Characterization of a Membrane-Bound Insect Transferrin

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Abstract

Transferrins are extracellular proteins that bind iron. Vertebrate transferrins have well-characterized roles in iron transport and immunity, but the functions of transferrins in most other animals are poorly understood. The goals of this study are to identify the functions of transferrin-3 (Tsf3) from *Drosophila melanogaster* (fruit fly), and to determine whether Tsf3 is conserved in other species of insects. Our experimental approach has been to predict important features of the protein, analyze gene expression, perform RNAi-mediated knockdown in cultured cells, and use phylogenetic analysis to identify Tsf3 orthologs. An analysis of the Tsf3 amino acid sequence predicts that Tsf3 is extracellular and anchored to the plasma membrane. Putative iron-binding residues are present in the carboxyl-lobe, but iron-binding residues appear to be lacking in the amino-lobe; therefore, we predict that only the carboxyl-lobe binds iron. Tsf3 is expressed at a low level throughout development in many tissues, but it is very highly expressed in prepupal salivary glands. Cells of the prepupal salivary glands are exceptional because of their extensive DNA replication and because they synthesize large amounts of glue proteins that are used by the insect to attach itself to a solid surface prior to pupation. High expression of Tsf3 in the prepupal salivary glands suggests that Tsf3 may participate in the uptake of iron in these glands, possibly to be used as a cofactor for enzymes required for DNA synthesis, or perhaps to be used in the glue biosynthesis process. To test the hypothesis that Tsf3 is used for iron uptake by insect cells, we are in the process of evaluating the effect of RNAi-mediated knockdown of Tsf3 on cellular iron content. Toward this goal, we verified that Tsf3 is expressed in cultured insect cells, synthesized dsRNA that targets Tsf3, and optimized a method to measure cellular iron content. Finally, we did a phylogenetic analysis of insect transferrins and identified orthologs of Tsf3 in insects from 10 different orders. Our findings demonstrate that Tsf3 is likely to be an extracellular, membrane-bound, iron-binding protein that may be involved in cellular iron uptake in diverse species of insects.

Transferrin 3 Amino Acid Sequence

Prediction of the signal peptide, transmembrane region, and iron binding residues.

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>DmTsf3
MQWLTLILVGLFASFLSISMCVAVQPPDDGKLRVCVVESRGVYRKTPKFCPLLEAKSNIE
CVIGVDRLDCVRRIHKGTAHFGVLTSEDLVAARWASVEILVASELRSHESHFEYEIVAVV
DNHANIHTVHDLRGARLCHPGYGLGNHWTEVLANYFEAAMVSKTCDPEMTVTEDRIASTA
KYFGPSCKAGPWVPDPKQDRILKNRYPSLCEMCYEPDSCDQTDKHWGRRGALYCLTSGGG
NVAWARLDDVRSHFGFSGIPAQSNPSDFSYLCPDGHLQPLNASQPCVWVAKPWPVVAARR
SHAAQVQRLVTGLNHDEPDSWQNALLSLLETYHVFTVPLDNVIAIDDYLDQATAFQSAYS
FPECNPPRSIVFCTTSIIQHIKCSWLQEASQVYGVQPNIQCVRTMDEQQCLDNTKFKETD
VVLVDQEMRVKAQRDYNLVPLLYEFAADMHDRYVTIALVHKDAKFESFRDLKGARACLPS
FEGAAHLSVQETIVNATGKVQSLHSYFHRDSCLWNLQSGRKCPLHYQGDEGALRCLSEGA
DVAFLSSDVYKKYVVGNLTSNWLTPGNHKDFRVLCPYGGIEKRSNFEYCYLHWTTRGHLM
THNSSLTRRNEIYNSLRDMDQLFGRKYKSETRPFTLYGIFDKRNNVLFRDDTDGLLGLQE
LHRDNAKRVMEHIYDRYANTQYYRNFDESGAMKSSHNLWILLLVCLLFVVGQPFG
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Signal P predicts a signal peptide, in bold, with a cleavage site at CVA-VQ. TMPred predicts a C-terminal transmembrane region, which is HNLWILLVCLLFVVGQPFG. The amino acids highlighted in yellow are predicted to be iron binding.

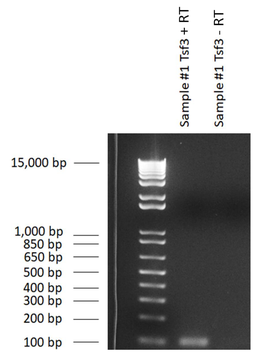
Expression of Transferrin 3

This graph from Flybase shows the expression of Tsf3 in different tissues, during different developmental stages of *D. melanogaster*.

- Tsf3 is expressed in many different tissues of *D. melanogaster.*
- Tsf3 is expressed through all the developmental stages of *D. melanogaster.*
- This suggests that Tsf3 expression is consistent with iron uptake throughout *D. melanogaster.*
- White prepupae have the highest expression out of all stages in development.
- The salivary gland in white prepupae has the highest expression when compared to other tissues in *D. melanogaster.*
- At the white prepupae stage large amounts of glue proteins are being produced. If this process requires iron, Tsf3 may help provide it.

Sg4 Cells Express Transferrin 3

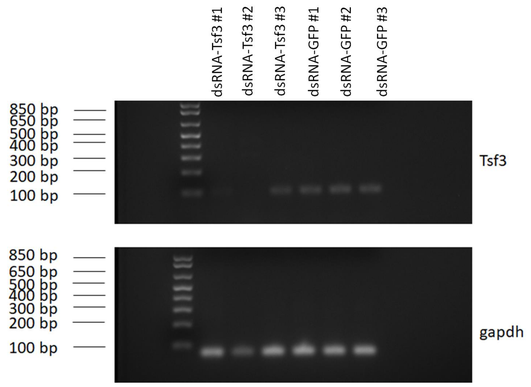
We want to study the function of Tsf3 in cultured *D. melanogaster* cells. We did RNA isolation, cDNA synthesis, PCR and agarose gel electrophoresis to verify that the Sg4 cells express Tsf3.



The sample with reverse transcriptase and Tsf3 primers showed a band at the expected size of 103 bp. The comparable sample without reverse transcriptase did not have a band at a larger size (372 bp), which confirmed that there was no genomic DNA contamination in the samples. These results demonstrate that Sg4 cells express Tsf3.

Knockdown of Transferrin 3

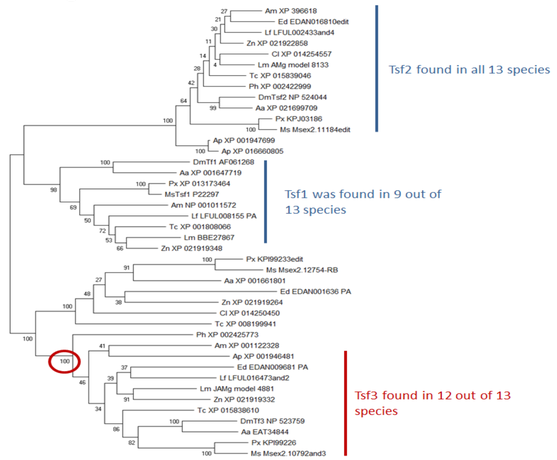
We want to study the effect of insufficient Tsf3 in the Sg4 cells. We did RNA interference, RNA isolation, cDNA synthesis, ran PCR and ran an agarose gel to determine if Tsf3 was knocked down successfully. Gapdh was used as the reference gene.



Two out of three samples treated with dsRNA-Tsf3 had strong knockdown and one sample had weak knockdown suggesting that knockdown efficiency varies between each sample.

Phylogenetic Analysis of Insect Transferrins

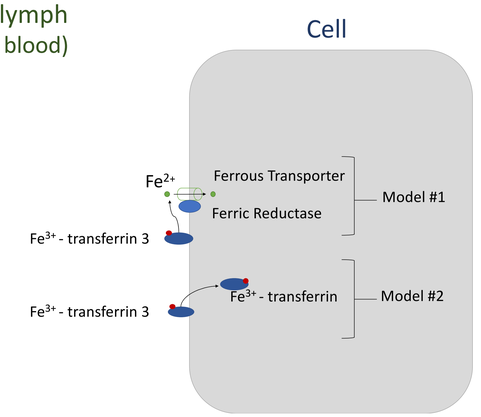
We wanted to determine if other insects had Tsf3 orthologs.



The gene cluster that contains DmTsf3 includes a sequence from all but one of the insect species analyzed. This cluster is supported by a bootstrap value of 100; therefore, it is likely that most species of insects contain a Tsf3 ortholog. Orthologous proteins tend to have the same biological function; therefore, anything we learn about the function DmTsf3 has a high possibility that it applies to other insects as well.

Possible Iron Uptake Pathways

This diagram shows two possible pathways that insect cells may use to uptake the iron bound to Tsf3. Model #1 shows the reduction of ferric ions bound to Tsf3 into ferrous iron, which is then transported into the cell through a ferrous transporter. Model #2 shows full uptake of the iron-bound Tsf3.



Conclusions

- Tsf3 is predicted to be an extracellular membrane-bound protein that can bind to iron.
- The salivary glands in the white prepupae have the highest expression of Tsf3 suggesting that transferrin 3 maybe involved in the uptake of iron in these glands.
- RNAi-mediated knockdown can successfully knockdown Tsf3 in Sg4 cells.
- Many insect species have a Tsf3 ortholog, which suggest anything we discover about Tsf3 in *D. melanogaster* may apply to other insects.

Future Research

- We will be performing a ferrozine-based assay to determine if knockdown of Tsf3 has an effect on iron concentration in Sg4 cells.
- If results from the ferrozine-based assay are positive, the next step would be to determine whether model #1 or model #2 is involved in the uptake of iron bound to Tsf3.

Acknowledgements

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