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Assessing dPrestin & NaDC1 (Indy) Interaction on Calcium Oxalate Crystal Formation in a Drosophila Model of Kidney Stones

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Abstract

Calcium oxalate (CaOx) crystals are one of the most common constituents in kidney stones found and synthesized in the human renal system. While several factors contribute to the aggregation of these stones, elucidating the role of anion transporter activity leads to a better understanding of this phenomenon. Using a *Drosophila* model to study the formation and inhibition of CaOx crystals in the fly malpighian tubule (MT), oxalate transport via **dPrestin**—the fly **Slc26a6 Cl^-/Ox^{2-}** exchanger was studied using both electrophysiology and MT dissection with CaOx birefringence assays. Here, the fly model suffices as it recapitulates renal oxalate function. In addition to dPrestin, the mammalian citrate transporter **NaDC1 (Indy)** was shown to have a protein-protein interaction with Slc26a6 such that oxalate transport is increased above normal [Ohana et. al] and further pursued in this study with the fly system[1]. In order to more faithfully control the perfusion of the fly MT in these studies, a **toner-transfer microfluidic device** was developed to better assess renal function via a variety of genetically encoded pH and voltage sensors. Preliminary results from ex-vivo MT CaOx assays reveal an increase in crystal count with dPrestin and INDY knockdown (RNAi) alone, however statistically insignificant. Electrophysiology experiments demonstrate the co-expression of **dPrestin** and **dINDY** in Xenopus oocytes increase oxalate transport, with possible voltage-dependent activity. **This work investigates the mechanisms of CaOx formation in the renal system via two transporters**. Further work includes developing a fully functional microfluidic platform for assessing the formation of CaOx in a physiologically accurate renal tubule system.

Background

Kidney Stone Formation (Nephrolithiasis) & Drosophila Model:

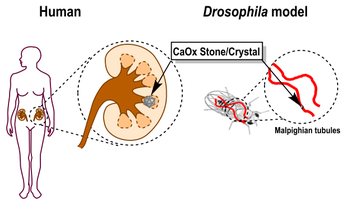


Fig.1: Physiological process of CaOx kidney stone replicated in the fly, along with consensus sequence.

- Rapid CaOx stone formation
- Genetic Manipulation: Use of the UAS/GAL4 promoter/driver with corresponding RNAi of interest

Relevance of NaDC1 (Indy) & Slc26a6 (dPrestin):

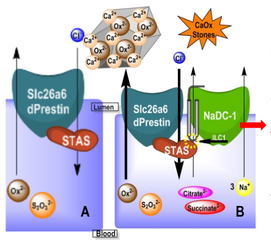


Fig.2: (A) Function of dPrestin alone; (B) Proposed protein-protein interaction between dPrestin and INDY in the fly.

- Indy: Na^+ coupled dicarboxylate transporter
- dPrestin: anion exchanger, channel protein important in renal function→ **Cl^- /oxalate exchanger**
- **Hypothesis:** INDY proposed to have a protein-protein interaction with Slc26a6 such that oxalate transport is increased

Methods

1) Drosophila Genetics: 3 pairs of autosomes, 1 pair of sex chromosomes
→Use of the UAS/GAL4 promoter/driver with corresponding **RNAi** of interest.
→***Ex-vivo MT Experiments:***

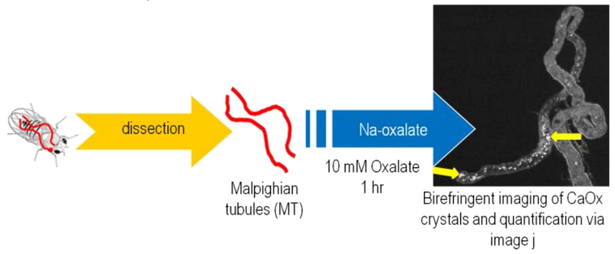


Fig.3: Protocol for MT CaOx crystallization studies.

2) Electrophysiology Experiments: By holding the membrane voltage at a controlled value, the kinetics and morphology of the induced currents through a particular ion transporter can be observed and analyzed.

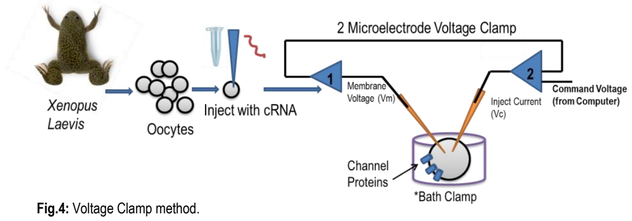


Fig.4: Voltage Clamp method.

3) Microfluidic Fabrication: prototype created via toner-transfer method to solve problems related to the current limitations (separating apical and basolateral sides) in MT perfusion and secretion assays.

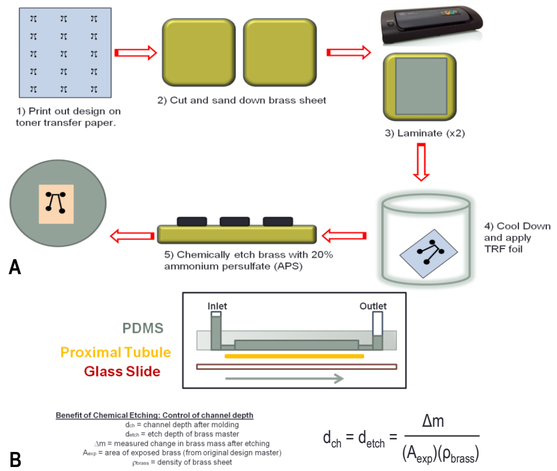


Fig.5: (A) Protocol (adapted from Easley, et.al) for microfluidic device. **(B)** Equation for controlling depth of etching.

Results

1) Ex-vivo 1 hour 10mM Oxalate MT tubule bath:

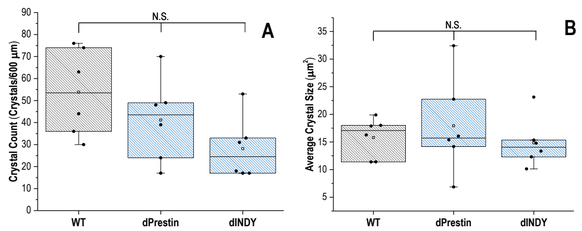


Fig. 6: (A) Crystal count of wild type (WT), dPrestin, dINDY knockdown flies with MT tubules soaked in 1 hr of 10mM oxalate Ringer's solution. (B) Average crystal size analysis. Statistical analysis done with 1-way ANOVA.

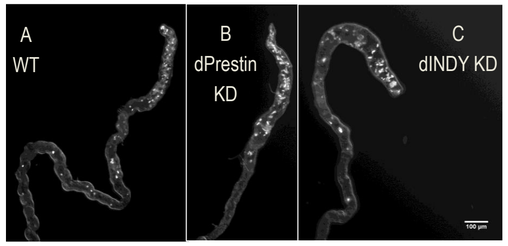
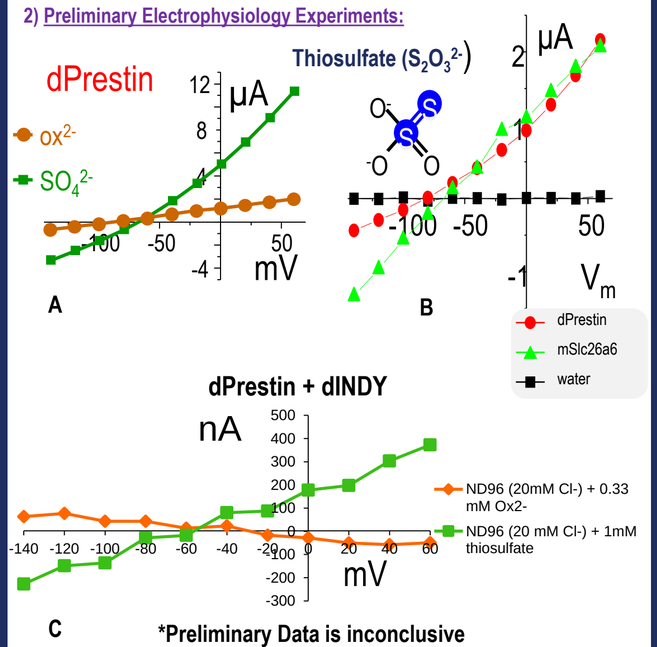


Fig. 7: (A) WT malpighian tubule birefringence imaging with CaOx stones illuminating white, (B) dPrestin knockdown fly MT crystal imaging, (C) dINDY knockdown MT analysis.

2) Preliminary Electrophysiology Experiments:



***Preliminary Data is inconclusive**

Fig. 8: (A) Previously published data from the lab showing Ox^{2-} and $SO4^{2-}$ elicited currents with dPrestin oocyte expression, and **(B)** mSlc26a6 (mammalian dPrestin) expression. **(C)** Co-expression data is currently inconclusive.

Microfluidics

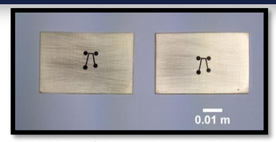


Fig.9: (A) Design 1 for ex-vivo MT perfusion experiments, with circular areas the inlets and outlet ports.

- **Addresses limitations in current MT CaOx stone** assays—allows for perfusion and separation of apical and basolateral sides of the tubule for more physiologically relevant conditions.

Conclusions

1) Fly MT CaOx Birefringence Assays: crystal decreases with either dPrestin or INDY knockdown (RNAi) alone
2) Electrophysiology: still need to identify transporter activity of interaction between Slc26a6 & NaDC1
-Preliminary data shows a decrease in oxalate transport with coexpression of dINDY + dPrestin, ***however*** dINDY is thought to be **non-electrogenic based on past experiments.**
3) Microfluidics (Future Direction): Many applications with pH and voltage sensors, cell culture, secretion assays
-Develop fully functional microfluidic device for variety of applications in assessing renal function *in-vitro* and with *ex-vivo* tissue.→ ***Greater applications in drug delivery***

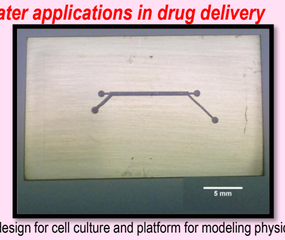


Fig. 10: Microfluidic design for cell culture and platform for modeling physiological renal system.

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