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Membrane Trafficking of Aquaporin 1 Is Mediated by Protein Kinase C via Microtubules and Regulated by Tonicity†

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ABSTRACT: It is well-known that the rapid flow of water into and out of cells is controlled by membrane proteins called aquaporins (AQPs). However, the mechanisms that allow cells to quickly respond to a changing osmotic environment are less well established. Using GFP−AQP fusion proteins expressed in HEK293 cells, we demonstrate the reversible manipulation of cellular trafficking of AQP1. AQP1 trafficking was mediated by the tonicity of the cell environment in a specific PKC- and microtubule-dependent manner. This suggests that the increased level of water transport following osmotic change may be due a phosphorylation-dependent increase in the level of AQP1 trafficking resulting in membrane localization.

Water can pass through cell membranes by diffusion, but the rapid control of the flow of water into and out of cells in continually changing osmotic environments is mediated by a family of membrane proteins called aquaporins (AQPs)[1] (*1*). In mammals, there are at least 13 members of this family with a wide-ranging tissue distribution (*2*). Discovered more than 15 years ago (*1*), AQPs are involved in diverse physiologies and pathophysiologies and have consequently been highlighted as key drug targets (*3*). Despite the availability of several AQP crystal structures (*4*−*7*), knowledge of how aquaporins actually facilitate the flow of water into and out of cells is less well established across the family. This is particularly true of the short-term regulation of AQP function in the context of the various osmotic conditions that routinely challenge cells. We present data showing that surface localization of AQPs can be regulated by a trafficking mechanism in response to osmotic stimuli, thereby controlling cellular transport of water across the membrane. Specifically, we suggest that following changes in the osmotic environment, increased water transport across the membrane is due to PKC- and microtubule-dependent AQP1 trafficking to the plasma membrane in response to tonicity.

Most AQPs are generally assumed to be constitutively expressed in cell-surface membranes, and recent studies have provided molecular details of how AQP1 (*8*), AQP3 (*9*), AQP5 (*6*), and AQP8 (*10*) can be localized to the cell surface. The best-studied exception is the regulation of AQP2 which requires protein kinase A (PKA) phosphorylation for cell-surface trafficking from intracellular vesicles (*11*). This results in the reabsorption of water from urine (*11*). It is believed that water then subsequently exits the cell via constitutively expressed AQPs (*12*). This constitutive cell-surface expression model is not consistent with the phosphorylation-dependent control of water transport already established for AQP2 (*11*, *12*), and an explanation is therefore needed to describe the mechanism for short-term regulation of other AQPs in response to rapid osmotic changes.

The trafficking mechanisms of functional AQP1, which is expressed in many tissues, including kidney, brain, erythrocytes, and lungs (*13*−*15*), are poorly understood. Although protein kinase phosphorylation of AQP1 has previously been shown to cause an increase in the water permeability of cells (*8*, *16*), it is not clear whether this increase in permeability was due to increased AQP1 activity, increased AQP1 levels, or another property of AQP1. Furthermore, it has also been observed that in rat cholangiocytes expressing the secretin receptor, AQP1 was translocated to the plasma membrane upon secretin stimulation (*17*).

Here we show that AQP1 can be trafficked to and from the plasma membrane in response to altered tonicity in a PKC- and microtubule-dependent manner. We created C-terminal GFP fusion proteins of AQP0, AQP1, and AQP2 and studied real-time localization and trafficking of these proteins in live HEK293 cells by confocal microscopy: fusion of GFP to the C-terminus of AQP1 has already been shown not to interfere with AQP1 function (*18*). The level of AQP1 membrane localization was rapidly increased by exposure to hypotonic conditions. This trafficking was blocked by both PKC and microtubule inhibitors but not by PKA or actin inhibitors. The initial AQP1 distribution profile was restored on returning to normal physiological conditions.

To measure increases in membrane localization, line intensity profiles were generated of confocal images of individual live cells. A minimum of three line profiles distributed at regular intervals covering the plasma membrane and the cytosol, but avoiding the nucleus, were measured. The fluorescence intensity over this distance was also measured, and the difference between the peak and the plateau of fluorescence was divided by the maximum fluorescence along the line scan to calculate the percentage of fluorescence at the membrane (*19*). Identification of the plasma membrane was achieved through staining with FM4-64 and overlaying the GFP images. Nuclei were identified via DAPI staining.

The overlay of the GFP image with either the bright-field image or the red fluorescence emitted by FM4-64 clearly indicated integration of GFP-tagged AQP1 and AQP0 at the plasma membrane as well as in the cytoplasm of HEK293 cells

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[1]Abbreviations: AQP, aquaporin; PKC, protein kinase C; PKA, protein kinase A; DMEM, Dulbecco's modified Eagle's medium; GFP, green fluorescent protein; V_2R, vasopressin V_2 receptor.

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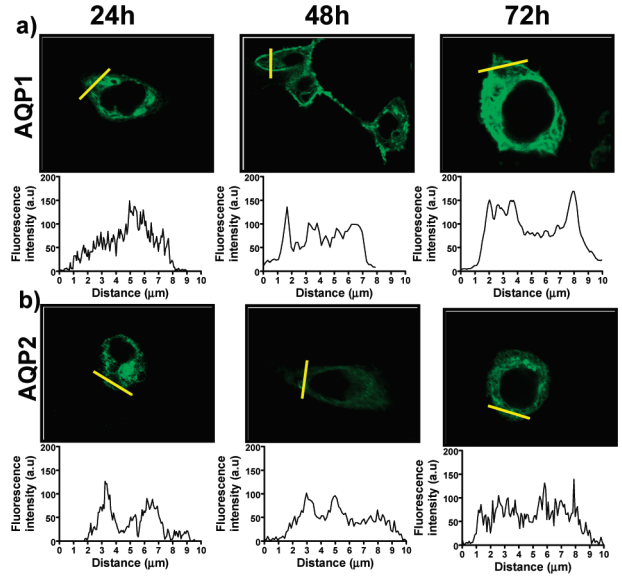

FIGURE 1: Expression profiles of (a) AQP1−GFP and (b) AQP2−GFP fusion proteins in HEK293 cells. The time post-transfection is indicated above each panel. Line scans below each image are indicated with a yellow line.

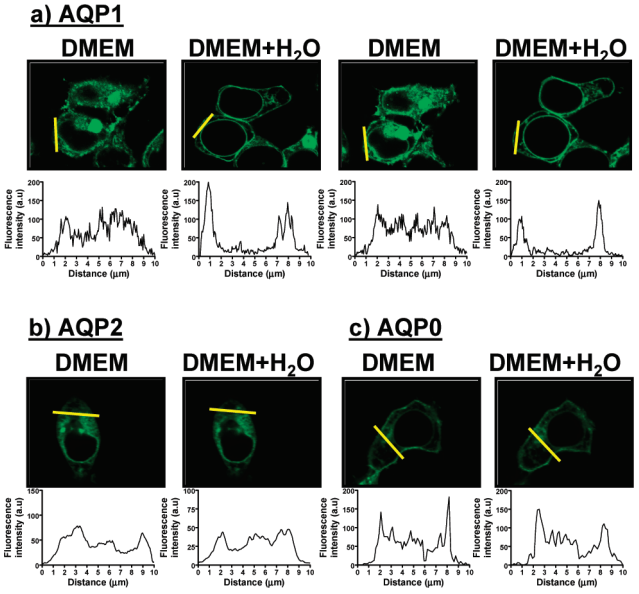

FIGURE 2: Expression profiles of (a) AQP1−GFP, (b) AQP2−GFP, and (c) AQP0−GFP fusion proteins in HEK293 cells. For each AQP, the cells shown are the same cells exposed sequentially, from left to right, to different tonic conditions. Line scans below each image are indicated with a yellow line. DMEM has an inorganic salt concentration of 120 mM, a glucose concentration of 25 mM, and an osmolality in the range of 322−374 mosM/kg of H_2O. DMEM+H_2O denotes a dilution of DMEM by a factor of 3 with water.

(data not shown). The relative membrane localization of AQP1 was 5 ± 2% at 24 h post-transfection, increasing to 26 ± 3 and 28 ± 6% (n = 3) at 48 and 72 h post-transfection, respectively (Figure 1a). A similar expression pattern was seen for AQP0, with membrane expression of 7 ± 2% at 24 h post-transfection, increasing to 26 ± 4 and 27 ± 5% at 48 and 72 h post-transfection, respectively (data not shown). In contrast, AQP2 was retained in the cytosol of HEK293 cells up to 72 h post-transfection (Figure 1b). This is consistent with other AQP2 expression studies, as HEK293 cells have no vasopressin V_2

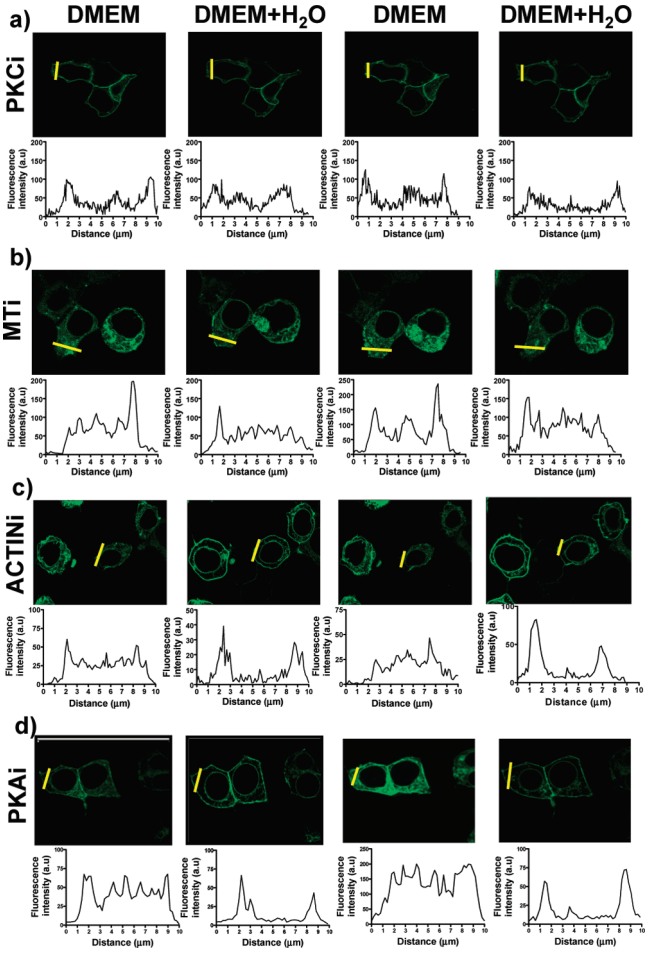

FIGURE 3: Expression profiles of the AQP1−GFP fusion protein in HEK293 cells 48 h post-transfection. Line scans below each image are indicated with a yellow line. PKCi (a) is a specific PKC inhibitor. MTi (b) is a microtubule inhibitor. ACTINi (c) is an actin inhibitor. PKAi (d) is a specific PKA inhibitor. Shown are the same cells under different tonic conditions, sequentially from left to right, for each inhibitor.

receptor required for trafficking of AQP2 to the membrane in kidney cells.

Membrane localization of AQP1 increased in a time-dependent manner when the cells were exposed to dilutions of DMEM by a factor of 1, 2, or 3 with water (data not shown). Dilution of DMEM by a factor of 3 was used in all subsequent experiments to induce trafficking while limiting the time of hypotonic exposure to the cells. On a return to DMEM, AQP1 localization resembled the original profile (Figure 2a). Membrane localization of AQP1, 48 h post-transfection in DMEM, was 26 ± 3%. Following replacement of DMEM with diluted DMEM, the relative level of membrane expression in the same cells was 77 ± 2%. All changes in membrane localization occurred within 10 s. The same cells showed a membrane expression profile similar to that initially observed (relative level of membrane expression of 26 ± 3%) following removal of diluted DMEM and addition of DMEM (Figure 2a). In contrast, the distribution profile of AQP0 remained unaltered in HEK293 cells 48 h post-transfection regardless of tonicity. AQP2 was retained in the cytosol, and the distribution profile of AQP2 remained the same in HEK293 cells 48 h post-transfection, also regardless of tonicity.

The tonicity-mediated increase in AQP1 membrane localization was found to be blocked by co-incubation with PKC and microtubule inhibitors: the relative level of membrane expression

of AQP1 in HEK293 cells 48 h post-transfection remained similar [27 ± 3% in DMEM and 25 ± 5% in diluted DMEM (Figure 3a)] to the original AQP1 relative membrane expression profile (26 ± 3%) regardless of tonicity when the cells were preincubated with the specific PKC inhibitor myr-PKC 19−27 at 50 μM (*20*) (Figure 3a) or the tubulin-disrupting microtubule inhibitor demecolcine at 2 μg/mL (*21*) (Figure 3b). In contrast, neither the PKA inhibitor nor the actin filamentation inhibitor affected tonicity-mediated membrane localization. Relative membrane expression values of AQP1 in HEK293 cells with either the actin inhibitor cytochalasin D at 2 μM (*22*, *23*) (Figure 3c) or the specific PKA inhibitor myr-PKA 14−22 at 50 μM (*24*) (Figure 3d) were not significantly different from the relative membrane expression values of AQP1 alone under the equivalent osmotic conditions.

Clearly, the specificity of this reversible effect is not simply due to whole cell morphology changes as it is not seen with AQP0, which has an expression profile similar to that of AQP1 in DMEM. Our data are also consistent with the previous observation that direct PKC phosphorylation of AQP1 has been shown to cause an increase in water permeability (*16*). To conclude, we demonstrate here that altering osmotic conditions induces a kinase-dependent increase in AQP1 membrane localization. We suggest therefore that an increase in the level of water transport in response to changing osmotic conditions in the cellular environment may be due to a PKC-dependent increase in AQP1 membrane localization. This explanation unifies the published body of data on water transport in humans and establishes a model for further investigation of its control.

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SUPPORTING INFORMATION AVAILABLE

Further experimental detail. This material is available free of charge via the Internet at http://pubs.acs.org.

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