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# A GFP Retinal Prion Protein's Intracellular Trafficking via Endocytic Intermediates

**Tarunkanti Mondal***

*Department of Clinical Diabetes and Research, University of Bhubaneswar, India*

## Abstract

The prion protein (PrP) is a key molecule implicated in neurodegenerative disorders. Understanding its intracellular trafficking is essential for unraveling its physiological and pathological functions. This article focuses on the intracellular trafficking of a green fluorescent protein (GFP) tagged PrP in retinal cells, specifically exploring its transport via endocytic intermediates. Endocytic pathways, including clathrin-mediated endocytosis, caveolae-mediated endocytosis, and macropinocytosis, play a crucial role in PrP internalization and trafficking. Endocytic intermediates, such as early endosomes, late endosomes, and lysosomes, are involved in sorting, recycling, and degradation of endocytosed proteins. Live-cell imaging and colocalization studies have provided insights into GFP-PrP's movement and localization within these organelles. Studies reveal rapid internalization of PrP via clathrin-mediated endocytosis, with subsequent transport to early endosomes. A fraction of PrP is recycled, while the remainder progresses to late endosomes and lysosomes for degradation. Elucidating the intracellular trafficking of GFP-PrP and its association with endocytic intermediates enhances our understanding of PrP's functions and implications in neurodegenerative diseases. Further investigations are needed to explore the molecular machinery involved and the impact of disease-associated mutations. This knowledge may contribute to potential therapeutic strategies for prion diseases.

**Keywords:** GFP; Retinal; Prion protein; Lysosomes; Neurodegenerative

## Introduction

The prion protein (PrP) is a fascinating molecule that plays a crucial role in the development and function of the nervous system. Abnormal folding of PrP has been implicated in the pathogenesis of prion diseases, a group of fatal neurodegenerative disorders. Understanding the intracellular trafficking and localization of PrP is essential for unraveling its physiological and pathological functions. In this article, we focus on the intracellular trafficking of a green fluorescent protein (GFP) tagged PrP in retinal cells, specifically exploring its transport via endocytic intermediates.

**Endocytic pathways and prp trafficking:** The endocytic pathway serves as a major route for the internalization and intracellular trafficking of various molecules, including cell surface receptors and signaling proteins. Several endocytic routes exist [1], such as clathrin-mediated endocytosis, caveolae-mediated endocytosis, and macropinocytosis. Understanding the specific endocytic pathway(s) involved in the internalization and trafficking of PrP is essential for elucidating its physiological functions and potential contributions to disease.

**Role of endocytic intermediates in prp trafficking:** Endocytic intermediates, including early endosomes, late endosomes, and lysosomes, play critical roles in the sorting, recycling, and degradation of endocytosed proteins. These organelles possess distinct protein and lipid compositions, allowing them to perform specific functions during intracellular trafficking. By studying the behavior of GFP-tagged PrP within these endocytic intermediates, researchers gain insights into the dynamics and fate of PrP within the cell [2].

**Experimental approaches:** Researchers have utilized various experimental techniques to investigate the intracellular trafficking of GFP-tagged PrP in retinal cells. Live-cell imaging using confocal microscopy provides valuable information on the movement of PrP within different endocytic compartments over time. Colocalization studies using immunofluorescence techniques and specific markers for endocytic organelles allow researchers to pinpoint the subcellular localization of GFP-PrP and its interaction with various endocytic intermediates [3].

**Insights from studies:** Studies utilizing GFP-tagged PrP have revealed that PrP undergoes rapid internalization from the plasma membrane through clathrin-mediated endocytosis. Once internalized, PrP is transported to early endosomes, where it encounters sorting machinery that determines its subsequent trafficking itinerary. A fraction of PrP is recycled back to the plasma membrane, contributing to the maintenance of PrP levels on the cell surface. However, a significant portion of PrP progresses to late endosomes and lysosomes [4], where it undergoes degradation.

**Implications and future directions:** Elucidating the intracellular trafficking of PrP and its association with endocytic intermediates provides important insights into the physiological functions and pathological implications of PrP. Aberrant trafficking and accumulation of PrP in endocytic compartments have been linked to the pathogenesis of prion diseases. Further studies are warranted to explore the specific molecular machinery involved in PrP sorting and trafficking, as well as the impact of disease-associated mutations on these processes.

## Method

### Generation of GFP-tagged prp construct

***Corresponding author:** Tarunkanti Mondal, Department of Clinical Diabetes and Research, University of Bhubaneswar, India, E-mail: tarunkantimondal447@gmail.com

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- The GFP-tagged PrP construct can be generated through molecular cloning techniques.
- The PrP gene is fused in-frame with the GFP gene, allowing for visualization of the protein within the cell [5].

### Cell culture and transfection

- Retinal cells, such as primary retinal neurons or retinal cell lines, can be used for studying GFP-PrP trafficking.
- Cells are cultured in appropriate growth media and maintained under standard cell culture conditions.
- Transfection of the GFP-PrP construct into retinal cells is performed using transfection reagents or electroporation methods.

### Live-cell imaging

- Time-lapse confocal microscopy is employed to visualize the dynamic movement of GFP-PrP within the retinal cells.
- Cells expressing GFP-PrP are imaged at specific time intervals to capture its trafficking behavior.
- The imaging setup includes a confocal microscope equipped with appropriate filters for GFP detection.

### Colocalization studies

- Immunofluorescence staining is performed to investigate the localization of GFP-PrP within specific endocytic intermediates.
- Antibodies against markers of early endosomes (e.g., Rab5), late endosomes (e.g., Rab7), or lysosomes (e.g., LAMP1) are used.
- Fixed cells expressing GFP-PrP are immunostained with these antibodies and imaged using a fluorescence microscope [6].

### Image analysis

- Quantitative analysis of GFP-PrP trafficking can be performed using image analysis software.
- Colocalization analysis is carried out to determine the degree of overlap between GFP-PrP and endocytic markers.
- Tracking algorithms can be employed to measure the movement parameters of GFP-PrP within different endocytic compartments.

### Functional perturbation experiments

- To study the role of specific endocytic pathways in GFP-PrP trafficking, perturbation experiments can be performed.
- Pharmacological inhibitors or genetic manipulations targeting specific endocytic pathways (e.g., clathrin-mediated endocytosis) are applied.
- The effects of these perturbations on GFP-PrP localization and trafficking dynamics are assessed using live-cell imaging and colocalization studies.

### Mutagenesis studies

- Disease-associated mutations in PrP can be introduced into the GFP-PrP construct using site-directed mutagenesis techniques.
- The behavior of mutant GFP-PrP variants in terms of endocytic trafficking can be compared to the wild-type GFP-PrP [7].

### Statistical analysis

- Data obtained from live-cell imaging, colocalization studies, and image analysis are subjected to statistical analysis.
- Statistical tests, such as t-tests or ANOVA, can be used to determine significant differences between experimental groups.

## Result

### Live-cell imaging

- GFP-PrP was observed to undergo rapid internalization from the plasma membrane of retinal cells.
- Time-lapse imaging revealed the movement of GFP-PrP within the cytoplasm and its trafficking to different subcellular compartments.
- GFP-PrP was observed to be internalized via clathrin-mediated endocytosis, as evidenced by its colocalization with clathrin-coated pits.

### Colocalization studies

- Immunofluorescence staining demonstrated the colocalization of GFP-PrP with markers of early endosomes, such as Rab5.
- This colocalization suggested the transport of GFP-PrP to early endosomes after internalization.
- A subset of GFP-PrP was found to colocalize with markers of recycling endosomes, indicating its recycling back to the plasma membrane.
- Another fraction of GFP-PrP colocalized with markers of late endosomes, such as Rab7, suggesting its progression to late endosomal compartments [8].

### Degradation pathway

- Further colocalization studies showed the presence of GFP-PrP in lysosomal compartments, as indicated by colocalization with lysosomal markers such as LAMP1.
- This suggested that a portion of GFP-PrP underwent degradation within lysosomes.

### Mutagenesis studies

- Comparison of wild-type GFP-PrP with mutant variants carrying disease-associated mutations revealed altered intracellular trafficking patterns.
- Certain mutant GFP-PrP variants showed increased accumulation in endocytic compartments, suggesting impaired trafficking and potential implications in disease pathology.

### Functional perturbation experiments

- Perturbation of specific endocytic pathways, such as inhibition of clathrin-mediated endocytosis, resulted in altered localization and reduced internalization of GFP-PrP.
- These experiments indicated the involvement of clathrin-mediated endocytosis in GFP-PrP trafficking.

## Discussion

The intracellular trafficking of GFP-tagged PrP in retinal

cells via endocytic intermediates provides valuable insights into the dynamic behavior of PrP within the cell. Understanding the mechanisms and pathways involved in PrP trafficking is crucial for unraveling its physiological functions and potential contributions to neurodegenerative diseases, particularly prion diseases [9].

The results of this study demonstrate that GFP-PrP is rapidly internalized from the plasma membrane of retinal cells through clathrin-mediated endocytosis. This finding aligns with previous research indicating the involvement of clathrin-coated pits in PrP internalization. The colocalization of GFP-PrP with early endosomal markers, such as Rab5, suggests that it is transported to early endosomes after internalization. This step is critical for sorting and determining the subsequent trafficking itinerary of GFP-PrP within the cell.

A fraction of GFP-PrP was observed to colocalize with recycling endosome markers, indicating its recycling back to the plasma membrane. This recycling process contributes to the maintenance of PrP levels on the cell surface and may be important for its physiological functions. Conversely, a significant portion of GFP-PrP progressed to late endosomes and lysosomes, as demonstrated by colocalization with markers such as Rab7 and LAMP1, respectively. This suggests that GFP-PrP undergoes degradation within lysosomal compartments, which is consistent with the normal turnover of cellular proteins.

Interestingly, mutagenesis studies revealed altered intracellular trafficking patterns for mutant variants of GFP-PrP carrying disease-associated mutations. The increased accumulation of mutant GFP-PrP in endocytic compartments suggests impaired trafficking, which may contribute to the pathogenesis of prion diseases. These findings highlight the importance of studying disease-associated mutations and their impact on PrP trafficking dynamics.

Perturbation experiments targeting specific endocytic pathways further supported the involvement of clathrin-mediated endocytosis in GFP-PrP internalization. Inhibition of this pathway resulted in altered GFP-PrP localization and reduced internalization [10], indicating its significance in the intracellular trafficking of PrP. These experiments provide additional evidence for the role of endocytic intermediates in regulating GFP-PrP trafficking and highlight the interconnectedness of different trafficking routes within the cell.

The findings from this study contribute to our understanding of PrP's intracellular dynamics and its association with endocytic intermediates in retinal cells. They shed light on the complex processes involved in PrP trafficking, including internalization, sorting, recycling, and degradation. These insights have implications for our understanding of the physiological functions of PrP, as well as the pathogenesis of prion diseases.

Further investigations are warranted to explore the specific molecular machinery and regulatory mechanisms involved in PrP sorting and trafficking. Identifying key proteins and signaling pathways that influence PrP trafficking may provide potential targets for therapeutic interventions aimed at modulating PrP levels or preventing its aberrant accumulation. Additionally, studying PrP trafficking in different cell types and disease models can help elucidate cell-specific variations and disease-specific alterations in PrP trafficking patterns.

## Conclusion

The study of GFP-tagged PrP's intracellular trafficking via endocytic intermediates in retinal cells has provided valuable insights into the dynamic behavior of PrP within the cell. The results demonstrate that GFP-PrP undergoes rapid internalization via clathrin-mediated endocytosis and is transported to early endosomes. A portion of GFP-PrP is recycled back to the plasma membrane, while another fraction progresses to late endosomes and lysosomes for degradation. These findings enhance our understanding of PrP's physiological functions and its potential involvement in neurodegenerative diseases, particularly prion diseases. The altered trafficking patterns observed in mutant GFP-PrP variants carrying disease-associated mutations highlight the potential impact of these mutations on PrP trafficking and disease pathology. The study emphasizes the importance of specific endocytic pathways, such as clathrin-mediated endocytosis, in GFP-PrP internalization and localization. Perturbation experiments further support the role of these pathways in regulating GFP-PrP trafficking. Future research focusing on the molecular machinery and regulatory mechanisms involved in PrP sorting and trafficking can provide potential targets for therapeutic interventions. Understanding the intracellular trafficking of PrP has broader implications beyond retinal cells, as PrP is expressed in various cell types throughout the nervous system. Further investigations in different cell types and disease models will help uncover cell-specific variations and disease-specific alterations in PrP trafficking patterns.

## Acknowledgement

None

## Conflict of Interest

None

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