Cell-Specific Localization of *CAPA* mRNA in the Central Nervous System and Midgut of the Mosquito, *Aedes aegypti*

Christine Paputsis and Jean-Paul Paluzzi

Department of Biology, York University, ON, Canada. Email: paputsis@my.yorku.ca

ABSTRACT

Using a hybrid experimental approach combining fluorescence *in situ* hybridization and immunohistochemistry using fluorescent probes, we aimed to localize neuroendocrine cells in the central nervous system as well as prospective endocrine cells in the midgut that produce *CAPA* gene products in the disease vector, *Aedes aegypti*. In agreement with the expression pattern of the *CAPA* gene in other insects, we found a medially localized pair of CAPA-producing cells in the first five abdominal ganglia, and in addition, an anterolateral cell pair in the terminal abdominal ganglia. In light of previous peptidomic studies that detected CAPA peptides associated with midgut tissue extracts, *CAPA* mRNA was not detected in the midgut suggesting that CAPA peptides are not produced here. Our findings provide a powerful technique for future studies to visualize the spatial expression patterns of neuropeptide transcripts in neuronal as well as other cell and tissue types.

INTRODUCTION

Blood-feeding insects face an array of challenges following blood meal engorgement including the control of water and ion titres in their haemolymph to maintain homeostasis. A model organism used in studying the hormonal regulation of hydromineral balance is the medically-important mosquito, *Aedes aegypti,* which is a vector of several human diseases including Zika, dengue and yellow fevers. In this mosquito and other dipterans, the *CAPA* gene encodes three peptides whose functions include roles in osmoregulation[1,2].

Previous studies using peptidomic approaches and immunohistochemistry in *A. aegypti* have incompletely determined the spatial expression of CAPA-like peptides in the central nervous system and midgut[3]. Moreover, no studies have yet elucidated the cell-specific localization of *CAPA* gene expression. Based on the aforementioned findings and studies that have localized *CAPA* mRNA in the central nervous system of other insects[2,4,5], we hypothesized that a single medial pair of neuroendocrine cells express the *CAPA* gene in each abdominal ganglion. We also hypothesized that endocrine cells in the midgut also express the *CAPA* gene based on previous immunohistochemistry studies and peptidomic analyses.

METHODOLOGIES

Dissections

- Adult mosquitoes (<1 week post-emergence) were collected and the ventral nerve cord and midgut were dissected in nuclease-free PBS

Fluorescence *in situ* hybridization

- Anti-sense and sense probes specific for the *A. aegypti* CAPA mRNA were prepared using linearized recombinant plasmids as templates
- Anti-sense (experimental) and sense (control) probes were hybridized to the dissected tissues using a FISH procedure described earlier[6]

Immunohistochemistry

- A rabbit anti-RhoprCAPA2 (EGGFISFPRV-NH_2) polyclonal antibody was used to detect CAPA-like (or FPRV-NH_2-like) immunoreactivity
- FITC- or Cy3-labeled secondary antibodies were used to visualize immunoreactive staining

RESULTS

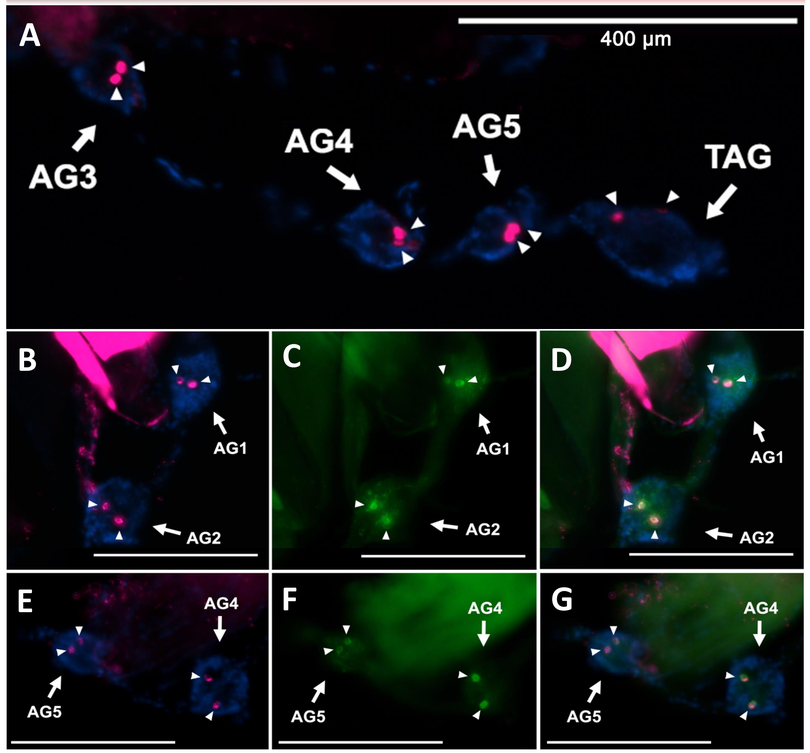

Figure 1. CAPA transcript expression (pink) and FPRV-amide immunoreactivity (green) in the abdominal ganglia (AG) of adult *A. aegypti* (**A**), including the terminal abdominal ganglion (TAG). The arrowheads point to two strongly stained medial cells that are present in the first five AG and one lateral cell pair in the TAG. The stained cell pair in each abdominal ganglion identified by the RNA probe (**B, E**) and anti-FPRV antibody (**C, F**) colocalize (**F**, **G**). Scale bars correspond to 200 µm.

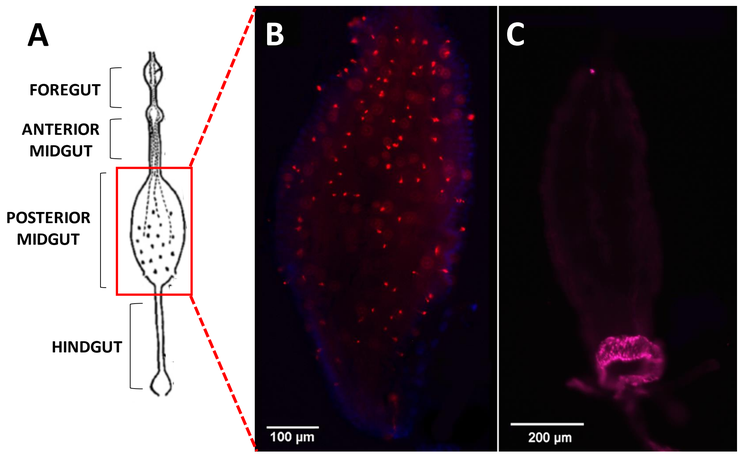

Figure 2. Schematic of the alimentary canal (**A**), CAPA-like immunolocalization (**B**) and CAPA transcript expression (**C**) in the adult *A. aegypti* posterior midgut. An abundance of CAPA-like immunoreactivity is found in midgut endocrine cells, whereas the *in situ* hybridization probe failed to detect any specific cells, indicating that the endocrine cells do not produce CAPA peptides but instead a structurally related peptide.

CONCLUSIONS

Our results confirm that a medial cell pair in each abdominal ganglion produces CAPA peptides, aside from the terminal ganglion, which contains a pair of anterolateral CAPA-producing cells. While CAPA-like immunostaining was detected in the posterior midgut, we did not find evidence of CAPA transcripts in the midgut, suggesting that midgut endocrine cells do not produce CAPA peptides. With an improved understanding of the neurobiology and endocrine system in *A. aegypti*, enhanced strategies to better manage this important disease vector may be realized.

REFERENCES

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