

Abstract

Basement Membranes (BM) are important for normal development and tumor progression. In order to get a better understanding of BM dynamics we identified genes that encoded BM interacting proteins. One such gene is predicted to be involved in vesicle-mediated transport in *Drosophila melanogaster*. Here we characterize this gene by utilizing molecular biology techniques like immunohistochemistry, RNA in situ hybridization, and Western blot analysis utilizing antibodies generated in the laboratory. Western blot analysis identified this protein to be ~30.8 Kilo Daltons in size. Anti-body staining indicates tissue and cell specific localization pattern for this protein. This pattern is similar to RNA in situ hybridization pattern observed in various tissues. Data related to this proteins' involvement in vesicle-mediated transport will be presented.

Introduction

Surfeit locus protein 4 is hypothesized to be involved in vesicle mediated transport. Here we show observations that were made to provide proof for the localization of *surf4*. Extraction of this protein from 3rd instar larvae of *Drosophila melanogaster* to identify its size compared to the proteome of the animal. This protein is studied, because of the potential pathways in which this coding gene can be involved in. For example, its connection with the proteins between Endoplasmic reticulum and Golgi apparatus can bear a lot of data.

Methods & Materials

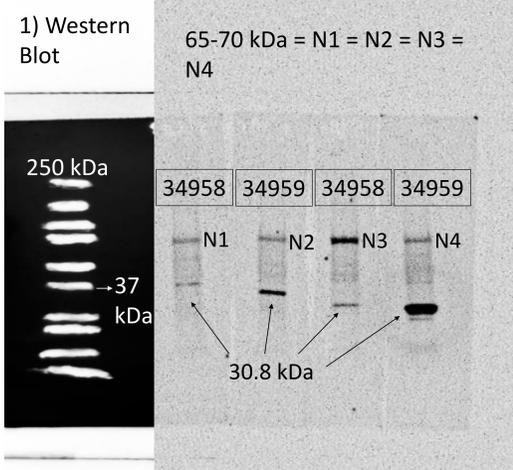
1) Western Blot: The protein extract from larvae was run through polyacrylamide gel and the protein fragments were separated by size using 120 volts of electric potential difference for 1 hour and 20 minutes. The separated proteins were transferred to a sheet of membrane and the membrane was washed with antibodies against *surf4*, so that the antibody binds *surf4*, confirming the presence of the protein product amongst the proteome of *Drosophila melanogaster*. The signal picked up by the primary antibody (1:2000) was visualized by the use of secondary antibodies(1:20000).

2) Anti-body Staining: In this experiment, developed anti-bodies against *surf4* protein were spread across the larval tissues of *Drosophila melanogaster* after fixation and a series of washing steps with PBTA to be able to detect *surf4* proteins in tissues if present. After treating the tissues with the primary anti-body (1:500 and 1:800), a green fluorescing secondary anti-body (1:500 and 1:800) was attached to the primary antibody for the detection of *surf4*.

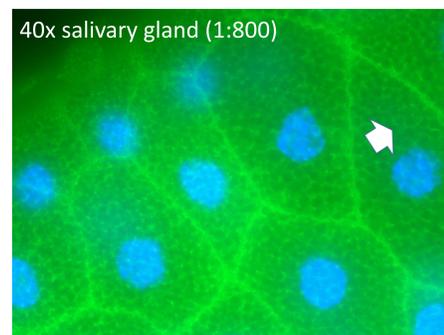
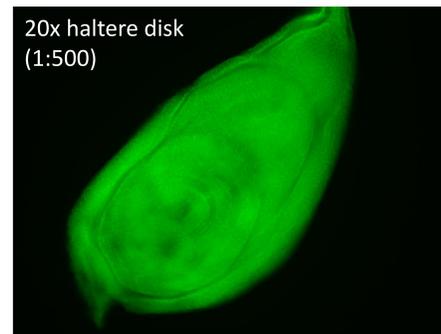
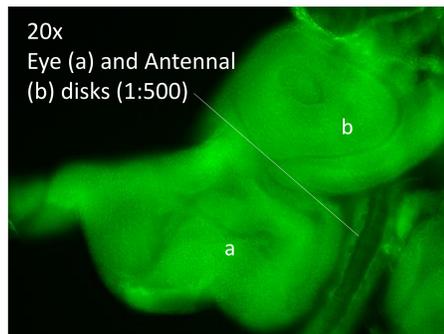
3) RNA in Situ Hybridization: *Surf4* RNA sequence was labeled with digoxigenin and sent through the larval tissues till the labeled RNA binds the complementary mRNA in the nucleus of the cell. Afterwards the labeled RNA was reacted with Anti-Digoxigenin-Alkaline phosphatase to create a purple color indicating the presence of targeted mRNA. The Anti-DIG-AP was used to detect digoxigenin.

Results

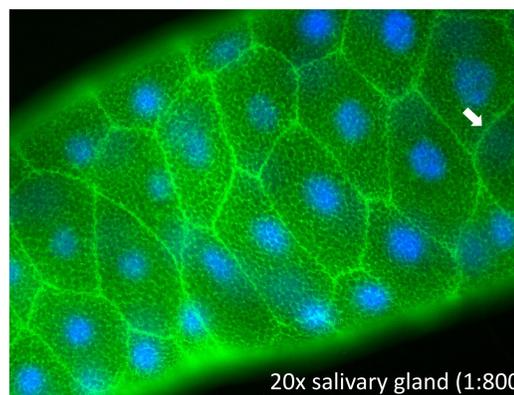
1) Western Blot: The experiment detected the *surf4* protein and with the highest signal seen in sample 34959 from rabbit number 2. non-specific band was detected at about 70 kDa. We intend to repeat the experiment with purified antibodies to eliminate any non-specific effects.



2) Antibody Staining



2) Anti-body Staining: The green color indicates the localization of Surf4 in the larval tissues of *Drosophila melanogaster*. This protein was expressed in the majority of tissues. One specific thing about the expression of this protein is that it is expressed in almost all of the cells of tissues. The blue color indicates the nucleus of the cells. *Surf4* protein is highly expressed in the phospholipid bilayer of the cells of the salivary gland proving the high activity of this protein in the membrane.



Additionally, it is also expressed in internal membranes (shown with thick arrowhead).

Results: Continued

3) RNA in Situ Hybridization: The Anti-DIG-AP was able to specifically detect digoxigenin labeled RNA sequences of *surf4*. the results of the experiment mimicked that of antibody staining results confirming each other's expected results. This experiment needs to be repeated to confirm this pattern of expression. Also, we need to look at the dark stains on the tissues and troubleshoot the procedures to make sure those dark spots are not contamination but specific staining of tissues.

For the stain to be developed: the *surf4* RNA infested tissues labeled with digoxigenin were washed with anti-body developed against diigoxigenin for 60 minutes in darkness. The images then were taken using bright filed microscopy.



Conclusion

Surf4 was successfully detected at the plasma membrane of the salivary gland tissue, which increases the possibility that *Surf4* protein is involved in the vesicle mediated transport of the Endoplasmic Reticulum and Golgi apparatus. The size of this protein was identified as 30.8 kDa. This information can be used in future experiments that include mutations in this protein to deeper explore the known history of this protein. Both immunohistochemistry and RNA in situ hybridization indicated that the *surf4* protein and mRNA is localized to tissues including salivary gland, wing disk, eye and antennal disk, leg disks, haltere disks, brain tissue, and the adipose tissue.

Acknowledgements

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References

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