RAB14:
ROLE IN CELL POLARITY AND JUNCTION FORMATION

By
NATASHA SINHA

A Thesis Submitted to The Honors College
In Partial Fulfillment of the Bachelors degree
With Honors in
Molecular and Cellular Biology
THE UNIVERSITY OF ARIZONA
August 2010

Dr. Jean Wilson
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ABSTRACT:
The establishment of polarity in epithelial cells is dependent on the proper distribution of polarity proteins to the apical or basolateral domain. In addition, these cells are dependent on the necessary trafficking of junctional proteins to cells junctions for cell-cell interactions. Rab GTPases are involved in a number of membrane trafficking pathways in the cell. Here we show Rab14’s role in regulating the establishment of polarity and junction formation in epithelial cells. Cyst culture was used to show inactive-Rab14 affects single lumen formation and targeting of gp135, an apically targeted protein, thus showing Rab14 may regulate epithelial polarity. In addition, calcium-switch experiments were used to show that not only do cells expressing inactive Rab14 take longer to lose their cell-to-cell contact, but they also regain their contacts more quickly, indicating Rab14 may be playing a role in junction formation. This was further supported by immunostaining experiments examining the distribution of junctional proteins; increased amounts of these proteins at the junction in inactive-Rab14 at the end-point showed that Rab14 may be involved in endocytosis or recycling of junctional components.

INTRODUCTION:
Eukaryotic cells have a highly specialized system of membrane trafficking. Cargo proteins are transported through the cell via vesicles, which continuously bud from the original organelle, are transported to their destination, and fuse with a target organelle. Specificity of membrane transport is provided by monomeric GTPases called Rab (Ras-related in brain) proteins [1]. Rab proteins are central regulators of vesicle budding, motility and fusion. On the cytosolic side of each organelle there is at least one Rab
GTPase and there are at least 60 known members of the Rab family. Many of these Rabs can be subdivided into subfamilies that show similar sequence identity and related function [1].

Rab proteins, like all GTPases, switch between a GDP-bound state (in the cytosol) and GTP-bound state (membrane-bound). GDP/GTP exchange factors (GEF) are responsible for the exchange of GTP for GDP in Rab GTPases, while GTPase-activating proteins (GAP) are involved in the hydrolysis of GTP to GDP. A Rab escort protein (REP) delivers the Rab protein to its proper membrane location. The GTP-bound Rab is the active form, and when the Rab protein is in its GTP-bound state, it interacts with a Rab effector protein that facilitates docking of the vesicle onto a membrane. The release of the Rab from the membrane is mediated by a Rab GDP-disassociation inhibitor (GDI), which also serves as a recycling factor that allows Rabs to function multiple times.

Mutations in Rab proteins and their effectors can cause serious health effects. For example, Griscelli syndrome, an autosomal recessive disorder, is known to cause partial albinism and immunological defects [2]. This disease is caused by a missense mutation in the gene coding for Rab 27a. Rab 27a is responsible for the movement of melanosomes, thus the mutation causes partial albinism, and regulation of lytic granule secretion in cytotoxic T lymphocytes, thus the mutation compromises the immune response. In addition, Griscelli syndrome patients with a mutation in the motor protein myosin Va, a Rab 27a effector that moves melanosomes along actin filaments also exhibit immunological symptoms [2]. Another disease, choroideremia, causes blindness through
“degeneration of the retinal pigment epithelium and the adjacent choroids and retinal photoreceptor cell layers” [1]. A mutation in REP-1 (a Rab escort protein involved in guiding the Rab protein to the geranylgeranyl transferase) causes a lack of function of Rab27a in the retinal pigment epithelium. Degeneration may be caused by a lack of melanosome transport, which reduces protection needed to block harmful light exposure.

Polarity in epithelial cells is dependent on the proper sorting of membrane proteins and ligands to the apical or basolateral domain of the cell [3]. In addition, several complexes are essential for the establishment and maintenance of cell polarity. These include the junctional polarity complex Par3/Par6/aPKC complex, the apical Crumbs/PALS1/PATJ complex, and the lateral Scribble/DLG/LGL complex [4]. These proteins involved in polarity have distinct distributions along the apex of the lateral membrane in the apical junctional complex (AJC). Rab proteins are also important in polarized membrane trafficking in epithelial cells. Conflicting data of the role of Rab proteins in trafficking to the apical or basolateral domain exists for several different Rab GTPases. For example, Rab 8 has been shown to regulate the movement of newly synthesized proteins to the basolateral surface in epithelial cells [5], while also being involved in targeting of apical proteins in in-vivo models [6]; thus showing that Rab 8 has essential roles in the establishment of polarity in epithelial cells. Rab 8 localizes at, or near recycling endosomes and is involved in the TGN-recycling endosome-plasma membrane pathway [5]. Rab 8 plays an important role in the development of polarity in polarized epithelial cells, however once cell polarity has been established, it is no longer associated with delivery to the basolateral membrane or endocytic recycling [5], thus showing that Rab 8
most likely is involved in the transport step from the TGN to the recycling endosome. In vivo evidence also indicates that Rab 8 is involved in targeting of apical proteins in intestinal epithelial cells. In mice deficient in Rab 8, apical peptidases and transporters were mislocalized to lysosomes causing their degradation and death of the mice due to a decrease in absorption rate of nutrients [6]. Therefore, it can be seen that Rab 8 may also be involved in the apical targeting of proteins. This confusion involving Rab 8’s role in either apical or basolateral transport also is evident with Rab 11, which has not only been shown to be involved in apical transport through recycling endosomes [7], but has also been shown to be critical in the export of a basolateral marker [8]. Similarly, Rab14 has been shown the play a role in both the biosynthetic and recycling pathway between the Golgi and endosomal compartments [9], as well as the targeting of the apical protein, VIP/MAL [10], indicating that Rab14 also plays a role in the development or maintenance of cell polarity.

Polarity in epithelial cells is also established through the proper formation of adherens junctions and tight junctions [4]. Tight junctions function as barriers to the diffusion of membrane proteins between the apical and basolateral domains of the plasma membrane, and they seal neighboring cells together, limiting paracellular transport. Each tight junction is comprised of a row of adhesion transmembrane proteins. Claudins are essential for tight junction formation and function, while occludins have been to shown to be necessary in tight junctions, but their function is still not understood. Zonula Occludins (ZO) proteins are intracellular peripheral membrane proteins that anchor tight junction sealing strands to the actin cytoskeleton [4]. In contrast to tight junctions,
adherens junctions form a strong membrane-spanning structure that is tethered to the tension-bearing filaments of the cytoskeleton and form a continuous adhesion belt just below tight junctions. These junctions are held together by cadherin proteins, which are calcium-dependent transmembrane proteins. E-Cadherin is specifically involved in epithelial junctions. Importantly, tight junction assembly is dependent on prior formation of adherens junctions.

The formation of both tight and adherens junctions is dependent on the polarized transport of vesicles, which is mediated by Rab GTPases [3]. Rab3B and Rab13 are two proteins that have been shown to localize at tight junctions [11]. Rab13 is involved in the assembly of functional tight junctions and Rab13 has a distinct role in vesicular transport from the TGN to the cell surface [12]. Furthermore, it was shown that Rab13 and a Rab13-binding protein, JRAB, are essential regulators of occludin recycling to the cell surface[13]. JRAB, in association with Rab13, might be involved in binding occludin-containing vesicles to the actin cytoskeleton of the plasma membrane, which then regulates the dynamic turnover of tight junctions [14]. Since Rab14 has been shown to regulate trafficking from the Trans-Golgi Network to apical endosomes, it may also play a role not only in maintaining epithelial polarity, but also in junction formation.

Here, we report that Rab14 regulates both epithelial polarity and junction formation. We have found that Rab14, when mutated, disrupts epithelial polarity in three-dimensional, cyst culture. In addition, the rate of junction formation and cell-to-cell contact differs
between the Rab14 wild-type and inactive Rab14. These results suggest that Rab14 negatively regulates junction formation.

MATERIAL AND METHODS:

Materials

Type I, Madin-Darby Canine Kidney (MDCK) cells were transfected with GFP, GFP-tagged Rab14, or GFP-tagged Rab14-S25N and grown in MDCK-G418 Media, containing DMEM, FBS, and G418. A gp135 antibody was used for labeling apical membranes in cyst experiments, along with an αMouse Cy3 (red) secondary antibody. Calcium-switch experiments were performed in calcium-free, spinner media and labeled with either an occludin antibody with the αRb Cy3 (red) secondary antibody, or decma (e-cadherin) antibody with αRt-Alexa (red) secondary antibody. All experiments used Topro for nuclear staining.

Methods

Cyst Culture

Cells of each genotype were grown to confluency in 25cm² flasks. Chilled, 8-well chamber slides were covered with 6µl of Matrigel in each well. Once cells were split, 2000-5000 cells were added to each well, along with .5ml of MDCK-G418 media and 2% Matrigel. Cysts were grown for 10 days and were observed daily for lumen formation.

Gp135 Labeling
Cysts were fixed in 4% Paraformaldehyde/1xPBS, quenched in 50mM NH₄Cl, and blocked in .05% saponin/10% FBS/PBS. Gp135, the primary antibody, was then added at a 1:20 concentration and then a secondary antibody, αMouse Cy3 at a 1:400 concentration and 1:100 concentration of Topro was used. After a final rinse with water, a coverslip was placed on the slide to prepare for observation under the Leica LSM Laser Scanning system.

**Calcium-Switch Preparation**

Cells of each genotype were grown to confluency on glass coverslips. Once confluent, the coverslips were placed in an individual well of a 24 well plate and were grown for 3 additional days.

**Calcium-Switch, Live-Cell**

Images were taken, using an Olympus microscope equipped with Hoffman optics using a 20x objective, at steady-state for each genotype before calcium-free media was placed in each well, after a PBS wash. Images were taken every 15 minutes of a marked area on the coverslip, to allow for images to be taken of the same region at each time-point. MDCK-GFP and MDCK-R14WT were in calcium-free media for 30 minutes, and MDCK-R14S25N were in calcium-free for a total of 45 minutes. Then complete, MDCK-G418 Media replaced the calcium-free media and images were again taken every 15 minutes for a total of 60 minutes.

**Calcium-Switch, Occludin and E-cadherin Labeling**
Coverslips were prepared as in the live-cell calcium-switch experiments. At each timepoint, one coverslip was removed and fixed and processed as described above. After blocking, the cells were labeled with either occludin or e-cadherin (decma) antibody at 1:100 concentration. They were then placed in αRb Cy3 (1:800) or αRt-Alexa (1:250), respectfully, secondary antibody. The coverslips were placed on microscope slides and images were obtained using a Leica LSM Laser Scanning system.

RESULTS:

Cysts culture show establishment of polarity is compromised in inactive Rab14

To determine if Rab14 mediates the establishment of polarity in epithelial cells, we grew MDCK-GFP, MDCK-R14WT, and MDCK-R14S25N in three-dimensional culture with Matrigel, which provided cues for cyst formation. When sufficient single lumen cysts were observed (about 70%-80%) under the light microscope (approximately 10 days after plating), the cysts were fixed and labeled for gp135, an apically targeted protein. As shown in Figure 1, in the MDCK-GFP controls, a red band was seen on the luminal plasma membrane showing proper localization of gp135 in the apical region. Similarly, the MDCK-R14 cysts contained a red circular band outlining the luminal region of the cyst, indicating correct targeting of gp135. However, in the MDCK-R14-S25N cysts, though there appeared to be many, large, single lumens under the phase contrast microscope, no true single lumen formation was seen. Nuclear labeling showed that cells grew within the lumen region, and gp135 labeling indicated the presence of multiple lumens. These results indicate that Rab14 is involved in establishment of polarity and trafficking of the apically targeted protein, gp135.
Calcium-switch and live-cell imaging shows Rab14 is involved in the maintenance of cell-to-cell contact in polarized epithelial cells

To test the role of Rab14 in the maintenance of junctions, we performed calcium-switch experiments [15]. In these experiments, we tested the rate of loss of cell-to-cell contact after the calcium-switch. MDCK cells of the three different genotypes were grown on coverslips to confluency before calcium-switch experiments were performed. Cells were placed in calcium-free media and images were taken every 15 minutes to see differences in contact loss between the different cell types.

As shown in figure 2, at steady-state, the cells’ surfaces are smooth and homogenous and no difference is apparent between the cell types. As shown in figure 3a, after 15 minutes in calcium-free media, the MDCK-GFP and MDCK-R14WT cells had rounded up and some of them had lost cell-to-cell contact. In contrast, the MDCK-R14S25N cells appear more resistant to loss of cell-to-cell contact and remained flattened and maintained their cell-to-cell interaction. After 30 minutes, in both the MDCK-GFP and MDCK-R14WT, the cells had all rounded up and had completely lost their cell-to-cell contacts. However, the MDCK-R14S25N cells had regions where some cells still remained flattened. In fact, the MDCK-R14S25N required an addition 15 minutes in calcium-free media for the cells to completely separate (a 30-minute time-point image of MDCK-R14S25N is shown in Figure 3b). It is important to note that the same region of cells was visualized at each time point, eliminating the possibility that these differences are due to variability on the coverslip.
Rab14’s involvement in the establishment of cell-to-cell contact was seen through calcium-switch and live-cell imaging.

The previous experiment tested the role of Rab14 in dissolution of cell-to-cell contact. To test whether Rab14 is also involved in the establishment of cell-to-cell contact after opening the junctions through calcium depletion, images were taken every 15 minutes once the cells were placed back in complete media. As in the previous experiments, the same cells were visualized at each time point. In Figure 4, the cells of each genotype at time 0 appeared completely separated from cell-to-cell contact. After 15 minutes in complete media both the MDCK-GFP and MDCK-R14S25N showed minimal gain of contact between neighboring cells, and thus appeared completely rounded up. However, the MDCK-R14S25N cells were more spread and many areas contained cells that had reestablished contact. After 30 minutes, some of the MDCK-GFP cells and MDCK-R14WT cells had reestablished cell-to-cell contact, but the degree of contact formation remained greater in the MDCK-R15S25N genotype. The images in figure 4 show that while MDCK-GFP and MDCK-S25N cells have a majority of rounded up cells, the MDCK-R14S25N cells have mostly flattened out by this time-point. After 45 minutes, many cells in the MDCK-GFP and MDCK-R14WT coverslips had regained their contact with each other though some cells still remained rounded up, and the MDCK-R14S25Ns appeared to have completely formed their contacts and flattened out. In contrast, it took up until the 60-minute time-point for the MDCK-GFP and MDCK-R14WT cells to complete their contact formation and become completely flat. These results show that not
only does it take longer for the MDCK-R14S25N cells to lose their cell-to-cell contact, but they also reform these contacts more quickly than the other genotypes.

*Rab14 regulates trafficking of e-cadherin and occludin to the junctions*

In order to define how Rab14 was affecting junction formation between neighboring cells, we next tested junction assembly over time in cells expressing the different forms of Rab14. We utilized e-cadherin labeling to identify adherens junction formation and occludin to assess tight junction formation.

We first examined the steady-state distribution of these markers on confluent cells. As shown in figures 5a and 6a, the distribution and intensity of both e-cadherin and occludin labeling at the junctions appeared similar throughout each genotype.

These experiments also utilized calcium-switch to assess junction assembly. Cells were placed in calcium-free as before to disassemble junctions completely. Figure 5a shows E-cadherin labeling at different time intervals for a total time of 60 min. At time 0, each of the cell types had broken their contact with neighboring cells and appeared similar. After 30 minutes, the MDCK-GFP and MDCK-R14WT contained low levels of e-cadherin at their junctions, while MDCK-R14S25N cells showed extensive levels of lateral e-cadherin. Furthermore, at 60-minutes the MDCK-GFP and MDCK-R14WT had more e-cadherin localized at their junctions, but the intensity of labeling was further increased in the MDCK-R14S25N cells. This difference is more evident when the cells are examined
at higher magnification, shown in figure 5b. These results indicate that Rab14 is playing a role in the trafficking of e-cadherin to adherens junctions in polarized epithelial cells.

To test if Rab14 is involved in tight junction assembly, we performed another calcium-switch experiment using occludin targeting as a marker of tight junction assembly. As mentioned above, occludin labeling was relatively consistent between the different genotypes at steady state (Figure 6a). At time 0, the different genotypes showed the same degree of loss of cell-to-cell contact. By 30 minutes, the MDCK-R14S25N cells began to show some occludin at the junctions, while the MDCK-R14WT and MDCK-GFP showed minimal occludin between cells. By 60 minutes, all genotypes had occludin labeling at the junctions between cells, but the MDCK-R14S25N cells had more defined occludin labeling at the junctions. At 120 minutes, though all cells demonstrated occludin labeling, the intensity of occludin in the MDCK-R14S25N was greater in comparison to the other two genotypes. Similar to the e-cadherin results, these results also indicate that Rab14 is playing a role in the trafficking of occludin to the tight junctions.

**DISCUSSION:**

Rab proteins are important regulators of many membrane events, including vesicle formation, cargo transport, and vesicle docking [1]. For example, Rab22 has been shown to regulate internalization, while Rab7 has been shown to be involved in transport from late endosomes to lysosomes. Here, we tested to see if Rab14 regulates the establishment of polarity and junction formation in epithelial cells. We find that inactive Rab14 prevented the formation of single lumens in three-dimensional cyst culture and affected
the targeting of gp135, an apically localized protein. These results showed that Rab14 is playing a role in the establishment of epithelial polarity. In addition, with the use of calcium-switch experiments, through both live-cell imaging and immunostaining, we show that Rab14 has a role in trafficking junctional proteins to their lateral membranes. These results suggest that Rab14 is involved in trafficking proteins involved in junction formation between neighboring cells.

*Does Rab14 modulate junction formation through the regulation of endocytosis of junctional proteins?*

Rab14 could regulate junction formation through modulation of several membrane trafficking events. It is possible that Rab14 mediates junction assembly through endocytosis of junctional components. A number of Rab GTPases have been involved in endocytic processes. Rab5 has been shown to be involved in clathrin-mediated endocytosis of molecules from the cell surface [16], while Rab4 is involved in recycling of receptors at the early endosome [17]. Endocytosis of e-cadherin is likely to occur through the clathrin-mediated pathway and once it is internalized it accumulates in early endosomes associated with Rab5 [18]. Previous studies of Rab14 have shown that overexpression of active-Rab14 or wild-type Rab14 causes a shift of distribution to the endosomal compartments [9], while overexpression of inactive-Rab14 causes a shift towards the TGN. Thus it was shown that Rab14 is involved in the transport pathway from the TGN to early endosomes, but not necessarily endocytosis. Our observations suggest that Rab14 may be playing a role in the endocytosis of junctional proteins from the plasma membrane to intracellular compartments. Because inactive-Rab14 causes a
slower loss of cell-to-cell contact in comparison to the Rab14 wild-type cells, it could be that Rab14 mediates the uptake of these junction proteins. Since Rab14 is inactivated, fewer proteins are being brought into the cell and thus remaining at the junctions, causing those cells to be less resistant to the loss of calcium. Previous results showing that some Rab14 is localized at the plasma membrane also supports this hypothesis [9].

Does Rab14 regulate junction formation through recycling of junctional proteins?

Another Rab regulated process is recycling. Rab11-recycling endosomes have been shown to be involved in the initial targeting of newly synthesized e-cadherin to the basolateral plasma membrane [8]. This process is important for junctional remodeling [19]. Rab13 is also involved in the regulation of tight junction integrity by regulating endocytic recycling of claudins and occludin [12, 14]. While it has been shown that Rab14 does not regulate transferrin receptor recycling [10], our experiments suggest that Rab14 may play a role in inhibiting recycling of junctional proteins. In cells expressing inactive-Rab14, recycling of these proteins may be increased, causing more proteins to be trafficked to the junctions, as demonstrated by the increased labeling at the cellular junctions in these Rab14 cells. When adhesion proteins associate with proteins in neighboring cells, their adhesion is greater, causing the cells to be resistant to loss of cell-to-cell contact. Since the immunostaining for both occludin and e-cadherin showed more of these proteins at the junctions in the inactive-Rab14, the results also support Rab14’s role in recycling.

Is Rab14 regulating junction formation through degradation of junctional proteins?
Rab proteins also regulate trafficking to lysosomes, and previous studies have shown that Rab14 is present on phagosomes and may play a role in phagocytosis [20]. If Rab14 is mediating lysosomal targeting of junctional components, then it would be predicted that overexpression of inactive-Rab14 would result in more junctional proteins. This would explain why inactive-Rab14 cells are more resistant to losing to their cell-to-cell contacts. However, a western blot of the different genotypes probed for the junctional proteins showed that there are equal amounts of both occludin and e-cadherin in all three genotypes, showing that Rab14 is not involved in regulating degradation of junctional proteins.

Is Rab14 involved in the regulation of junctional proteins through biosynthetic pathways?

When exogenous Rab14 is expressed in a cell, it has been shown to localize to biosynthetic compartments [9, 10]. In addition, previous studies have shown that Rab14 is involved in apical trafficking from the TGN to apical endosomes, and Rab14 vesicles fuse with early endosomes and the plasma membrane [10]. Furthermore, different Rab GTPases have been shown to be involved in the transport of newly synthesized proteins from biosynthetic compartments to other areas of the cell. As described above, Rab11 is involved in the trafficking of newly synthesized e-cadherin to the basolateral membrane [7]. Rab13 has also been shown to be involved in regulating delivery of cargo to recycling endosomes from the TGN during biosynthetic delivery [21]. Although Rab14 has also been shown to localize at the TGN and may be involved in trafficking of newly synthesized proteins [9, 10], the length of time of the calcium-switch experiments...
described here was not enough to account for the time it would take for synthesis of new proteins and their transport to the cell junctions.

We show that Rab14 is most likely to be involved in either endocytic or recycling pathways of junctional proteins. To further investigate Rab14’s role in endocytosis an endocytic assay can be performed. Junctional cell surface proteins can be biotinylated and incubated for indicated periods of time to allow endocytosis. Biotinylated cargo proteins can be isolated, and the samples analyzed using an immunoblot assay. The values for biotinylated cargo proteins protected from treatment are normalized to total cargo proteins expressed in the cells. These values can be compared in cells expressing wild-type Rab14 and inactive-Rab14 to see if the levels of biotinylated cargo proteins differ. Similarly, to test Rab14’s role in recycling, a recycling assay can be conducted. Cell surface proteins are biotinylated and incubated for the indicated periods of time to allow endocytosis. Cells are then incubated for the indicated periods of time to allow recycling of endocytosed cargo proteins back to the cell surface. The total amount of cargo proteins expressed in the cells is determined and then the biotinylated cargo proteins are isolated. These different experiments can be conducted to further investigate Rab14’s role in recycling and endocytosis.
Figure 1. gp135 labeling shows disruption of lumen formation with expression of Rab14-S25N.

MDCK-GFP cells form a single lumen and apically target gp135 (left panel). MDCK-R14-WT cysts (middle panel) also form single lumen and target gp135 to the lumenal plasma membrane. MDCK-R14-S25N expressing cells (right panel) are unable to form single lumen and mistarget gp135. Green, GFP. Red, gp135. Blue, Topro (nuclei).
Figure 2. Distribution of cells at steady-state in live-cell imaging appear similar for all cell types. MDCK-GFP, MDCK-R14-WT, and MDCK-R14-S25N cells at steady state demonstrate similar distribution of cells in complete media at confluency.
Figure 3. **Cells expressing Rab14 S25N lose cell-cell contact more slowly after calcium switch.** After 15 minutes in calcium-free media, cells expressing GFP and Rab14-WT have rounded up and some have lost cell-cell contact. MDCK-R14-S25N remain more flattened have fewer contacts lost (Figure 3a). Rab14-S25N cells retain their contact even after 30 minutes in calcium-free medium (Figure 3b).
Figure 4. Cells expressing Rab14-S25N reestablish cell-cell contacts sooner than other genotypes

Cells were incubated in calcium free media for 30 minutes (MDCK-GFP or Rab14-WT) or 45 minutes (Rab14-S25N) to completely separate the junctions and then placed in complete medium. Cells were imaged immediately (T0) and at 15 minute intervals. After 15 minutes, cells expressing GFP or Rab14-WT remain rounded. However, cells expressing Rab14-S25N have begun to spread and establish cell-cell contacts. At 30 minutes, both GFP and Rab14-WT expressing cells have begun to spread and reestablish cell-cell contacts, and Rab14-S25N cells have spread more and appear to have reformed junctions. After 45 minutes, cells expressing Rab14-S25N appear to have regained confluency, however, cells expressing GFP or Rab14 WT remain somewhat rounded and not completely adhered. By 60 minutes, the GFP and Rab14-WT cells appeared relatively flattened and adherent.
Figure 5. E-cadherin labeling at the adherens junctions is enhanced after calcium switch in Rab14-S25N expressing cells.

The steady state distribution of e-cadherin appears similar in all genotypes (left column). In Figure 5a, (top and middle panels), both MDCK-GFP and MDCK-R14-WT cells gain e-cadherin at the junctions by 30 minutes after calcium-switch and continue to target e-cadherin to adherens junctions up to 60 minutes after calcium-switch. MDCK-R14-S25N cells (bottom panel) have more e-cadherin at the junctions after both 30 and 60 minutes after calcium-switch than the other genotypes. Figure 5b is a higher magnification of the cells demonstrating the differences in labeling between the genotypes.
Figure 6. Occludin labeling at the tight junctions is enhanced after calcium switch in Rab14-S25N expressing cells.

Figure 6a shows that occludin labeling in all genotypes appears similar at steady state. Thirty minutes after calcium switch, the top and middle panels show MDCK-GFP and MDCK-R14-WT cells have begun to target occludin to the tight junctions. However, MDCK-R14-S25N cells have more occludin labeling at the tight junctions at this time point. Occludin reinsertion into the junctional region continues at 60 and 120 minutes after addition of complete media in all cell types. However, Rab14-S25N expressing cells continue to display enhanced occludin labeling.

Literature Cited


