

## Biochemistry I (MEDS 350)

Discussion/tutorial, Wednesday, November 9, 2005

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Hoashi, T, Watabe, H., Muller, J. Yamaguchi, Y., Viera, W.D., Hearing, V.J. (2005) J Biol. Chem. 280, 14006-14016, MART-1 Is Required for the Function of the Melanosomal Matrix Protein PMEL17/GP100 and the Maturation of Melanosomes

The authors of this paper faced a common problem: a gene without a clear function. They utilize a number of different approaches to try to figure out what MART-1 does. Each of you will specify a figure for which you want to prepare, focusing on the methods used, the quality of the data shown and the validity of the conclusion drawn. On Wednesday, you will be responsible for leading the discussion of the figure you selected.

Things to think about as you read this paper:

Background on melanosomes – what they are? what is melanin? Was this a problem worth studying?

Fig.1 – Focus on antibody specificity, subcellular fractionation and metabolic labeling

Fig.2 – Are you convinced that Pmel17 and MART-1 form a complex early in the secretory pathway?

Fig.3 – Do the immunofluorescence images add anything to the story?

Fig.4 – What conclusions are justified based on this figure?

Fig.5 – Does the rescue experiment presented make a strong case for what MART-1 is doing?

Fig.6 – What are we seeing in this figure? A picture of a normal melanosome would help.

Fig.7 – Immunofluorescence localization - needed?

Fig.8 – What does expression of MART-1 siRNA do to cells?

Fig.9 – Could this experiment be done without radioactively labeled amino acids.

Are any of the conclusions over-stated? What should be done next?