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HINDGUT EPITHELIAL ION TRANPORTERS IN *DROSOPHILA MELANOGASTER*

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Drosophila melanogaster is used as a model for mammalian renal function to study epithelial transport and regulation. Ion transporters in the Malpighian (renal) tubules are well studied and characterized. The hindgut is also an epithelial tissue in the fly renal system that is involved in water and ion regulation, but it is not as well studied or understood as the Malpighian tubules. The purpose of these experiments is to test the function of the ion transporters in the hindgut, specifically Inwardly Rectifying Potassium Channel 2 (*Irk2*) and Sodium Chloride Co-transporter 69 (*Ncc69*) in the hindgut.

The UAS-Gal4 system makes tissue specific knockdown possible to test these ion transporters. Gal4 is expressed in specific tissues using endogenous enhancers. The Gal4 then binds to an Upstream Activating Sequence (UAS) which allows the expression of interfering RNA (RNAi), which results in tissue specific knockdown of specific genes. The Hindgut-Gal4 expresses specifically in the hindgut and was used for these experiments.

The experiments conducted consist of an excretory drop assay, a water weight assay, and a 24hr dehydration survival assay. For all experiments the flies are prepared via starvation with access to water for 6 to 7 hours and are then fed blue food overnight. The blue food is either standard fly food or food containing 0.3M NaCl. In the excretory drop assay the flies are moved from the blue food and placed in an empty vial for two hours. Afterwards, the excretory drops on the vials are counted. In the water weight assay the flies are then weighed, baked for 2 days at 60^{o}C, and weighed again. The difference between the total weight and the dry weight divided by the number of flies gives the average water weight per fly. The dehydration survival assay is conducted by moving the flies from the food into empty vials for 24 hours. The living and dead flies are then counted to determine survival.

To test *Irk2* function in the hindgut, 3 independent RNAi lines were used: V108140, V4341, and Trip-Bl. Increased excretion was observed with *Irk2* knockdown in the hindgut using V4341. Decreased survival was observed in the 24-hour dehydration assay after high-salt feeding when *Irk2* was knocked down in the hindgut using V108140 and V4341.

Ncc69 was tested using $Ncc69^{r2}$—a germline (germline (full body) loss-of-function mutation—and two independent RNAi lines: V30000 and V30001. $Ncc69^{r2}$ flies showed a decrease in excretion in the excretory drop assay and a decrease in survival for female flies in the 24-hour dehydration assay. The water weight assay revealed that these flies had less water than control flies, indicating dehydration. The hindgut-specific knockdown of *Ncc69* using RNAi showed a trend towards increased excretion in the excretory drop assay and no significant change in overall survival in the 24-hour dehydration survival assay for both RNAi lines.

In conclusion knocking down *Irk2* in the hindgut has effects on excretion and survival in dehydrating conditions, but further experiments are needed to better define the role of *Irk2* in the hindgut and the relationship between excretory drop and dehydration survival phenotypes. Global loss of *Ncc69* results in decreased water content, variably decreased dehydration survival,

and a decrease in excretion. Decreased excretion may be a compensatory mechanism. *Ncc69* knockdown in the hindgut increases excretion without statistically significant effects on dehydration survival. More experiments are also needed to better determine the role of *Ncc69* in the hindgut.