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# Polarisation, key to good localisation

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**Abstract**

Polarisation of cells is crucial for vectorial transport of ions and solutes. In literature, however, proteins specifically targeted to the apical or basolateral membrane are often studied in non-polarised cells. To investigate whether these data can be extrapolated to expression in polarised cells, we studied several membrane-specific proteins. In polarised MDCK cells, the Aquaporin-2 water channel resides in intracellular vesicles and apical membrane, while the vasopressin-type 2 receptor, anion-exchanger 1 (AE1) protein and E-Cadherin mainly localise to the basolateral membrane. In non-polarised MDCK cells, however, Aquaporin-2 localises, besides plasma membrane, mainly in the Golgi complex, while the others show a dispersed staining throughout the cell. Moreover, while AQP2 mutants in dominant nephrogenic diabetes insipidus are missorted to different organelles in polarised cells, they all predominantly localise to the Golgi complex in non-polarised MDCK cells. Additionally, the maturation of V2R, and likely its missorting, is affected in transiently-transfected compared to stably-transfected cells. In conclusion, we show that the use of stably-transfected polarised cells is crucial in interpreting the processing and the localisation of membrane targeted proteins.


*Keywords:* Polarisation; Aquaporin-2; V2R; Principal cell; Kidney

## 1. Introduction

In the body, epithelial layers form the boundary between different compartments, separating the interstitium from the outside world, and are of vital importance in maintaining ionic homeostasis by allowing regulated vectorial transport of ions and solutes. Crucial in this process is the establishment of an asymmetric cell surface distribution, dividing the plasma membrane in two structurally and functionally different domains, known as the apical and the basolateral membrane. The process of cell polarisation is guided by different polarisation cues, induced by cell–cell and cell–extracellular matrix (ECM) contacts, resulting in the formation of tight junctions and a reorganisation of the cytoskeleton (reviewed in [1] and [2]). Typical is the reorganisation of the microtubule network to an apico-basolateral array, which allows the vectorial transport to and from the apical and basolateral domains, which is important for maintaining asymmetry, as well as for transepithelial transport [1].

Two of such proteins for which proper polarised expression is important are the water channel Aquaporin-2 (AQP2) and its upstream vasopressin type-2 receptor (V2R) [3], which are essential in the process of vasopressin-regulated concentration of urine. After synthesis and homotetramerisation in the endoplasmic reticulum and transport through the Golgi, AQP2 is stored in so-called storage vesicles localised close to the apical membrane [4–6]. Vasopressin binding by basolaterally-localised V2R leads to a transient rise in intracellular cAMP levels resulting in

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protein kinase A (PKA) activation and subsequent AQP2 phosphorylation at serine 256 (S256) [7]. Phosphorylation of minimally three monomers of the AQP2 tetramer results in an apical translocation of the protein, enabling reabsorption of pro-urinary water from the apical side [8]. The constitutively expressed basolateral water channels AQP3 and AQP4 enable the basolateral exit of reabsorbed water to the interstitium.

Recently, we generated polarised cell models for the regulation of V2R and AQP2. In MDCK cells, GFP-tagged V2R and untagged AQP2 were stably transfected. The resulting GFP-V2R was shown to be (mature) complex-glycosylated, localised to the basolateral membrane of polarised cells and was stimulated and internalised upon vasopressin stimulation, thereby closely resembling the in vivo situation [9]. Polarised MDCK-AQP2 cells show a subapical localisation of AQP2, which redistributes to the apical membrane after an increase in cellular cAMP after AVP stimulation or by forskolin [10]. AQP2 mutations found in nephrogenic diabetes insipidus (NDI), a disorder in which the kidney is unable to concentrate urine, often interfere with this routing, resulting in a different localisation of AQP2 [11–15]. Other often used mutations, though not NDI derived, are the S256A and S256D mutants, which represent constitutively non-phosphorylated and phosphorylated AQP2, respectively. These two mutations have been crucial in understanding the routing of the AQP2 molecule after vasopressin signalling [7,8,16]. In steady state and independent of forskolin stimulation, AQP2-S256A resides in intracellular vesicles. In contrast AQP2-S256D is constitutively localised in the apical membrane.

The collecting ducts of the distal nephron, roughly consists of two major cell types: the principal cells, which express AQP2 and V2R, and the α-intercalated cells. The polytopic chloride–bicarbonate exchanger AE1 is expressed in the basolateral membrane of the latter cell type and is involved in the regulation of our pH homeostasis [17]. Mutations in the gene for AE1 cause autosomal dominant distal renal tubular acidosis (ddRTA) and are characterised by defective trafficking [18–20].

Madin–Darby canine kidney (MDCK) cells provided a polarised cell model in which the routing of these specific apical and basolateral proteins could be studied in resembling organ physiology. However, in literature, several proteins, known to target specifically to either the apical or the basolateral membrane, are investigated in non-polarised cells models ranging from cells unable to polarise, like HEK293 or HeLa, to cells that can polarise, but are not grown as such (e.g., MDCK or LLCPK-1). In these studies localisation to the plasma membrane is often extrapolated as an apical or basolateral membrane localisation in polarised cells. To assess whether this extrapolation can be made, we investigated the consequence of using a non-polarised versus a polarised cell model on the localisation of the above-mentioned membrane proteins. In addition, we analysed whether maturation of these proteins differ between stably-transfected versus transiently-transfected cells.

## 2. Materials and methods

### 2.1. Cells

The stably transfected MDCK cell lines MDCK-AQP2 [10]; MDCK-AQP2-S256A [16]; MDCK-AQP2-S256D [16]; MDCK-AQP2-P262L [21]; MDCK-AQP2-R254L [22]; MDCK-GFP-V2R [9]; MDCK-HA-AE1 [23] were cultured as described [24]. Stable MDCK cells containing AQP2-E258K were constructed as described [24]. COS7 cells were maintained in Dulbecco's modified Eagle's medium (DMEM, Biowittaker, Verviers, Belgium) supplemented with 10% foetal calf serum (PAA Laboratories, Karlsruhe, Germany). As the original American Type Cell Culture MDCK cell line is known to be multiclonal, several groups have selected clonal MDCK cell lines. Here, MDCK high resistance (HRS) or type I cells [25] have been used throughout. AQP2 constructs have been transfected to MDCK-HRS cells with passage numbers between 90 and 120. In case a transfection had been repeated with a same construct in MDCK cells of a late passage number, localisation and variation thereof (see below) were similar. All AQP2 stable cell lines were derived from the same standard stock of cells. Stably-transfected cell lines were first tested for the expression of the respective gene of interest by immunoblotting. Then, at least four clones were analysed by immunocytochemistry to select a consensus localisation of the protein of interest. Selected clones were used for a limited number of passages (<20) as some of the clones lost expression when grown at higher passage numbers.

### 2.2. Immunocytochemistry and immunoblotting

Cells were seeded on polycarbonate filters (Costar, Cambridge, MA, USA) at a density of either $1.5\times10^5$ cells/cm$^2$ for polarised cells or $9.4\times10^3$ cells/cm$^2$ for non-polarised cells. Immunocytochemistry and confocal laser-scanning microscopy (CLSM) were performed as described [26]. As primary antibodies 1:50-diluted mouse anti-early endosomal antigen-1 (EEA1; BD Transduction Laboratories, Lexington, KY, USA), 1:50-diluted mouse anti-Golgi marker 58K (Sigma Aldrich, St. Louis, MO), 1:200-diluted rat anti-E-Cadherin (Sigma Aldrich, St. Louis, MO), 1:50-diluted mouse anti-HA (Sigma Aldrich, St. Louis, MO) and 1:50-diluted rabbit anti-AQP2 [27] were used. Anti-rabbit and anti-mouse secondary antibodies coupled to Alexa 488 or 594 (Molecular Probes, Leiden, The Netherlands) were used in a 1:100 dilution. For staining surface FLAG-tagged GFP-V2R, a slightly modified protocol was used: COS cells were washed and incubated with 1:100-diluted mouse anti-FLAG antibody (Sigma Aldrich, St. Louis, MO) at 4°. Subsequently, cells were fixed and immunocytochemistry and CLSM were continued as described above.

The glycosylation pattern of the transfected GFP-V2R was determined by immunoblotting as described [9].

## 3. Results

### 3.1. Localisation of wild-type Aquaporin-2 in polarised and non-polarised cells

To establish whether localisation in non-polarised cells can be extrapolated to a polarised situation as exists in vivo, we first studied the localisation of AQP2. For this, we seeded stably-transfected MDCK-AQP2 cells at low and high density. The cells were allowed to grow for 3 days, resulting in a polarised monolayer for the higher cell density only. Before fixation, the cells were treated with the adenylate cyclase activator, forskolin, to induce apical membrane localisation of AQP2. Without stimulation, AQP2 has been reported to be localised to the Golgi complex or to vesicles different from the Golgi [24,28–30]. Therefore, we performed a co-localisation experiment with the Golgi complex marker protein 58K [31].

Following immunocytochemistry, we saw a clear difference in AQP2 localisation between the two cell conditions. In the polarised cells, AQP2 mainly resided in the apical membrane. However, also some vesicular staining of AQP2 was observed, indicating that not all AQP2 was translocated to the apical membrane (Fig. 1, upper panel). These intracellular vesicles, however, were different from the Golgi complex, as no co-staining was observed with 58K. In non-polarised cells, however, AQP2 partially localises to the plasma membrane, but also appeared to reside in structures close to the nuclear membrane, which showed a clear co-localisation with 58K (Fig. 1, lower panels). This indicated that in non-polarised MDCK cells AQP2 partially resides in the Golgi-complex. Notably, the xz scans in Fig. 1 also show a clear difference in height between the two conditions confirming the difference in polarisation status.

### *3.2. Localisation of AQP2 mutants in polarised cells versus non-polarised cells*

To investigate whether polarisation is also crucial in determining the implication of AQP2 mutants, often impaired in their routing, we investigated the localisation of AQP2-S256A and AQP2-S256D, as these mutants respectively represent strictly intracellular or apical localisation. Stably transfected MDCK cells expressing these proteins were seeded in low and high density and immunocytochemically analysed in combination with subcellular marker proteins. Indeed, in polarised cells, AQP2-S256D staining expectedly showed a clear apical localisation, while the basolateral marker protein E-Cadherin revealed the basolateral membrane (Fig. 2A, upper left panels). In non-polarised MDCK-AQP2-S256D cells, however, AQP2-S256D did not localise to the plasma membrane, but was localised intracellularly. Similar to wt-AQP2, AQP2-S256D showed a considerable co-localisation with the 58K Golgi marker protein in non-polarised cells, while it is also present in other intracellular, but unknown, structures (Fig. 2A, lower left panels).

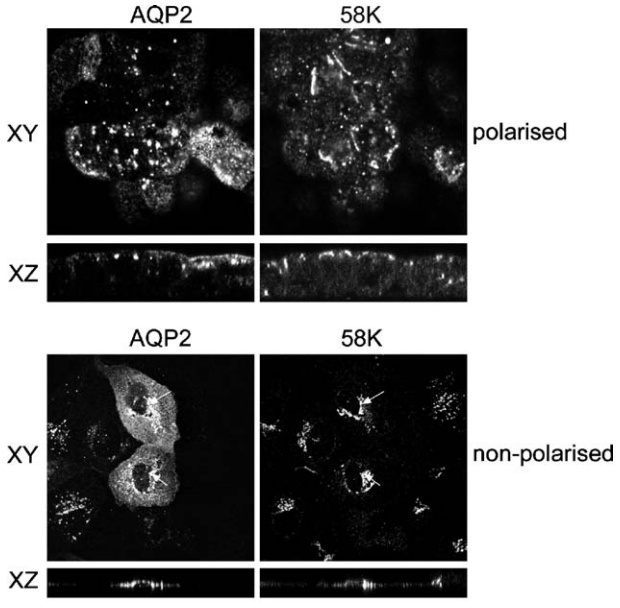


Fig. 1. Localisation of Aquaporin-2 in polarised and non-polarised MDCK cells. MDCK cells, stably transfected with wild type AQP2, were grown to full confluency (top panels) to obtain a polarised cell layer or sparsely seeded (lower panels) to obtain non-polarised cells. The left panels show AQP2 staining, while the right panels represents staining of the Golgi complex marker protein 58K. Arrows indicate co-staining. The xz view is taken at approximately midway of the xy panel. Please note the difference in height between the top and bottom xz panels, indicative of the difference in polarisation.

Examining AQP2-S256A, we found that in polarised cells this protein is localised in vesicles in the cytosol, which co-stained to a low extent with the early endosome marker EEA1 (Fig. 2A, upper right panels). Non-polarised cells showed a similar intracellular distribution for AQP2-S256A without any co-localisation with the Golgi complex marker protein 58K (Fig. 2A, lower right panels). This may indicate that the subcellular localisation of AQP2-S256A was not affected by the difference in cellular polarisation.

As missorting of naturally-occurring AQP2 mutants is of fundamental importance for our understanding of dominant NDI, we also investigated the localisation of NDI-causing AQP2 mutants. AQP2-E258K has been reported to be retained in the Golgi complex in oocytes, but to late endosomes/lysosomes in polarised liver cells [32,33]. Upon analysis in polarised MDCK cells, stably-expressed AQP2-E258K indeed localised to an intracellular compartment, which was distinct from the Golgi complex, indicated by the lack of co-localisation with 58K (Fig. 2B, upper left). In non-polarised MDCK cells, however, AQP2-E258K co-localised with 58K to a great extent (Fig. 2B, lower left). Recently, we also reported that in polarised MDCK cells, AQP2-P262L resides in the basolateral membrane and intracellular vesicles, distinct from the ER and Golgi [21], and that AQP2-R254L localises to intracellular vesicles [22]. Co-localisation studies in polarised cells confirmed this localisation for AQP2-P262L (Fig. 2B, upper middle panel), while co-staining with 58K revealed that AQP2-R254L did not localise to the Golgi complex (Fig. 2B, upper right). In non-polarised cells, however, both AQP2-P262L and AQP2-R254L showed considerable co-localisation with 58K (Fig. 2B, lower middle and right), indicating that in non-polarised cells, these proteins localise to a great extent to the Golgi complex.

### *3.3. Polarised versus non-polarised: are the differences in sorting found for other directed membrane proteins?*

As these results could be a unique feature of AQP2, we next investigated whether the localisation of other membrane proteins was also depending on the extent of polarisation. Therefore, we seeded MDCK-V2R-GFP cells and examined the V2R localisation one and 3 days after seeding in conjunction with the basolateral marker protein E-Cadherin. At day 1, at which the cells are not confluent (note the flatness of the cells in the xz plane), E-Cadherin was diffusely expressed throughout the cell, and showed no plasma membrane staining (Fig. 3A, upper panel). In these cells, some plasma membrane localisation for V2R-GFP was found, but the majority of the staining was scattered throughout the cytosol. At day 3, however, full confluency and therefore polarisation was reached, as is shown

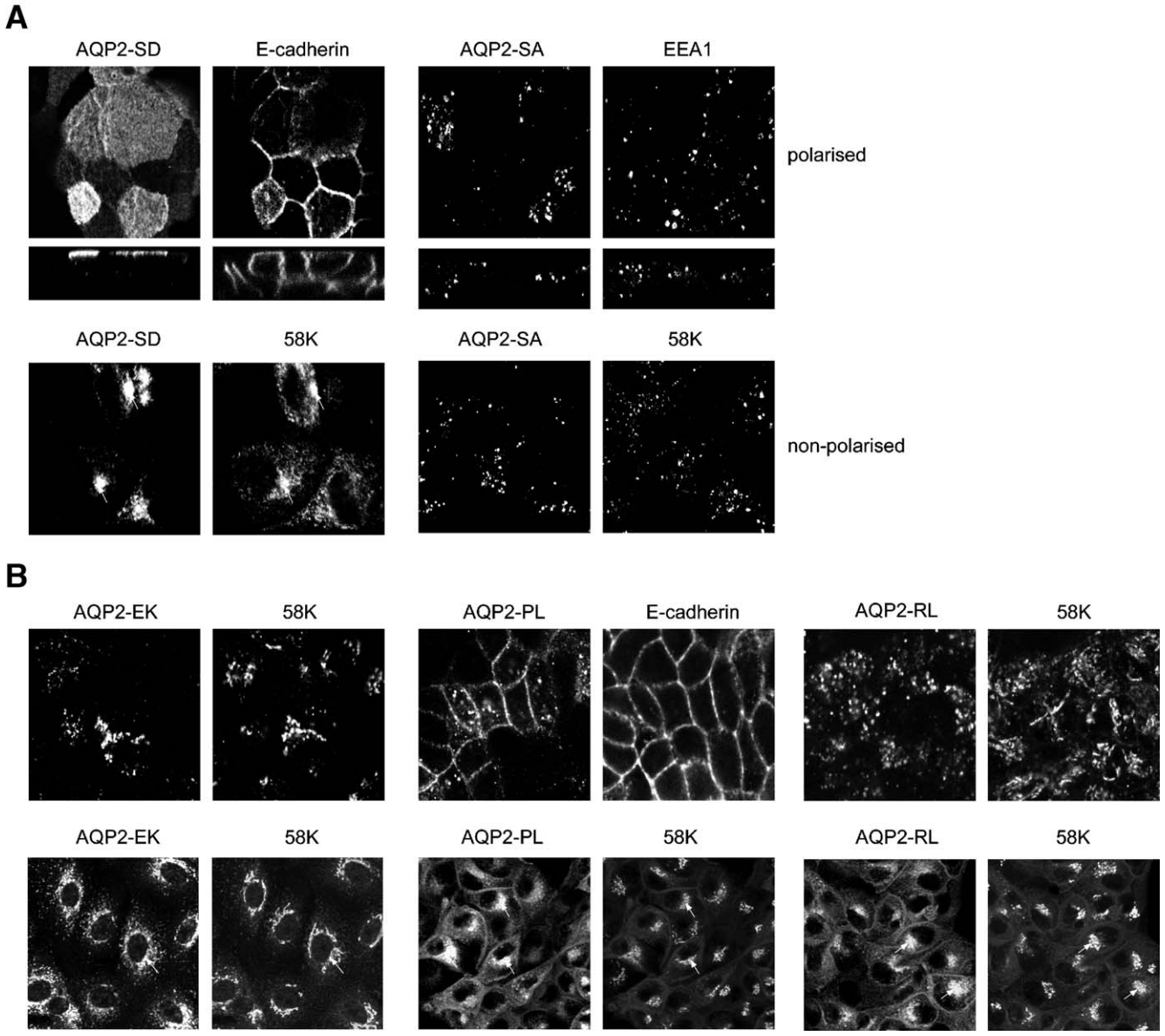


Fig. 2. Localisation of mutant AQP2 is dependent on polarisation. MDCK cells stably transfected with mutant-AQP2 (as indicated) were grown as described either to polarity or as non-polarised cells. The top panels represent the polarised cells; the lower panels represent non-polarised cells. The marking above the panels indicate for which AQP2 mutant was stained or for which subcellular marker protein. Arrows indicate co-staining. AQP2-SD, AQP2-S256D; AQP2-SA, AQP2-S256A; AQP2-EK, AQP2-E258K; AQP2-PL, AQP2-P262L; AQP2-RL, AQP2-R254L.

by the height of the cells in the xz plane. Analysis of the localisation now revealed a strong basolateral staining for E-Cadherin (Fig. 3A, lower panel). Additionally, besides some late endosome localisation [9], V2R-GFP was also mainly localised to the basolateral membrane, in a clear overlap with E-Cadherin (Fig. 3A, lower panel).

### *3.4. Proteins of non-principal cell origin also require polarity*

Besides V2R, we also analysed another integral membrane protein, being the chloride–bicarbonate exchanger (AE1), normally expressed in basolateral membrane of α-intercalating cells [20]. As confirmed in Fig. 3B (lower panel), AE1 indeed localises to the basolateral membrane of stably-transfected polarised MDCK cells [20]. Grown non-polarised, however, results in the loss of the basolateral localisation (Fig. 3B, upper panel). Instead, these cells show an intracellular and apical staining for AE1. Similar results have been obtained using rat IMCD cells, endogenously expressing AE1 (data not shown).

### *3.5. Expression of membrane proteins in transiently-transfected cells*

In the experiments described above we employed stably transfected MDCK cells, partly because these cells are not efficiently transfected in a transient fashion. For this reason, transfection of COS and HEK293 cells is popular, as these cells can be transfected transiently to a high extent. Concurrently,

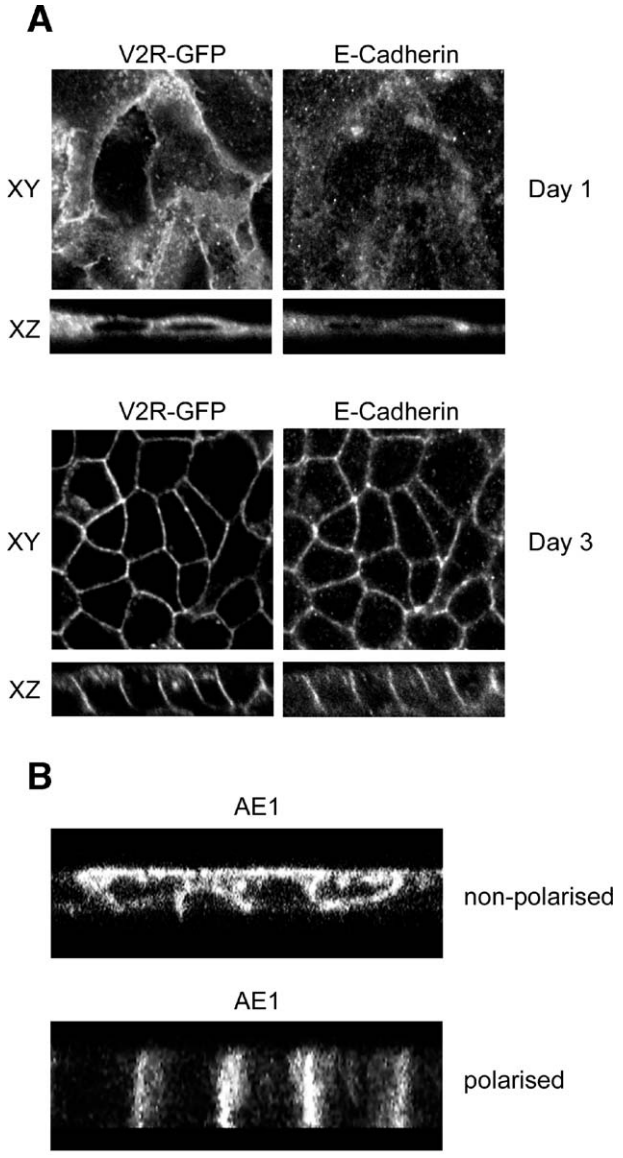


Fig. 3. Polarisation leads to correct basolateral localisation of V2R. (A) MDCK-V2R-GFP cells at day 1 (top panels) and day 3 (lower panels) after seeding, representing non-polarised and polarised cells, respectively. Left panels show the GFP signal, right panels show E-Cadherin. The xz view is taken at approximately midway of the xy panel. Note the difference in height between the cells on day 1 and day 3 in the xz panels. (B) xz planes of MDCK-AE1 cells grown to polarity (top panel) or as non-polarised cells (lower panel), stained for AE1.

however, these cells are unable to polarise. To determine whether transient expression can also be of influence on the localisation of membrane proteins, we decided to study V2R in transiently-transfected COS cells. In its itinerary to the plasma membrane, V2R-GFP is high mannose glycosylated in the endoplasmic reticulum, resulting in a protein of 60–62 kDa. In the Golgi complex, this glycosylation is changed for complex-glycosylation, resulting in a mature protein of 75 kDa, which is not changed upon its further route to the plasma membrane [34]. Immunoblot analysis of the V2R-GFP, stably expressed in polarised grown-MDCK or transiently expressed in non-polarised COS cells revealed a striking difference. In transiently-transfected COS cells, the ER-glycosylated form of V2R-GFP was much more pronounced than the complex-glycosylated form, when compared to these bands in stably-transfected cells (Fig. 4A). This indicates that V2R-GFP matured better in stably transfected cells compared to transiently transfected cells and that maturation depends on polarisation of the cells. To determine whether the extent of polarisation makes a difference in the level of maturation, we analysed the level of maturation of V2R-GFP in polarised versus non-polarised MDCK cells. We employed the seeding and harvesting conditions as described for Fig. 3. Immunoblot analysis of equal protein amounts revealed similar maturation of V2R-GFP at days 1–3 (Fig. 4B). This indicated that the reduced level of maturation in COS cells is due to the transient expression of V2R-GFP in COS cells and not due to differences in polarity. Similarly, with transient expression of AQP2 in COS, HEK293 or HeLa cells, the ER-

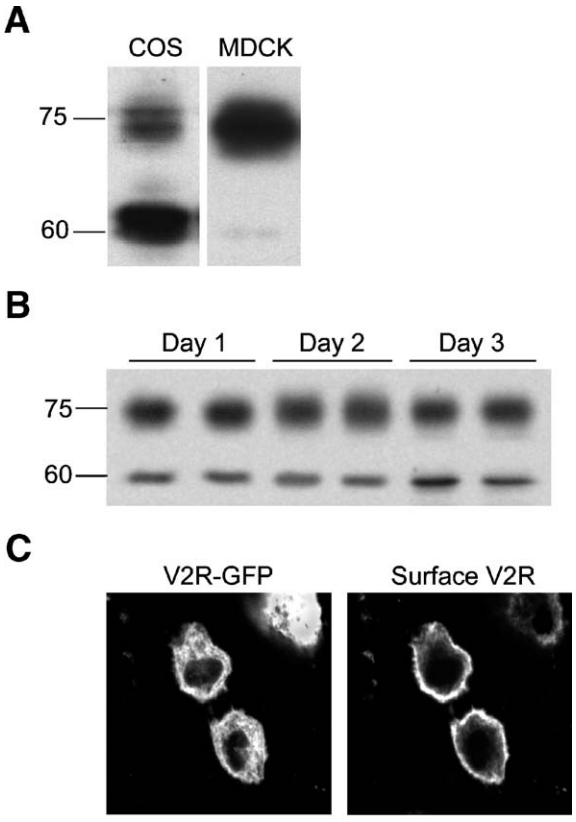


Fig. 4. Transient transfection leads to aberrant localisation and maturation. (A) Immunoblot for GFP-V2R of transiently transfected COS cells (left panel) and stably transfected MDCK cells (right panel). The 62-kDa protein band represents the core protein, while the 75-kDa protein band represents the mature, complex glycosylated protein. All lanes were of equal protein loading. Please note the high amount of core protein in the left lane compared to the right lane. B) Immunoblot for V2R-GFP in MDCK-GFP-V2R lysates. Lysates were taken at different time-points after seeding, representing the change in polarisation status (see Fig. 3A). The 60-kDa protein band represents the core protein, while the 75-kDa protein band represents the mature, complex glycosylated protein. All lanes were of equal protein loading. Please note the lack of difference between the different days of polarisation. (C) Immunocytochemistry of non-permeabilised transiently-transfected COS cells. The left plane shows the GFP signal of total GFP-V2R. The right panel represents the FLAG-signal, which represents due to lack of permeabilisation, surface GFP-V2R only.

glycosylated form is the most predominant form (data not shown). Consistent with the immunoblot data, parallel immunocytochemistry revealed a strong intracellular expression of V2R-GFP in transiently transfected COS cells (Fig. 4C). As the cells were transfected with a GFP-V2R construct bearing an extracellular FLAG tag, we could discriminate surface from intracellular V2R by staining for the FLAG tag in non-permeabilised cells. Staining for surface expression, revealed that a fraction of GFP-V2R reached the plasma membrane, which is consistent with the partial maturation found on immunoblot (Fig. 4A and C). These experiments indicate that, next to the lack of polarisation, transient expression also influences the localisation of membrane proteins.

## 4. Discussion

Polarisation is recognised as an important feature of epithelial cells. The establishment of asymmetry in epithelial cell layers allows regulated vectorial transport of ions and solutes, crucial for maintaining ion homeostasis. Hence, specialised epithelial layers form the boundary between different compartments in the body, and between the body and the outer environment. The kidney and more specifically nephrons consist of a series of tubules lined with epithelium specialised in ion transport. Polarisation of these cells and the concomitant reorganisation of the cytoskeleton ensures the correct transport of ions either towards the urine for secretion or the blood for reuptake [1,2]. Here, we studied the effect of polarity and the localisation of several renal proteins.

### *4.1. The use of non-polarised cells hides the true localisation of AQP2*

We show that in non-polarised cells, the localisation of AQP2 is not maintained to intracellular vesicles and plasma membrane, but then also localises to the Golgi complex (Fig. 1). In vivo, AQP2 has been localised to multivesicular bodies and smaller vesicles, but not to the Golgi complex [5], indicating that fully polarised MDCK cells are a better physiological model than non-polarised cells to study trafficking of AQP2.

The necessity of using polarised cells to investigate the routing and function of AQP2 is demonstrated further by the experiments described in Fig. 2. We show that the constitutively active mutant AQP2-S256D does not localise to the plasma membrane in non-polarised cells, while in polarised it is found in the apical membrane. The routing of the AQP2-S256A mutant, on the contrary, does not seem to be different between the two experimental conditions used (Fig. 2). As the vesicular staining only partially co-localises to the endosomal marker EEA1, AQP2-S256A is probably retained in storage vesicles. Translocation to the apical membrane of AQP2 is induced by phosphorylation of the S256 residue [5,7]. As the phosphorylation is impaired in the AQP2-S256A mutant, no translocation can take place, either in polarised or in non-polarised cells. The apparent lack of difference for AQP2-S256A localisation between polarised and non-polarised cells could indicate that its localisation in storage vesicles is not depending on the polarisation status of the cells. This leads to the speculation that in non-polarised cells, translocation from storage vesicles after cAMP signalling is directed towards the Golgi complex. Indeed the Golgi complex has been recognised as a critical cellular organ involved in protein trafficking [2]. Low et al. [35], have also shown that upon losing polarity, a shift in membrane protein trafficking towards intracellular compartments could be seen. This could also explain the difference between the storage vesicle localisation of AQP2-S256A and the Golgi-like staining of wild type AQP2 in non-polarised cells. It needs to be noted, however, that even in fully-polarised LLC-$PK_1$ cells, we found that stably-expressed AQP2 is also partially expressed in the Golgi complex (not shown). This is consistent with data from Brown and co-workers, who also (partially) localised AQP2 to the Golgi complex in stably-transfected unstimulated LLC-$PK_1$ cells [36]. At present, it is unclear whether this difference is due to the tubular origin of the cells (LLC-$PK_1$ from proximal tubules; MDCK from collecting duct) or is cell-type dependent. Clearly, however, our data reveal that for studying protein trafficking in a physiological relevant setting, it is important to search for a cell model that mimics the in vivo localisation and regulation mostly closely.

### *4.2. The molecular cause of dominant NDI: polarity makes the difference*

In Fig. 2B, we investigated the mutants AQP2-E258K, -P262L and -R254L, all of which cause NDI by defective routing to different organelles [14,21,22,33]. In polarised cells, AQP2-E258K is missorted to the Golgi complex and late endosomes/lysosomes, AQP2-P262L resides in intracellular vesicles of unknown identity, but which are different from the Golgi complex or early endosomes, while AQP2-R254L co-localises partly with early endosomes and, due to its resemblance in localisation with AQP2-S256A and its inability to be phosphorylated after forskolin stimulation, likely resides in genuine AQP2 storage vesicles [7,8,16,21]. In non-polarised cells, however, all these mutants mainly localise to the Golgi complex, a location indiscernible from that of wild type AQP2 (Fig. 1). These data indicate that analysis of the sorting of these mutants in polarised cells was essential to identify the cellular cause underlying dominant NDI.

### *4.3. Missorting due to a lack of polarisation is a general phenomenon*

The experiments described in Fig. 3 suggest that the mislocalisation in non-polarised cells is a common feature for proteins that have a regulated basolateral or apical transport. V2R is a predominantly basolaterally localised protein, which is largely retained intracellular in non-polarised cells. As shown in Fig. 3B, AE1 shows a similar sensitivity with regard to the polarisation status of the cell. Concurrently, we found that the basolateral marker protein E-Cadherin was also dispersed throughout the cell when grown non-polarised, whereas it was localised in the basolateral membrane in the confluent state (Fig. 3A).

### 4.4. Transient expression affects proper maturation

Compared to stably-transfected MDCK cells (Fig. 4B), transiently-transfected COS cells showed a defective maturation of V2R-GFP, while the extent of maturation of V2R-GFP was not changed by the extent of polarity of MDCK-V2R-GFP cells. Because the expression levels are usually very high in transient transfection assays, overloading of the capacity of the ER and Golgi complex to fold and assemble these proteins properly is a likely explanation for this. This hypothesis is supported by the observed correlation between the total expression of an ER-retained AQP2 mutant in *Xenopus* oocytes and the increased levels of unglycosylated versus high-mannose glycosylated bands [37]. While the V2R-GFP is already mislocalised in stably-transfected cells grown non-polarised, the affected maturation of V2R-GFP in transiently-transfected non-polarised cells (here COS cells) likely adds to its mislocalisation. Our data, therefore, indicate that caution should be attained when using transiently-transfected cells to study membrane proteins.

In polarised cells, the co-localisation of the molecular motors dynein and dynactin with AQP2 [38] indicates that the transport of AQP2 bearing vesicles is mediated through microtubules, the organisation of which is crucial for proper transport. Additionally, AQP2 was found to bind to a multiprotein motor complex [39]. Knepper and co-workers have shown by screening AQP2 bearing vesicles for associated proteins in renal inner medullary collecting duct that several myosin family members are associated with AQP2 vesicles [40]. This indicates that the transport of AQP2 vesicles is also mediated by actin-based motors. Indeed, AQP2 has been shown to interact with actin [41] and the organisation of the actin cytoskeleton controls the apical membrane insertion of AQP2 [42,43]. In cancer, loss of polarisation is one of the first signs of malignancy. Depolarisation leads to a change in the intracellular cytoskeleton and a rearrangement of microtubules [1]. Therefore, routing to and from organelles is organised differently. Furthermore, also components of the plasma membrane fusion machinery, normally localised apically, redistribute to intracellular localisations when MDCK cells lose polarity [35]. In this respect, the missorting of the membrane proteins in non-polarised cells as found here might be due to a changed organisation of the microtubular and/or cytoskeletal network, which will be the subject of future research.

In conclusion, our study shows that for studying the routing of specifically sorted apical or basolateral membrane proteins it is crucial to use a polarised cell model and that the use of non-polarised cell models can cloud the interpretation of mutant phenotype, as is shown here for the regulated water channel AQP2.

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