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Michelle Coca

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Pilot Studies of Two Possible Iron Uptake Mechanisms in Insect Cells

Michelle E. Coca, Diana G. Najera, Maureen J. Gorman

Department of Biochemistry and Molecular Biophysics

Kansas State University

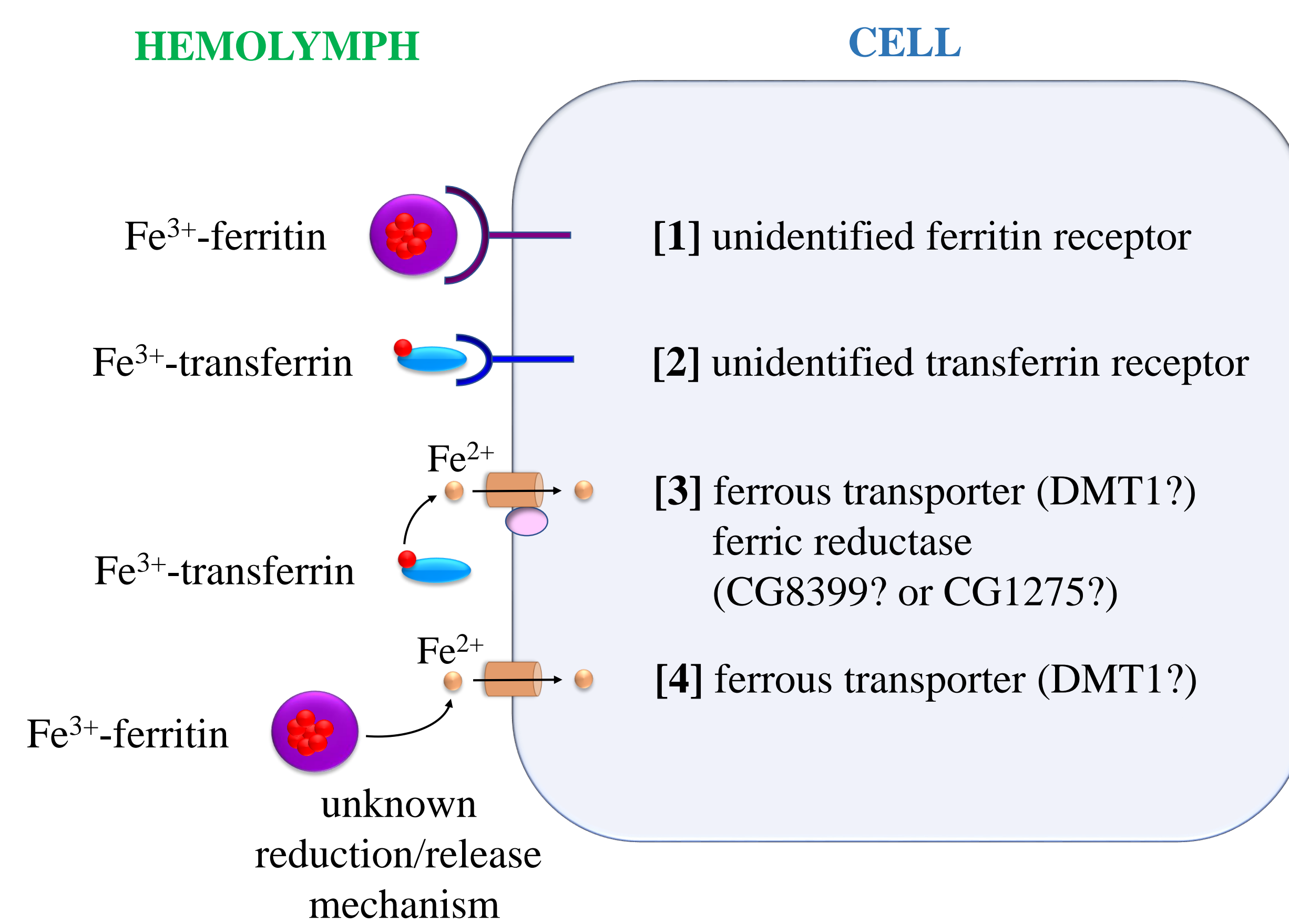
Developing Scholars Program



Background

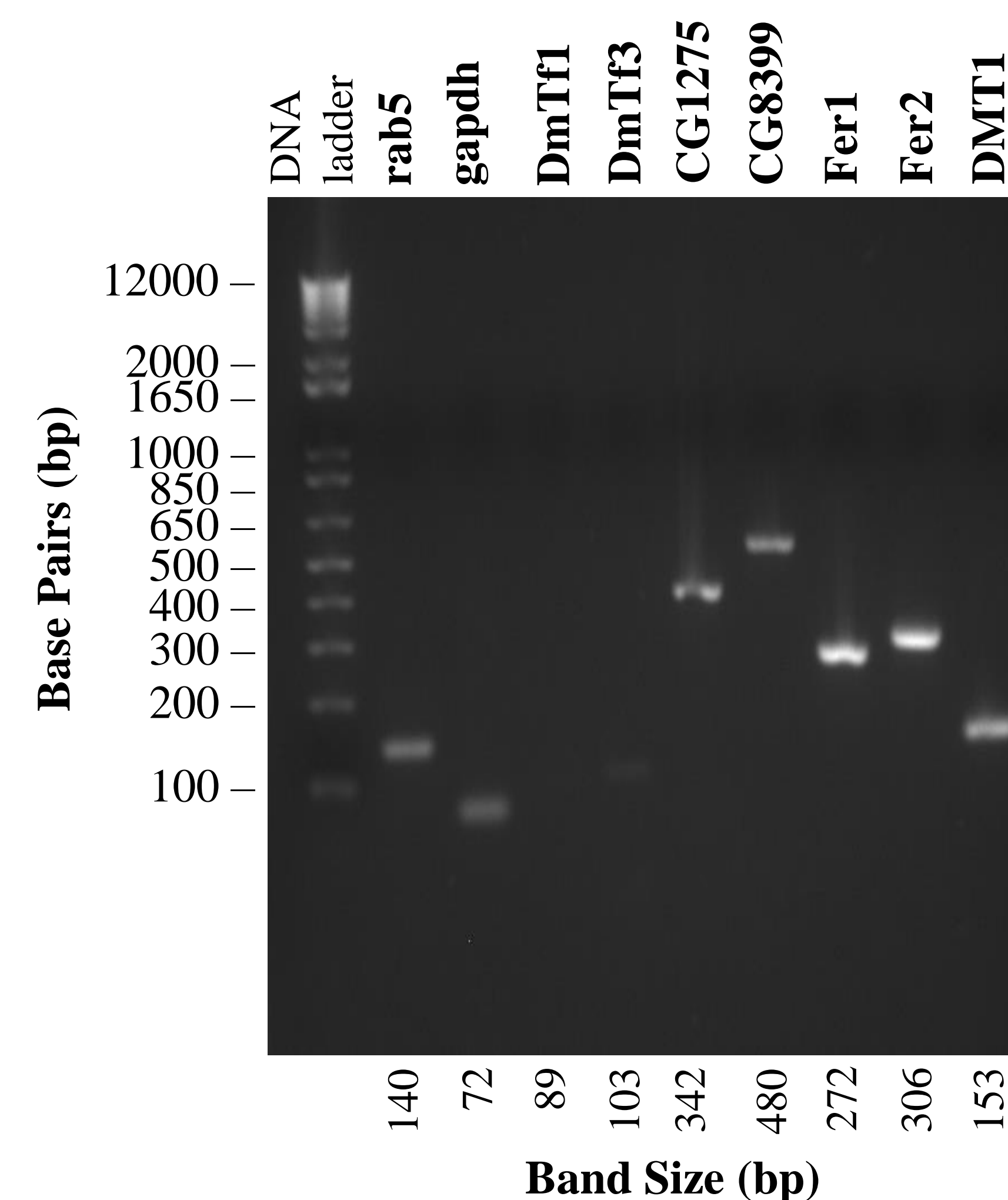
Iron is an essential nutrient in all organisms because it plays an important role in many biological processes. In insects, iron is involved in many processes including energy metabolism, detoxification of pesticides and plant defense compounds, and geomagnetic orientation. However, just as it is important, iron can also be toxic. It is for this reason that its uptake must be strictly regulated. Unlike in insects, in mammals there is a well-understood pathway for iron transport. It involves the transport of iron from cell to cell by transferrin, a protein that binds up to two ferric ions. Transferrin is then taken in by another cell through receptor-mediated endocytosis. However, in insects, transferrin may play a very small role. The majority of iron is transported out of the cell by ferritin, a protein that can store high amounts of iron. Since there are no identified ferritin or transferrin receptors in insect cells, it is not known how iron enters the cell.

Models of Iron Uptake



Iron-related Gene Expression in Sg4 Cells

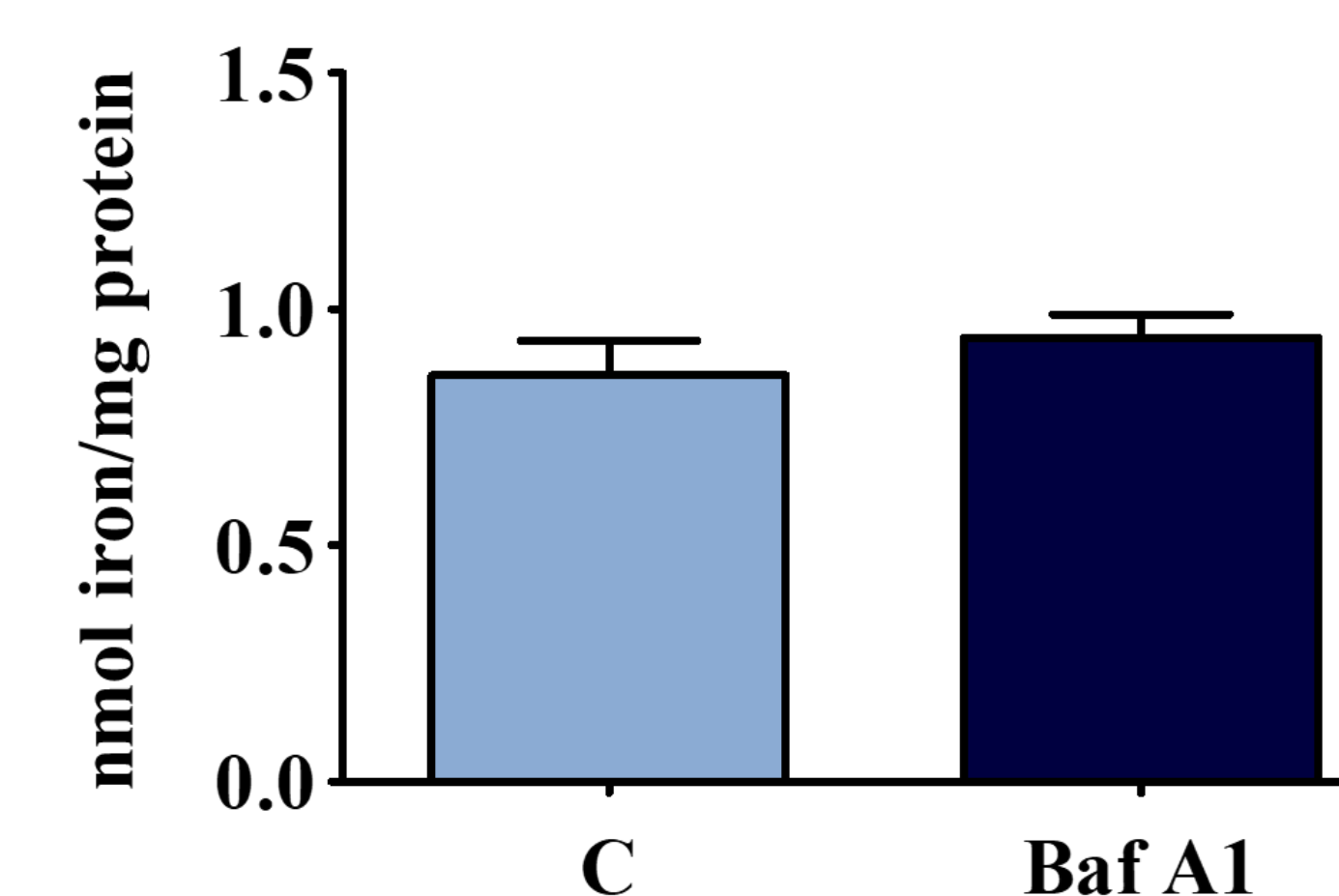
- ▷ PCR was performed to determine if Sg4 cells express rab5, Transferrin 1, Transferrin 3, two ferric reductases (CG1275 and CG8399), Ferritin 1, Ferritin 2, and Divalent Metal Transporter 1 (DMT1).
- ▷ The results suggest that Sg4 cells express all the iron-related genes we tested except Transferrin 1. Expression for Transferrin 3 was very low.
- ▷ All PCR products were the expected size.



Endocytosis Inhibitor Did Not Block Iron Uptake

- ▷ A pilot study showed that treating Sg4 cells growing in Express Five medium with 8 nM of BafA1 for 72 hours does not block iron uptake.
- ▷ Sg4 cells growing in Schneider's medium supplemented with 10% fetal bovine serum were treated with either 8 nM of Baf A1 or with DMSO (control) for 72 hours.
- ▷ Cellular iron content was measured using a Ferrozine-based assay.
- ▷ The results showed that treating Sg4 cells with BafA1 had no effect on cellular iron content (measured as nmol iron per mg protein).

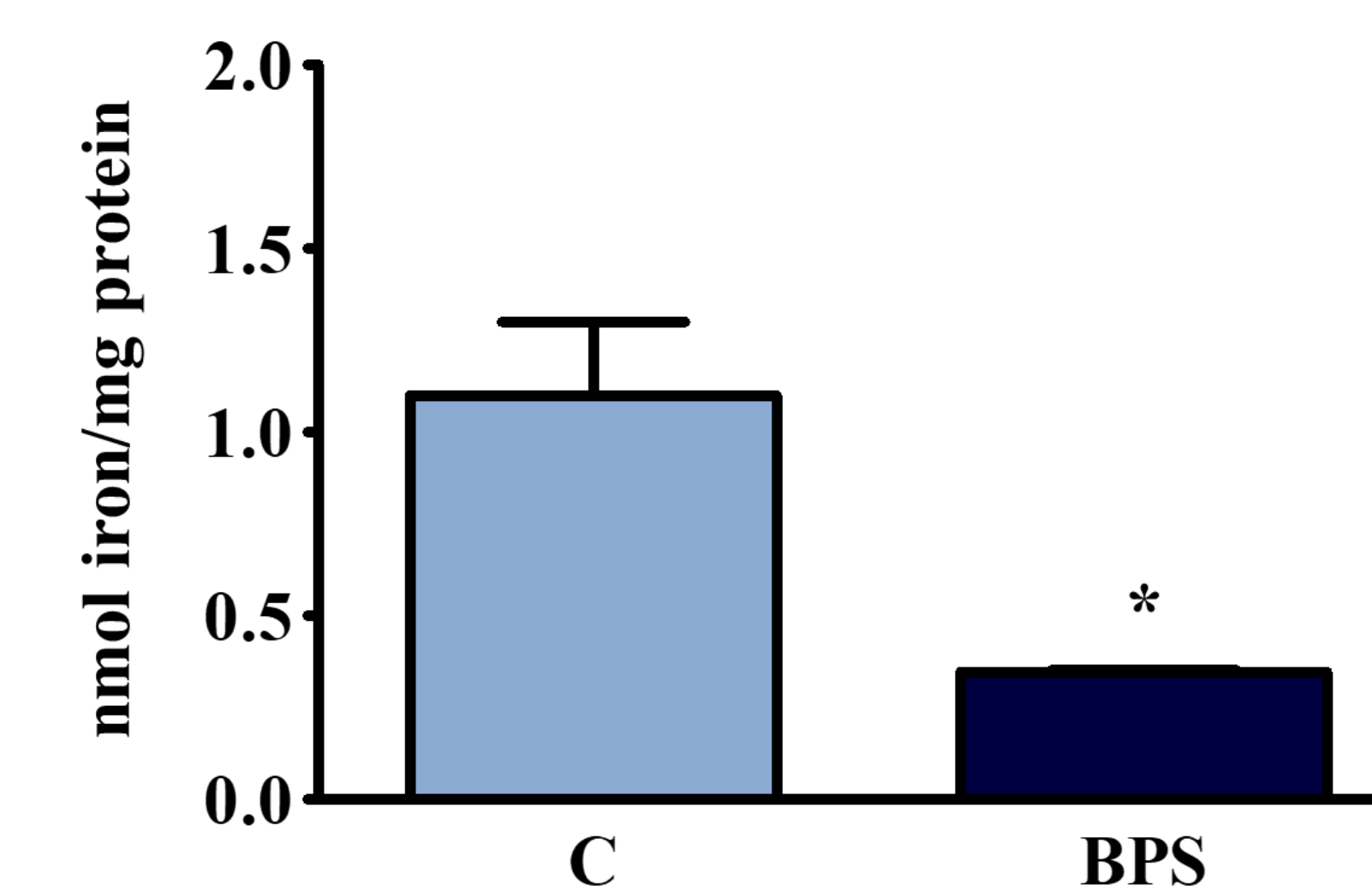
Effect of Endocytosis Inhibitor on Iron Content



Ferrous Chelator Blocked Iron Uptake

- ▷ Sg4 cells growing in Schneider's medium supplemented with 10% fetal bovine serum were treated with either 40 μM of BPS or sterile water (control) for 96 hours to determine if the ferrous form of iron is involved in iron uptake.
- ▷ Cellular iron content was measured using a Ferrozine-based assay.
- ▷ The results showed a significant decrease in cellular iron content (measured as nmol iron per mg protein) in cells treated with BPS compared to control cells.

Effect of Ferrous Chelator on Iron Content



Objective

- The purpose of this project was to test four models of iron uptake by cultured Sg4 cells, an isolate of the *Drosophila melanogaster* S2 cell line.
- ▷ **Models [1] and [2]** involve endocytic uptake of protein-bound iron. To test these models, cells were treated with Bafilomycin A1 (Baf A1), an endocytosis inhibitor, and the effect on cellular iron content was measured.
 - ▷ **Models [3] and [4]** involve iron uptake through a ferrous transporter. To test these models, cells were treated with bathophenanthrolinedisulfonic acid (BPS), a ferrous chelator, and the effect on cellular iron content was measured.
- The results of this project could lead to better insect control strategies and provide insight into less understood iron uptake mechanisms of mammals.

Summary

- ▷ Sg4 cells express rab5, ferritin, two ferric reductases and DMT1. Lack of transferrin expression suggests models 2 and 3 are unlikely iron uptake mechanisms, while expression of ferritin supports models 1 and 4.
- ▷ Treating cells with Bafilomycin A1, an endocytosis inhibitor, had no effect on cellular iron content, suggesting that endocytosis is not involved.
- ▷ Alternative explanations: endocytosis was not blocked by Baf A1 or another pathway compensated for the lack of endocytosis.
- ▷ Treating cells with BPS, a ferrous chelator, decreases cellular iron content, indicating that the ferrous form of iron is involved in the uptake process and a ferrous transporter may be involved.

Future Research

- ▷ Knock down DMT1 by RNA interference to determine if iron uptake is blocked.
- ▷ Design and perform an endocytosis assay to confirm that endocytosis was successfully blocked.

Acknowledgments

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