PTEN-Mediated Apical Segregation of Phosphoinositides Controls Epithelial Morphogenesis through Cdc42

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SUMMARY

Formation of the apical surface and lumen is a fundamental, yet poorly understood, step in epithelial organ development. We show that PTEN localizes to the apical plasma membrane during epithelial morphogenesis to mediate the enrichment of *PtdIns(4,5)P_2* at this domain during cyst development in three-dimensional culture. Ectopic *PtdIns(4,5)P_2* at the basolateral surface causes apical proteins to relocalize to the basolateral surface. Annexin 2 (Anx2) binds *PtdIns(4,5)P_2* and is recruited to the apical surface. Anx2 binds Cdc42, recruiting it to the apical surface. Cdc42 recruits aPKC to the apical surface. Loss of function of PTEN, Anx2, Cdc42, or aPKC prevents normal development of the apical surface and lumen. We conclude that the mechanism of PTEN, *PtdIns(4,5)P_2*, Anx2, Cdc42, and aPKC controls apical plasma membrane and lumen formation.

INTRODUCTION

Most organs consist of epithelial tubes (Lubarsky and Krasnow, 2003). Despite diversity of developmental mechanism, tubular organs and related structures, such as alveoli and cysts, share a common organization, with the apical (AP) surface lining the central lumen and a basolateral (BL) surface attached to adjoining cells and extracellular matrix (ECM). A key gap in our knowledge of how cells assemble into tubules is how the AP surface and lumen are formed.

Culture systems that recapitulate tube morphogenesis have been useful in understanding the mechanism of tubulogenesis. The three-dimensional (3D) Madin-Darby canine kidney (MDCK) cell system is an excellent model of epithelial morphogenesis in vitro (Debnath and Brugge, 2005; Lubarsky and Krasnow, 2003; O'Brien et al., 2002). MDCK cells embedded in a gel of ECM form cysts, spherical epithelial monolayers enclosing a central lumen (Montesano et al., 1991). Results from MDCK and other systems have led to a general model for tube morphogenesis (Lubarsky and Krasnow, 2003; O'Brien et al., 2002). In this model, the cell interprets an extracellular cue from the ECM and transduces it into a signal to generate the axis of polarity. Following this initial event, the most crucial step is the formation of the central lumen and AP plasma membrane (PM).

Cdc42 plays a central role in polarization from yeast to mammals. In metazoa, one target of Cdc42 is Par6, part of the Par3/Par6/aPKC complex, a master regulator of polarity. Cdc42 is associated with formation of tight junctions (TJs) and the regulation of traffic to the PM (Kroschewski et al., 1999; Musch et al., 2001). However, activation of Rac1, but not Cdc42, is needed for epithelial TJ formation (Mertens et al., 2005). Despite this controversy, Cdc42 is activated upon cell-cell contact, suggesting a role for this protein during epithelial morphogenesis (Kawakatsu et al., 2002; Kazmierczak et al., 2004). Interestingly, FDG1, a guanine nucleotide exchange factor (GEF) that regulates Cdc42 activity, is needed for lumen formation in vivo (Suzuki et al., 2001).

Here, we elucidate a molecular mechanism central to the formation of the AP PM and lumen during morphogenesis in the 3D MDCK system.

RESULTS

PtdIns(4,5)P_2 and PTEN Are at the AP PM

To determine the localization of *PtdIns(4,5)P_2* in MDCK cysts, we stably expressed the pleckstrin homology (PH) domain of phospholipase Cδ1, a high-affinity marker for *PtdIns(4,5)P_2* (Rescher et al., 2004), fused to GFP (PHD-GFP). PHD-GFP was distributed mainly to the AP PM of MDCK cysts, where it largely colocalized with the AP marker gp135/podocalyxin (Figure 1A, arrowheads); a smaller degree was found in the BL PM, with a distribution similar to that of actin (see Figure S1A in the Supplemental Data available with this article online). Using

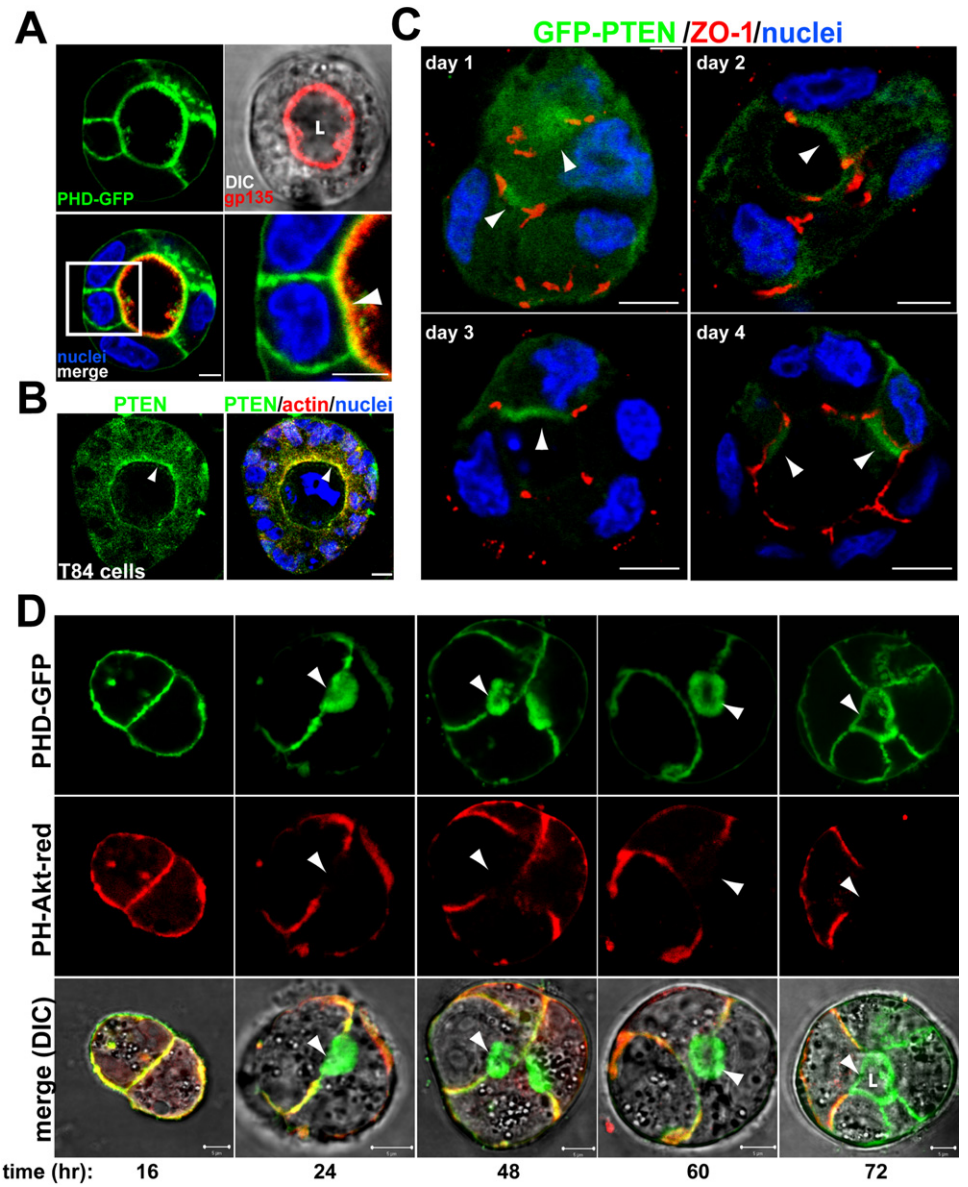

Figure 1. *PtdIns(4,5)P_2* and PTEN Localize to the AP PM in Cysts

(A) *PtdIns(4,5)P_2* is enriched in the AP PM. MDCK PHD-GFP cysts were stained for nuclei (blue) and gp135 (red, top right, merged with DIC). In all micrographs, single confocal sections through the middle of cysts are shown and nuclei are stained blue unless otherwise indicated. Bottom right panel shows a magnification of the indicated region of the merged panel (bottom left). Arrowhead indicates the colocalization of PHD-GFP and gp135 at the AP PM.

(B) PTEN localizes to the AP PM in T84 human colon cysts. Endogenous PTEN could not be localized in MDCK cysts but could be localized in T84 cysts. T84 human colon cells were allowed to form cysts for 8 days; stained for PTEN (green), actin (red), and nuclei; and visualized. Arrowheads indicate PTEN at the AP PM.

(C) GFP-PTEN localizes to the AP PM. MDCK GFP-PTEN cells form cysts at 1, 2, 3, and 4 days. Cells were stained for ZO-1 (red). Arrowheads indicate GFP-PTEN at the AP PM.

(D) *PtdIns(4,5)P_2* segregates from *PtdIns(3,4,5)P_3* during cystogenesis. MDCK PHD-GFP (top) and PH-Akt-GFP (middle, merged with DIC in bottom row) were plated to form cysts for 4 days. Live cells were analyzed by confocal microscopy at 1, 2, 3, or 4 days. In this and all other micrographs, "L" indicates lumen.

Scale bars are 5 μm.

PH-Akt-GFP, a probe for products of PI3K (*PtdIns[3,4]P_2* and *PtdIns[3,4,5]P_3*; we refer to both collectively as *PtdIns[3,4,5]P_3*), we found *PtdIns(3,4,5)P_3* highly enriched at the BL PM and absent from the AP PM of MDCK cysts in Matrigel (Figure S1B), confirming previous data in collagen (Yu et al., 2003).