The G-Signaling Protein Rcp Controls the Polarized Basement Membrane Deposition in Epithelial Cells

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Abstract

Epithelial tissues are the most common type of tissue in the human body, forming the outer layer of the skin and most organs. They are composed of epithelial cells and rely heavily on their cellular architecture, which is organized by an apical-basal polarity, for their function. One critical component for the establishment and maintenance of the epithelial cell architecture is the proper placement of the basement membrane. The basement membrane is a specialized sheet within the extracellular matrix that lines the basal side of epithelial cells, and there is a biological pathway that is responsible for the proper placement of the basement membrane at the basal side. This pathway controls the production of basement membrane proteins within epithelial cells and basally secretes them from these cells to the basement membrane. Despite the basement membrane’s important role in epithelial cell organization and polarity, the biological pathway dedicated to the polarized secretion of basement membrane proteins is poorly understood. To study basement membrane deposition, we use the follicular epithelium of the *Drosophila* ovary as a model system. In a genetic screen looking for genes involved in the proper placement of basement membrane proteins, we identified a new gene, *Rcp* (Receptor component protein), which has been shown to be involved in G-protein signaling. It has been shown that the loss of *Rcp* leads to the mislocalization of basement membrane proteins to the apical side of epithelial cells. We identified one *Rcp* mutant line, *Rcp^{R552}* , that frequently displayed mislocalization of basement membrane proteins to the apical side of epithelial cells. Additionally, using immunostaining and super-resolution microscopy, we determined that *Rcp* localizes in the cytoplasm and nucleus of epithelial cells, suggesting that *Rcp* has a role in gene expression. Altogether, our results identified one *Rcp* mutant line that affected the normal polarized deposition of basement membrane proteins and identified the intracellular localization of *Rcp* within epithelial cells.
Introduction

Epithelial tissues are the most common type of tissue in the human body, forming the outer layer of the skin and most organs. These epithelial tissues are composed of epithelial cells, which have a specific apical-basal polarity that is critical for epithelial cell architecture, organization, and function. One key component of this apical-basal polarity is the proper placement of the basement membrane only at the basal side of epithelial cells. The basement membrane is a specialized sheet within the extracellular matrix that is composed of proteins that are produced within epithelial cells. Therefore, there is a biological pathway that is responsible for the polarized secretion of these proteins from inside the epithelial cells in which they are produced to the proper location of the basement membrane at the basal side of these cells.

However, the mechanisms involved in this biological pathway are not well understood, as only a few components involved in this pathway have been identified (Daley et al., 2012; Denef et al., 2008; Devergne et al., 2017; Devergne et al., 2014; Lerner et al., 2013).

This lack of knowledge surrounding this biological pathway that controls the proper placement of the basement membrane could be seen as surprising considering the pivotal role that the basement membrane plays in epithelial cell polarity, tissue organization, and organ morphogenesis (Daley et al., 2012; Yurchenco, 2011). The mislocalization of the basement membrane to the apical side of epithelial cells, rather than to the basal side, results in the loss of apical-basal polarity in these cells. The loss of this apical-basal polarity results in the loss of epithelial cell and tissue architecture, organization, and proper development, which can lead to harmful effects, such as developmental defects, the development of cancer, and tumor metastasis (Denef et al., 2008). Therefore, not only will research on this topic contribute to the understanding of the mechanisms involved in biological pathway that controls the proper
placement of the basement membrane, it can also contribute to the understanding of how the loss of apical-basal polarity and the mislocalization of the basement membrane can result in harmful effects, including improper organ morphogenesis and cancer.

In order to study the mechanisms involved in the biological pathway that controls the proper placement of the basement membrane in epithelial cells, the follicular epithelium of the *Drosophila*, or fruit fly, ovary was used as a model system (Figure 1). Using the follicular epithelium of the *Drosophila* ovary as a model system is beneficial for this kind of research since *Drosophila* and humans have many of the same genes, including those that are associated with human diseases, such as cancer. It is estimated that 75% of the genes that cause diseases in humans are also found in *Drosophila*, and therefore, *Drosophila* can be used as an effective model system to study the roles that genes play in human diseases (Pandey & Nichols, 2011). Additionally, *Drosophila* are useful model systems due to their short life cycles, low cost, ease at which they can be kept in labs, number of offspring produced per generation, and ease at which they can be genetically manipulated.

Using the follicular epithelium of the *Drosophila* ovary as a model system, one gene that has been found to play a critical role in the biological pathway that controls the proper placement
of the basement membrane is Rcp (Receptor component protein), which has been shown to be involved in G-protein signaling (Figure 2). Rcp has been shown to play an important role in this biological pathway, as the loss of Rcp has been shown to lead to the apical mislocalization of the basement membrane in epithelial cells (Figure 3).

However, the exact role that Rcp has in this biological pathway is still unknown. This research, therefore, looks to characterize Rcp by identifying how different Rcp mutations affect the proper placement of the basement membrane and by determining the intracellular localization of Rcp within epithelial cells in order to gain a better understanding of what role Rcp plays within these cells and in this biological pathway. Additionally, Rcp is a gene that is found in both Drosophila and humans, with hCRCP being the human homolog of Rcp. Therefore, by studying Rcp, a better understanding of how this gene functions

Figure 2: CGRP Complex. Rcp is known to play a role in G-protein signaling activity by interacting with the Calcitonin-Receptor Like Receptor (CLR) of the Calcitonin G-Protein Receptor Protein (CGRP). Rcp is involved in both CGRP and Adrenomedullin signaling to function in G-protein signaling and cAMP production. Rcp's activity can be cell type dependent.

Figure 3: The Loss of Rcp Leads to the Apical Mislocalization of Basement Membrane Proteins. Longitudinal section through egg chambers expressing Pcan-GFP (green) and stained for F-Actin (red) and DNA (blue). A and A’ represent the control, in which there is normal Rcp production. B and B’ show the follicular epithelium when it expresses Rcp RNAi, in which there is a loss of Rcp from the cells. In Rcp RNAi cells, the basement membrane proteins accumulate apically, rather than only basally (B’ arrows).
in humans is possible, which can be used to understand how the development of human diseases, such as cancer, may be associated with the loss of epithelial cell organization and polarity that results from the loss of \( Rcp \) in these cells. Overall, this research, therefore, contributes to knowledge surrounding cell polarity and epithelial tissue organization by investigating a gene that is important for basement membrane deposition and organization.

**Methods**

*The Characterization of Newly Generated Rcp Mutant Lines*

To gain a better understanding of the role that \( Rcp \) has within the biological pathway that controls the proper placement of the basement membrane, newly generated \( Rcp \) mutant lines were characterized in order to determine if these mutations affected the proper placement of the basement membrane. The follicular epithelium of the *Drosophila* ovary was used as a model system, and the various \( Rcp \) mutations were identified through a genetic screen. \( Rcp^{R17}, Rcp^{R28}, Rcp^{R55.2}, Rcp^{R63-1}, \) and \( Rcp^{FM60} \) were the five \( Rcp \) mutant lines that were generated. Each of these mutant lines represented a different mutation to the gene \( Rcp \). For example, the \( Rcp^{R55.2} \) mutation was a deletion mutation in which Exon 5 is missing from the gene \( Rcp \) (Figure 4). For each of these mutant lines, the FRT-FLP system was used to generate egg chambers through mitotic recombination that contained both mutant and wild-type cells in the follicular epithelium. Confocal microscopy was utilized to compare the phenotypes of these mutant and wild-type...
cells, and the clonal markers ubiGFP and ubiRFP were used in order to be able to differentiate between these mutant and wild-type cells, as these clonal markers are displayed in wild-type cells, but not in mutant cells.

Before being able to use confocal microscopy, however, Drosophila ovaries had to first be dissected and fluorescently stained. Genetic crosses were created in which females with a genotype containing one of the Rcp mutations, as well as the basement membrane marker VkgGFP, were crossed with males whose genotype contained either ubiGFP or ubiRFP. In total, there were ten crosses made, including ♀RcpR17;VkgGFP x ♂ubiRFP, ♀RcpR17;VkgGFP x ♀ubiGFP, ♂ubiRFP, ♀RcpR28;VkgGFP x ♀ubiGFP, ♀RcpR28;VkgGFP x ♂ubiGFP, ♀RcpR55.2;VkgGFP x ♀ubiRFP, ♀RcpR55.2;VkgGFP x ♀ubiGFP, ♀RcpR63-1;VkgGFP x ♂ubiRFP, ♀RcpR63-1;VkgGFP x ♀ubiGFP, ♀RcpFM60;VkgGFP x ♂ubiRFP, and ♀RcpFM60;VkgGFP x ♀ubiGFP. Progeny with the desired genotype of one Rcp mutation, one clonal marker, and VkgGFP were produced from these crosses. Female progenies were then dissected for their ovaries using PBS and a dissecting microscope (Figure 5). Following dissection, the ovaries were then fixed using 4% Paraformaldehyde, and then washed using PBST. Hoechst, a blue, fluorescent stain for DNA, and Phalloidin-Alexa 647, a violet, fluorescent stain for F-Actin, were both then added into the PBST so that the nuclei and edges of individual epithelial cells within the follicular epithelium could be visualized using confocal microscopy. After another wash using PBST, the ovaries were mounted onto microscope slides and could be looked at using

Figure 5: Dissection of Female Drosophila. Ovaries are removed from female Drosophila in order to use them as a model system for this research. Each ovary is composed of individual eggs.
confocal microscopy. Using the confocal microscope, images of the mutant cells, including the stains for DNA and F-Actin, the basement membrane marker VkgGFP, and the clonal markers ubiGFP or ubiRFP, were acquired.

The Characterization of the Intracellular Localization of Rcp in Epithelial Cells

In order to gain a better understanding of Rcp’s role in epithelial cells and in the biological pathway that controls the proper placement of the basement membrane, the intracellular localization of Rcp in these cells was characterized. The localization of a protein within a cell can help to reveal what kind of role that protein plays in that cell, and so therefore, determining the intracellular localization of Rcp in epithelial cells is an important step towards identifying its role. To conduct this research, the follicular epithelium of the Drosophila ovary was once again used as a model system, and three transgenic lines in which Rcp was tagged were used. These three transgenic lines were HA-Rcp, Rcp-HA, and Rcp-mCherry. An additional fourth transgenic line, hCRCP-HA, was used in order to see whether hCRCP, which is the human homolog of Rcp, has the same intracellular localization as Rcp. The HA and mCherry tags attached to Rcp and hCRCP in these transgenic lines are necessary due to the fact that Rcp is not fluorescent. Therefore, these tags are necessary to provide the fluorescence that Rcp lacks.

In order to express these tagged versions of Rcp and hCRCP in the follicular epithelium, the UAS-GAL4 system was utilized (Figure 6). In this system, the transcription factor GAL4 binds to the transcriptional activating sequence UAS in order to activate the transcription and expression of the tagged versions of Rcp and hCRCP within the progeny of genetic crosses that combine female Drosophila that have UAS and male Drosophila that have GAL4. There were four crosses made in total, which included ♀UAS-HA-Rcp x ♂PcanGFP, tjGAL4, ♀UAS-Rcp-HA x ♂PcanGFP, tjGAL4, ♀UAS-Rcp-mCherry x ♂PcanGFP, tjGAL4, and ♀UAS-hCRCP-HA
The female progeny from these crosses were then dissected using PBS and a dissecting microscope so that their ovaries could be studied.

Following the dissections, the ovaries needed to be fixed using 4% Paraformaldehyde and then washed using PBST. Ovaries expressing Rcp-mCherry were then stained with Hoechst and Phalloidin-Alexa 647 in order to stain the DNA and F-Actin within these ovaries, were then washed again with PBST, and then were mounted onto a microscope slide and imaged using confocal and super-resolution microscopy.

Ovaries expressing HA-Rcp, Rcp-HA, and hCRCP-HA, however, had to undergo immunostaining in order to become fluorescent, unlike mCherry which is already naturally fluorescent. For immunostaining, the ovaries expressing HA-Rcp, Rcp-HA, and hCRCP-HA were first fixed using 4% Paraformaldehyde, washed using PBST, and blocked using 5% BSA solution and PBS. Following this, a primary antibody solution made with 5% BSA and an anti-HA primary antibody was added. After washing the ovaries once again with PBST, a secondary antibody solution was added, which included PBST, 5% BSA, Hoechst, Phalloidin-Alexa 647, and an anti-rat secondary antibody. For some ovaries, fibrillarin, a stain for the nucleolus, was used instead of the Phalloidin-Alexa 647 stain for F-Actin. Following the addition of the secondary antibody solution, the ovaries were washed again with PBST, and then were mounted on microscope slides and were imaged using confocal and super-resolution microscopy.
Results

The Characterization of Newly Generated Rcp Mutant Lines

Images and data were acquired for each of the five newly generated *Rcp* mutant lines in order to determine if any of these mutations resulted in the mislocalization of the basement membrane to the apical side of the follicular epithelium. Data collected for each mutant line included the number of egg chambers that had clones, the number of clones within these egg chambers, and the number of times that apical mislocalization was observed within these clones. Clones represent the areas of the egg chamber where mutant cells are located. More than one clone can be present in an egg chamber. When wild-type cells are located in between mutant cells, the mutant cells on either side of the wild-type cells are considered separate clones. Apical mislocalization within the clones was identified by the presence of basement membrane proteins at both the apical and basal side of the cells, rather than only at the basal side.

For the *Rcp*<sup>R17</sup> mutant line (Figure 7), only 1 egg chamber with clones was found, with there being 2 clones within this egg chamber. Neither of these clones displayed apical mislocalization of the basement membrane. The *Rcp*<sup>R28</sup> mutant line (Figure 8) generated 13 egg chambers that had a total of 19 clones, but no apical mislocalization was observed for any of these clones. The *Rcp*<sup>R55.2</sup> mutant line (Figure 9), however, did display apical mislocalization of the basement membrane, as it generated 25 egg chambers that in total had 40 clones, and 12 of these 40 clones displayed apical mislocalization of the basement membrane. The *Rcp*<sup>R63-1</sup> mutant line (Figure 10), however, only had 2 egg chamber with clones, and only 2 clones within these egg chambers, with neither displaying apical mislocalization. Lastly, the *Rcp*<sup>FM60</sup> mutant line (Figure 11) generated 21 egg chambers that had a total of 28 clones, with 3 of these clones displaying apical mislocalization. These results show that the *Rcp*<sup>R55.2</sup> mutant line and its
frequent display of apical mislocalization of the basement membrane confirms that Rcp is involved in the proper placement of the basement membrane. The Rcp^{17} and Rcp^{R63-1} mutant lines did not have enough data to make any determinations about their effect on the basement membrane, and therefore, more data will need to be collected for these mutant lines.

Figure 7: The Rcp^{R17} Mutant Line Generated Only One Egg Chamber That Had Clones. Longitudinal section through egg chamber expressing VkgGFP (basement membrane, green) and ubiRFP (clonal marker, red), and stained for F-Actin (violet) and DNA (blue). Dashed lines separate mutant cells (-/-) from wild-type cells (+/+). ubiRFP is expressed only in wild-type cells. Only one egg chamber with clones was found, with there being only two clones, as shown in A-A”. As seen in A”, no basement membrane mislocalization was observed.

Figure 8: The Rcp^{R28} Mutant Line Does Not Result in Apical Mislocalization of the Basement Membrane. Longitudinal section through egg chamber expressing VkgGFP (basement membrane, green) and ubiGFP (clonal marker, green), and stained for F-Actin (violet) and DNA (blue). Dashed lines separate mutant cells (-/-) from wild-type cells (+/+). ubiGFP is expressed only in wild-type cells. The Rcp^{R28} mutant line generated 13 egg chambers that in total had 19 clones. However, as demonstrated by B’, none of these clones showed apical mislocalization of the basement membrane.
Figure 9: The $Rcp^{R55.2}$ Mutant Line Frequently Results in the Apical Mislocalization of the Basement Membrane. Longitudinal section through egg chambers expressing VkgGFP (basement membrane, green) and ubiGFP (clonal marker, green), and stained for F-Actin (violet) and DNA (blue). Dashed lines separate mutant cells (-/-) from wild-type cells (+/+). ubiGFP is expressed only in wild-type cells. The $Rcp^{R55.2}$ mutant line generated 25 egg chambers that in total had 40 clones, with 12 of these clones displaying apical mislocalization of the basement membrane. C-C’ and D-D’ show two examples of this apical mislocalization (arrows) of the basement membrane for $Rcp^{R55.2}$ mutant cells.
**Figure 10: The Rcp<sup>R63-1</sup> Mutant Line Only Generated Two Egg Chambers With Clones.** Longitudinal section through egg chamber expressing VkgGFP (basement membrane, green) and ubiGFP (clonal marker, green), and stained for F-Actin (violet) and DNA (blue). Dashed lines separate mutant cells (−/−) from wild-type cells (+/+) ubiGFP is expressed only in wild-type cells. Only two egg chambers with clones were found, with there being a total of two clones found in these egg chambers. As seen in E–E′, no apical mislocalization of the basement membrane was observed for the Rcp<sup>R63-1</sup> mutant cells.

**Figure 11: The Rcp<sup>FM60</sup> Mutant Line Displayed Apical Mislocalization of the Basement Membrane.** Longitudinal section through egg chamber expressing VkgGFP (basement membrane, green) and ubiRFP (clonal marker, red), and stained for F-Actin (violet) and DNA (blue). Dashed lines separate mutant cells (−/−) from wild-type cells (+/+) ubiRFP is expressed only in wild-type cells. The Rcp<sup>FM60</sup> mutant line generated 21 egg chambers that in total had 28 clones. Three of these clones displayed apical mislocalization of the basement membrane. One example of this apical mislocalization is shown in F–F″.
The Characterization of the Intracellular Localization of Rcp in Epithelial Cells

Images were acquired through confocal and super-resolution microscopy for HA-Rcp (Figure 12), Rcp-HA (Figure 13), Rcp-mCherry (Figure 14), and hCRCP-HA (Figure 15) in order to determine where Rcp and hCRCP localize within epithelial cells. Through these images, it is observed that Rcp-HA, Rcp-mCherry, and hCRCP-HA all localize in the cytoplasm and accumulate in the nucleus of epithelial cells. However, since the different HA-tagged Rcp do not overlap with a fibrillarin marker for the nucleolus, they appear to not be localized within the nucleolus, which is a structure inside the nucleus that is the site of ribosome biogenesis and has a role in the cell’s response to stress. The images for HA-Rcp were not as clear as were the others in terms of being able to see accumulation of HA-Rcp in the nucleus. This may be due to the addition of the HA tag at the N-terminus end of Rcp, as adding this tag to the N-terminus might have affected Rcp's localization more than it does when it is added to the C-terminus, as in Rcp-HA, Rcp-mCherry, and hCRCP-HA. Through the results seen for each of the four transgenic lines, it appears that both Rcp and hCRCP localize in the cytoplasm and accumulate in the nucleus, but not in the nucleolus, of epithelial cells, which suggests that Rcp has a role in gene expression.

Figure 12: Intracellular Localization of HA-Rcp in Epithelial Cells. Longitudinal section through egg chamber expressing HA-Rcp (red) and PcanGFP (basement membrane, green), and stained for F-Actin (violet) and DNA (blue). HA-Rcp is localized in the cytoplasm of these cells. No nuclear accumulation observed, however, small circular regions in which there is a lack of HA-Rcp (A’ circle) may be the nucleolus, which would indicate that HA-Rcp does not localize in the nucleolus.
Figure 13: Intracellular Localization of Rcp-HA in Epithelial Cells. Longitudinal section across the top of an egg chamber expressing Rcp-HA (red) and PcanGFP (basement membrane, green), immunostained for fibrillarin (nucleolus marker, violet), and stained for DNA (blue). Rcp-HA localizes in the cytoplasm and accumulates in the nucleus, but not in the nucleolus (marked by *), which is a structure located inside of the nucleus.
Figure 14: Intracellular Localization of Rcp-mCherry in Epithelial Cells. Longitudinal section across the top of an egg chamber expressing Rcp-mCherry (red) and PcanGFP (basement membrane, green), immunostained for fibrillarin (violet), and stained for DNA (blue). Rcp-mCherry localizes in the cytoplasm and accumulates in the nucleus, but not in the nucleolus (marked by *), which is a structure located inside the nucleus.
Discussion

The Characterization of Newly Generated Rcp Mutant Lines

In order to gain a better understanding of the role that Rcp has in epithelial cells and in the biological pathway that controls the proper placement of the basement membrane, newly generated Rcp mutant lines were characterized through identifying what affect, if any, they have on the placement of the basement membrane. It was found that the mutant lines Rcp$^{R17}$ and Rcp$^{R63-1}$ only generated a couple egg chambers that had clones, and no apical mislocalization of the basement membrane was observed in these clones. The mutant line Rcp$^{R28}$, on the other hand, generated 13 egg chambers with clones, with there being 19 clones in total, but no apical mislocalization of the basement membrane was observed. The mutant lines Rcp$^{R55.2}$ and Rcp$^{FM60}$, however, did display apical mislocalization of the basement membrane. Rcp$^{FM60}$ generated 21 egg chambers that together had a total of 28 clones, with 3 of these clones showing apical
mislocalization of the basement membrane. $Rcp^{R55.2}$ was the mutant line that not only generated the largest number of egg chambers that had clones, as well as the largest number of clones found in total from these egg chambers, but it also displayed the most apical mislocalization. The $Rcp^{R55.2}$ mutant line produced 25 egg chambers with clones, with there being 40 clones within these egg chambers. Of these 40 clones, there were 12 in which the apical mislocalization of the basement membrane was observed. 30% of the clones produced by $Rcp^{R55.2}$ displayed apical mislocalization, which is significantly higher than the 10% for $Rcp^{FM60}$ and the 0% for $Rcp^{R17}$, $Rcp^{R28}$, and $Rcp^{R63-I}$. Therefore, out of all five mutant lines, $Rcp^{R55.2}$ most likely has the largest impact on the proper functioning of the gene $Rcp$. This may be due to the fact that $Rcp^{R55.2}$ is a deletion mutation in which Exon 5 is missing from the gene $Rcp$. Therefore, since basement membrane mislocalization occurs when Exon 5 is missing due to this mutation, this exon may be important for the proper function of the gene $Rcp$ in epithelial cells. Since Exon 5 encodes for the 3’UTR of Rcp, it may have a role in the stability of the $Rcp$ mRNA, and the mRNA may become unstable and unable to properly function without this exon. Overall, the results for the $Rcp^{R55.2}$ mutant line confirms $Rcp$’s involvement in the proper placement of the basement membrane.

For future research on this topic, it will be important to acquire more data for the $Rcp^{R17}$ and $Rcp^{R63-I}$ mutant lines, as the little amount of data that was able to be acquired for these mutant lines is not sufficient enough to make any kind of determination about how these mutants affect the functioning of $Rcp$ and the proper placement of the basement membrane. More data should be acquired for all of these mutant lines, but especially for $Rcp^{R17}$ and $Rcp^{R63-I}$ since a significantly lower amount of data was found for those two mutants. However, if additional tries at finding egg chambers with clones for these two mutant lines proves unsuccessful, then maybe there is something else at play that is causing less clones to form for these mutant lines.
The Characterization of the Intracellular Localization of Rcp in Epithelial Cells

In order to gain a better understating of the potential roles that Rcp may have within epithelial cells and the overall biological pathway that controls the proper placement of the basement membrane, its intracellular localization was determined using HA and mCherry tagged versions of Rcp, as well hCRCP, which is the human homolog of Rcp. Through the use of these tagged versions of Rcp and hCRCP, it was determined that Rcp and hCRCP localize within the cytoplasm and accumulates in the nucleus of epithelial cells, but not in the nucleolus, which is a structure located inside of the nucleus. Since Rcp and hCRCP have the same intracellular localization, hCRCP may have the same role in humans as Rcp has in Drosophila. Additionally, this cytoplasmic and nuclear localization suggests that Rcp and hCRCP may play a role in gene expression.

For future research on this topic, the localization of Rcp could be confirmed through the use of antibodies that detect Rcp directly. By using antibodies that detect Rcp directly, it could potentially allow for a more accurate visualization of where Rcp is located than is possible when using the HA and mCherry tags. For example, one possible reason why the images for HA-Rcp were not as clear as those of the Rcp-HA, Rcp-mCherry, and hCRCP-HA is that the HA tag might have interfered with Rcp’s localization by being placed on the N-terminus instead of on the C-terminus. By using antibodies that detect Rcp directly, any issues like this would be avoided and may lead to a more specific determination of where Rcp is localized.

Conclusion

Despite the importance of the proper placement of the basement membrane at the basal side of epithelial cells, the biological pathway that controls this proper placement is not well-known. Therefore, this research aims to fill in gaps in knowledge surrounding this biological
pathway and epithelial cell polarity. \textit{Rcp} is a gene that has been found to play an important role in the proper placement of the basement membrane in epithelial cells, but its exact role is still unknown. However, through this research, the intracellular localization of \textit{Rcp}, as well as its human homolog \textit{hCRCP}, within epithelial cells was determined, which helps with suggesting possible roles that \textit{Rcp} could have within these cells based on where it localizes. Since it was determined that \textit{Rcp} is localized in the cytoplasm and nucleus of epithelial cells, it may play a role in gene expression. Additionally, this research characterized five newly generated \textit{Rcp} mutant lines in order to see how these different mutations impacted the proper placement of the basement membrane. From this, \textit{Rcp}^{R55.2} was found to be a mutation that resulted in the frequent mislocalization of the basement membrane to the apical side of the follicular epithelium. Therefore, due to the mislocalization that was displayed in cells with this mutation, \textit{Rcp}^{R55.2} may have a negative effect on the proper functioning of the gene \textit{Rcp}, potentially due to the fact that this mutation is a deletion mutation in which Exon 5 is missing from the gene \textit{Rcp}. Therefore, Exon 5 may have been identified as an important exon for the proper functioning of the gene \textit{Rcp}. Overall, \textit{Rcp} plays an important role in the proper placement of the basement membrane and in maintaining epithelial cell polarity, as without the proper functioning of this gene within epithelial cells, basement membrane mislocalization results.

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