Diabetic Complications Consortium

Application Title: Metabolic biosensor zebrafish transgenics

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1. Project Accomplishments:

During the funding period, we successfully generated transgenic zebrafish reporter lines incorporating genetically-encoded metabolic biosensors. We then validated the use of the redox and glucose biosensors.

2. Specific Aims:

AIM1: Establish versatile zebrafish UAS transgenic reporter lines for cell type-specific expression of genetically-encoded biosensors for mitochondria, ATP/ADP ratio, H_2O_2, NADH/NAD+ ratio, redox state and free glucose.

Results: We generated UAS transgenic reporter lines with following biosensors: Grx1-roGFP2 (cytosolic redox), mitoGrx1-roGFP2 (mitochondrial redox), PercevalHR (ATP/ADP), HyPer3 (H_2O_2), Peredox (NADH/NAD+) and Sweetie (Glucose, in collaboration with Dr. Jacob Keller at Janelia Research Campus). By crossing these Tg(UAS:biosensor) with either Tg(hsp70:Gal4) (global expression) or Tg(cdh17:Gal4) (renal tubule specific expression), we analyzed whether these biosensors could be expressed at levels sufficient to study corresponding metabolites. While Grx1-roGFP2, mitoGrx1-roGFP2, HyPer3 and Sweetie exhibited bright expression *in vivo*, PercevalHR and Peredox resulted in only weak expression in the F1 stable transgenic system (Fig. 1).

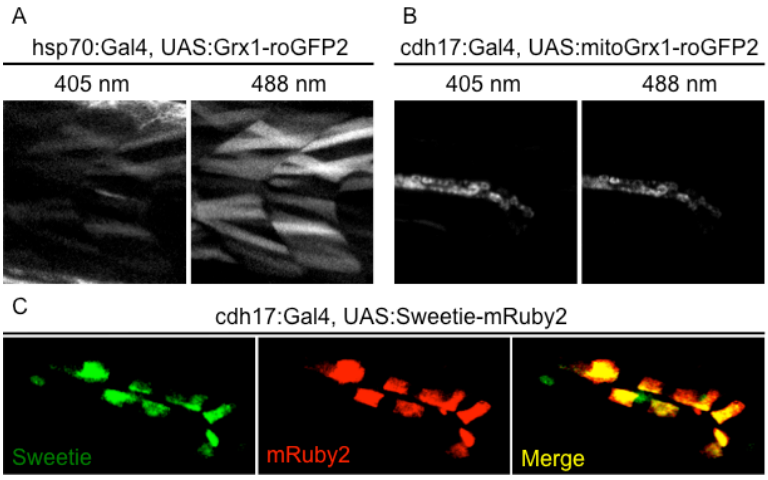

Figure 1. (A) Expression of Grx1-roGFP2 in muscle fibers driven by the global promoter hsp70 imaged by alternating excitation wavelengths of 405/488 nm. (B) Expression of mitoGrx1-roGFP2 in the distal portion of the pronephric nephron. (C) UAS-controlled Sweetie in tubular epithelia under cdh17:Gal4.

AIM2: Demonstrate *in vivo* sensitivity of biosensor lines using oxidant stress and diabetic conditions.

Results: The redox-sensitive fluorescent reporters (Grx1-roGFP2 and mitoGrx1-roGFP2) rapidly and reversibly responded to changes in cellular oxidative stress levels modulated by a pro-oxidant, tert butyl hydroperoxide (tBH), and a reducing agent, dithiothreitol (DTT) (Fig. 2A). The normalized ratios of signals from 405/488 nm excitations were greater in the cytosol than in mitochondria in the muscle fibers (Fig. 2B). This may indicate that mitochondria may be equipped with a mechanism to suppress oxidative stress levels. Further, we generated transgenic zebrafish lines, Tg(podo:Gal4, UAS:Grx1-roGFP2), Tg(podo:Gal4, UAS:mitoGrx1-roGFP2), Tg(cdh17:Gal4, UAS:Grx1-roGFP2) and Tg(cdh17:Gal4, UAS:mitoGrx1-roGFP2), that display the fluorescent reporters specifically in podocytes and tubular epithelia of the zebrafish pronephros. As in the muscle fibers, mitoGrx1-roGFP2 rapidly responded to the tBH treatment in podocytes (Fig. 2C).

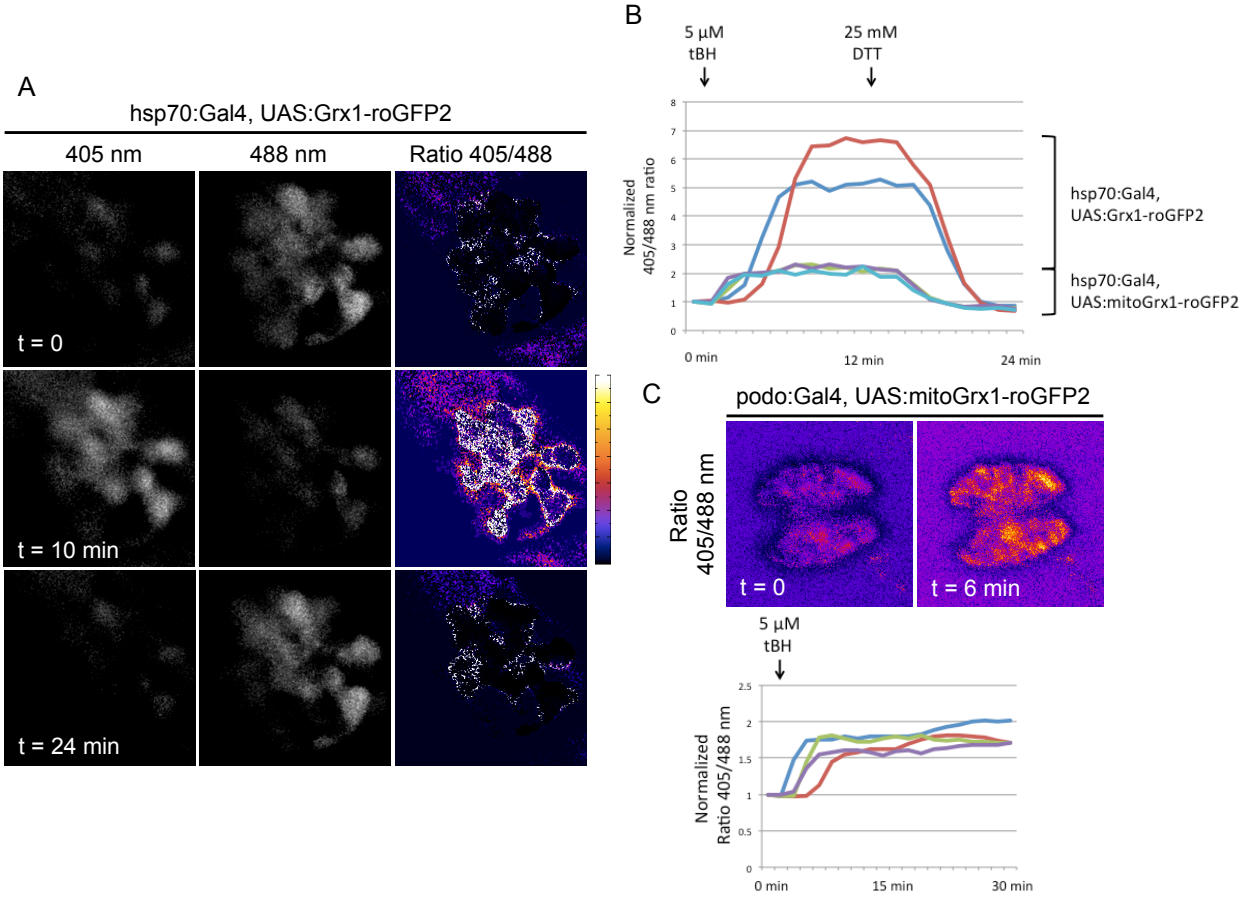

Figure 2. (A) Ratiometric imaging of Grx1-roGFP2 in the pineal gland of Tg(hsp70:Gal4, UAS:Grx1-roGFP2). tBH and DTT were added to the medium at t = 0 and t = 10 min, respectively. (B) Compartment-specific response of Grx1-roGFP2 to 5 µM tBH and 25 mM DTT in the muscle fibers. The ratiometric 405/488 changes were followed for 24 min. The values were normalized to the basal 405/488 ratio before treatment (set to 1.0). (C) Response of mitoGrx1-roGFP2 to 5 µM tBH in glomerular podocytes. tBH was added to the medium at t = 5 min.

The intensity of the glucose sensor, Sweetie, expressed in the muscle fibers in Tg(hsp70:Gal4, UAS:Sweetie-mRuby2), increased upon injection of 10 % glucose into the circulation. Furthermore, intravenous injection of insulin also induced the increase of the Sweetie intensity, validating the use of this sensor in monitoring intracellular glucose levels (Fig. 3).

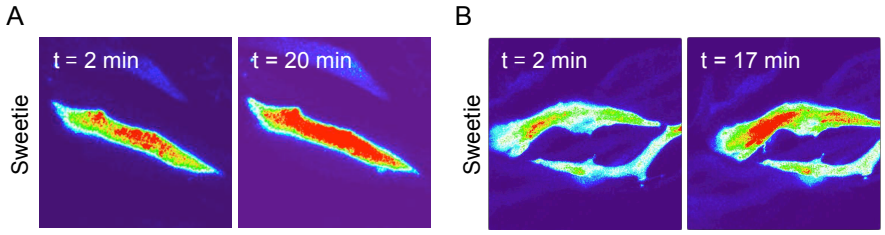

Figure 3. Transient expression of Sweetie in the muscle fibers under hsp70:Gal4. Injection of glucose (A) and insulin (B) into the circulation provoked an increase in Sweetie GFP intensity.

3. Publications:

Poster presentation at ASN Kidney Week 2017
"Optical analysis of mitochondrial dynamics and function in renal physiology and pathology *in vivo* using metabolic biosensor transgenic zebrafish"
Manuscript in preparation.