glycoproteins to the axon and dendrites of hippocampal neurons in culture. *Cell.* 62:63–72.

Dotti, C. G., and G. Banker. 1991. Intracellular organization of hippocampal neurons during the development of neuronal polarity. *J. Cell Sci.* 15(suppl.): 75–84.

Dotti, C. G., C. A. Sullivan, and G. A. Banker. 1988. The establishment of polarity by hippocampal neurons in culture. *J. Neurosci.* 8:1454–1468.

Evinger, C., and J. T. Richsen. 1986. Transsynaptic retrograde transport of fragment C of tetanus toxin demonstrated by immunohistochemical localization. *Brain Res.* 380:383–388.

Fabian, R. H. 1991. Retrograde axonal transport and transcytosis of immunoglobulins: implications for the pathogenesis of autoimmune motor neuron disease. *In* Advances in Neurology. 56: Amyotrophic lateral sclerosis and other motor neuron diseases. L. P. Rowland, editor. Raven Press Ltd. 433–444.

Ferguson, I. A., J. B. Schweitzer, and E. M. Johnston. 1990. Basic fibroblast growth factor: receptor-mediated internalization, metabolism, and anterograde axonal transport in retinal ganglion cells. *J. Neurosci.* 10:2176–2189.

Fletcher, T. L., P. Cameron, P. De Camilli, and G. Banker. 1991. The distribution of synapsin I and synaptophysin in hippocampal neurons developing in culture. *J. Neurosci.* 11:1617–1626.

Freier, S., M. Eran, and J. Faber. 1987. Effect of cholecystokinin and of its antagonist, of atropin, and of food on the release of immunoglobulin A and immunoglobulin G specific antibodies in the rat intestine. *Gastroenterology.* 93: 1242–1246.

Friden, P. M., L. R. Walus, G. F. Musso, M. A. Taylor, B. Malfroy, and R. M. Starzyk. 1991. Anti-transferrin receptor antibody-drug conjugates cross the blood-brain barrier. *Proc. Natl. Acad. Sci. USA.* 88:4771–4775.

Ghitescu, L., and M. Bendayan. 1992. Transendothelial transport of serum albumin: a quantitative immunocytochemical study. *J. Cell Biol.* 117:745–755.

Gonnella, P. A., K. Simonoski, R. A. Murphy, and M. R. Neutra. 1987. Transepithelial transport of epidermal growth factor by absorptive cells of suckling rat epithelium. *J. Clin. Invest.* 80:22–32.

Goslin, K., and G. Banker. 1989. Experimental observations on the development of polarity by hippocampal neurons in culture. *J. Cell Biol.* 108:1507–1516.

Goslin, K., and G. Banker. 1991. Rat hippocampal neurons in low-density culture. *In* Culturing Nerve Cells. G. Banker and K. Goslin, editors. MIT Press, Cambridge, MA. pp. 251–281.

Goslin, K., D. J. Schreyer, J. H .P . Skene, and G. Banker. 1990. The distribution of GAP-43 during the development of neuronal polarity. *J. Neurosci.* 10: 588–602.

Hansen, S. H., and J. E. Casanova. 1994. Gsα stimulates transcytosis and apical secretion in MDCK cells through cAMP and protein kinase A. *J. Cell Biol.* 126:677–687.

Hashida, R., A. Anamizu, J. Kimura, S. Ohkuma, Y. Youhida, and T. Takano. 1986. Transcellular transport of lipoprotein through arterial endothelial cells in monolayer culture. *Cell Struct. Funct.* 11:31–42.

Herzog, V. 1983. Transcytosis in thyroid follicle cells. *J. Cell Biol.* 97:607–617.

Hirt, R. P., G. J. Hughes, S. Frutiger, P. Michetti, C. Perregaux, O. Poulain-Godefroy, N. Jeanguenat, M. Neutra, and J.-P. Kraehenbuhl. 1993. Transcytosis of the polymeric Ig receptor requires phosphorylation of Serine 664 in the absence but not the presence of dimeric IgA. *Cell.* 74:245–255.

de Hoop, M. J., and C. G. Dotti. 1993. Membrane traffic in polarized neurons in culture. *J. Cell Sci.* 17(suppl):85–92.

de Hoop, M. J., L. A. Huber, H. Stenmark, E. Williamson, M. Zerial, R. G. Parton, and C. G. Dotti. 1994. The involvement of the small GTP-binding protein rab5a in neuronal endocytosis. *Neuron.* 13:11–22.

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

Huber, L. A., M. J. de Hoop, P. Dupree, M. Zerial, K. Simons, and C. G. Dotti. 1993b. Protein transport to the dendritic plasma membrane of cultured neurons is regulated by rab8p. *J. Cell Biol.* 123:47–55.

Hunziker, W., and I. Mellman. 1989. Expression of macrophage-lymphocyte Fc receptors in Madin-Darby Canine Kidney cells: polarity and transcytosis differ for isoforms with or without coated pit localization domains. *J. Cell Biol.* 109:3291–3302.

Hunziker, W., P. Mâle, and I. Mellman. 1990. Differential microtubule requirements for transcytosis in MDCK cells. *EMBO (Eur. Mol. Biol. Organ.) J.* 9: 3515–3525.

Hunziker, W., J. A. Whitney, and I. Mellman. 1991. Selective inhibition of transcytosis by brefeldin A in MDCK cells. *Cell.* 67:617–627.

Hunziker, W., J. A.Whitney, and I. Mellman. 1992. Brefeldin A and the endocytic pathway. Possible implications for membrane traffic and sorting. *FEBS Lett.* 307:93–96.

Ikonen, E., R. G. Parton, W. Hunziker, K. Simons, and C. G. Dotti. 1993. Transcytosis of the polymeric immunoglobulin receptor in cultured hippocampal neurons. *Curr. Biol.* 3:635–644.

Killisch, I., C. G. Dotti, D. J. Laurie, H. Lüddens, and P. Seeburg. 1991. Expression patterns of GABA_A receptor subtypes in developing hippocampal neurons. *Neuron.* 7:927–936.

King, G. L., and S. M. Johnson. 1985. Receptor-mediated uptake and transport of insulin by endothelial cells. *Science (Wash. DC).* 227:1583–1585.

Kraehenbuhl, J.-P., and M. R. Neutra. 1992. Transepithelial transport and mucosal defence II: secretion of IgA. *Trends Cell Biol.* 2:170–174.

Kühn, L., and J. P. Kraehenbuhl. 1983. Monoclonal antibodies recognizing the secreted and membrane domains of the IgA dimer receptor. *Ann. NY Acad. Sci.* 409:751–759.

LaVail, J., and T. P. Margolis. 1987. The anterograde axonal transport of wheat germ agglutinin as a model for transcellular transport in neurons. *In* Axonal Transport. R. S. Smith and M.A. Bisby, editors. Alan R. Liss, Inc., New York. pp. 311–326.

LaRocca, C. D., and R. G.Wiley. 1988. Monoclonal anti-Thy1 antibody (OX7) is axonally transported in rat nervous system. *Brain Res.* 449:381–385.

Liljeström, P., and H. Garoff. 1991. A new generation of animal cell expression vectors based on the Semliki Forest virus replicon. *Biotechnology.* 9:1356–1361.

van Lookeren Campagne, M., C. G. Dotti, E. R. A. Jap Tjoen San, A. J. Verkleij, W. H. Gispen, and A. B. Oestreicher. 1992. B-50/GAP43 localization in polarized hippocampal neurons in vitro: an ultrastructural quantitative study. *Neuroscience.* 50:35–52.

Mostov, K. 1993. Protein traffic in polarized epithelial cells: the polymeric immunoglobulin receptor as a model system. *J. Cell Sci. Suppl.* 17:21–26.

Mostov, K. E., and D. L. Deitcher. 1986. Polymeric immunoglobulin receptors expressed in MDCK cells transcytosis IgA. *Cell.* 46:613–621.

Mostov, K. E., M. Friedlander, and G. Blobel. 1984. The receptor for transepithelial transport of IgA and IgM contains multiple immunoglobulin-like domains. *Nature (Lond.).* 308:37–43.

Mostov, K., G. Apodaca, B. Aroeti, and C. Okamoto. 1992. Plasma membrane protein sorting in polarized epithelial cells. *J. Cell Biol.* 116:577–583.

Nelson, W. J. 1992. Regulation of cell surface polarity from bacteria to mammals. *Science (Wash. DC).* 258:948–955.

Okamoto, C. T., S.-P. Shia, C. Bird, K. E. Mostov, and M. G. Roth. 1992. The cytoplasmic domain of the polymeric immunoglobulin receptor contains two internalization signals that are distinct from its basolateral sorting signal. *J. Biol. Chem.* 267:9925–9932.

Okamoto, C. T., W. Song, M. Bomsel, and K. Mostov. 1994. Rapid internalization of the polymeric immunoglobulin receptor requires phosphorylation of serine 726. *J. Biol. Chem.* 269:15676–15682.

Olkkonen, V. M., P. Liljeström, H. Garoff, K. Simons, and C. G. Dotti. 1993. Expression of heterologous proteins in cultured rat hippocampal neurons using the Semliki Forest vector. *J. Neurosci. Res.* 35:445–451.

Pardridge, W. M. 1991. Advances in cell biology of blood-brain barrier transport. *Semin. Cell Biol.* 2:419–426.

Pickard, G. E., and A.-J. Silverman. 1981. Direct retinal projections to the hypothalamus, piriform cortex, and accesory optic nuclei in the golden hamster as demonstrated by a sensitive anterograde horseradish peroxidase technique. *J. Comp. Neurol.* 196:155–172.

Raphael, G. D., H. M. Druce, J. N. Baraniuk, and M. A. Kaliner. 1988. Pathophysiology of rhinitis. 1. Assessment of the sources of protein in metacho-line-induced nasal secretions. *Annu. Rev. Respir. Dis.* 138:413–420.

Rodman, J. S., J. W. Mercer, and P. D. Stahl. 1990. Endocytosis and transcytosis. *Curr. Opin. Cell Biol.* 2:664–672.

Rodriguez-Boulan, E., and S. K. Powell. 1992. Polarity of epithelial and neuronal cells. *Annu. Rev. Cell Biol.* 8:395–427.

Ruda, M., and J. D. Coulter. 1982. Axonal and transneuronal transport of wheat germ agglutinin demonstrated by immunocytochemistry. *Brain Res.* 249:237–246.

Schwab, M. E., and H. Thoenen. 1978. Selective binding, uptake and retrograde transport of tetanus toxin by nerve terminals in rat iris. *J. Cell Biol.* 77:1–14.

Schwab, M. F., K. Suda, and H. Thoenen. 1979. Selective retrograde transsynaptic transfer of a protein, tetanus toxin, subsequent to its retrograde axonal transport. *J. Cell Biol.* 82:798–810.

Siminoski, K., P. Gonella, J. Bernake, L. Owen, M. Neutra, and R. A. Murphy. 1986. Uptake and transepithelial transport of nerve growth factor in suckling rat ileum. *J. Cell Biol.* 103:1979–1990.

Simons, M., E. Ikonen, P. J. Tienari, A. Cid-Arregui, U. Mönning, K. Beyreuther, and C. G. Dotti. 1995. The intracellular routing of human APP: axonal delivery followed by transport to the dendrites. *J. Neurosci. Res.* 41:121–128.

Soda, R., and M. Tavassoli. 1984. Transendothelial transport (transcytosis) of iron-transferrin complex in the blood marrow. *J. Ultrastruct. Res.* 88:18–29.

Solari, R., L. Kühn, and J. P. Kraehenbuhl. 1985. Antibodies recognizing different domains of the polymeric immunoglobulin receptor. *J. Biol. Chem.* 260:1141–1145.

Sooranna, S. R., and S. F. Contractor. 1991. Vectorial transcytosis of immunoglobulin G by human term trophoblast cells in culture. *Exp. Cell Res.* 192:41–45.

Song, W., M. Bomsel, J. Casanova, J.-P. Vaerman, and K. Mostov. 1994. Stimulation of transcytosis of the polymeric immunoglobulin receptor by dimeric IgA. *Proc. Natl. Acad. Sci. USA.* 91:163–166.

Sztul, E. 1992. Transcytotic vesicle fusion with plasma membrane. *Methods Enzymol.* 219:44–52.

Wandinger-Ness, A., M. K. Bennett, C. Antony, and K. Simons. 1990. Distinct transport vesicles mediate the delivery of plasma membrane proteins to the apical and the basolateral domains of MDCK cells. *J. Cell Biol.* 111:987–1000.

Wilson, I. D., R. D. Soltis, R. E. Olson, and S. L. Erlandsen. 1982. Cholinergic stimulation of immunoglobulin A secretion in rat intestine. *Gastroenterology.* 83:881–888.

Wollner, D. A., K. A. Krzeminski, and W. J. Nelson. 1992. Remodeling the cell surface distribution of membrane proteins during the development of epithelial cell polarity. *J. Cell Biol.* 116:889–899.

Zurzolo, C., A. Le Bivic, A. Quaroni, L. Nitsch, and E. Rodriguez-Boulan. 1992. Modulation of transcytotic and direct targeting pathways in a polarized thyroid cell line. *EMBO (Eur. Mol. Biol. Organ.) J.* 11:2337–2344.