Galactosemia Research: finally flying forward

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*no conflicts of interest to disclose

In this breakout session…

- Why do we need a WHOLE ANIMAL genetic model for classic galactosemia?
- Meet *Drosophila melanogaster* (fruit fly)
- What have we done so far and what does it mean?
- What do we plan for the future?
- Time for questions and discussion

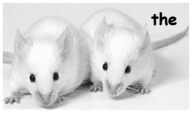
Why do we need a whole animal genetic model for classic galactosemia?

- To identify genetic and environmental modifiers of outcome, we need an animal that actually shows an outcome
- To address questions of timing and location, meaning when and where in development does the "damage" occur, we need a model (with organs) that develops
- To test new candidate interventions, we need a model with long-term as well as acute symptoms

A GALT knockout mouse?

In 1996, Dr. Nancy Leslie and her colleagues reported a GALT knockout mouse (Biochem Mol Med. 1996 Oct;59(1):7-12):

- these mice demonstrated the anticipated biochemical GALT defect, confirming that the "correct" gene had been disrupted, and
- they accumulated high levels of gal-1P upon exposure to dietary galactose, but
- these mice remained healthy and fertile despite exposure to high levels of dietary galactose, meaning they failed to mimic either acute or long-term outcomes experienced by patients with GALT-deficiency



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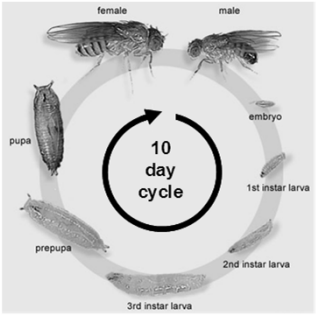
Meet *Drosophila melanogaster*…



Some benefits of using fruit flies…

- small, easy to maintain large populations, facile genetics, biochemistry, and development
- many metabolic pathways are highly conserved between flies and people
- established tools for testing learning and memory, movement, and reproduction
- established tools for genetic and pharmacologic screens

http://www.nobelprize.org/

Thomas Hunt Morgan
(1866-1945)
Nobel Prize in 1933 for discovering the chromosomal basis of inheritance, work conducted using fruit flies

Some other human conditions that have been modeled successfully in fruit flies

- metabolic (e.g. diabetes)
- neurodegenerative (e.g. Parkinson's and Huntington's)
- cognitive (e.g. Fragile X)
- inflammatory (e.g. asthma)
- behavioral (e.g. ethanol-related)

What have we done so far and what does it mean?

- demonstrated that fruit flies have an intact Leloir pathway of galactose metabolism
- created a GALT-null fruit fly and confirmed it genetically and biochemically
- demonstrated relevant acute outcome in GALT-null larvae and long-term outcomes in adults
- begun to identify genetic and environmental factors that modify those outcomes

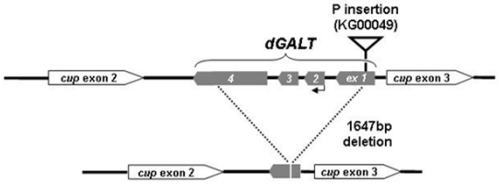
Fruit flies encode a functional Leloir pathway

Protein	Fly Gene	% Identity	% Conservation	% Gaps
dGALK	CG5288	27	43	20
dGALT	CG9232	57	72	0
dGALE	CG12030	60	76	0

Specific Activity
(pmol product/µg protein/min)

Enzyme	Larvae	Pupae	Adults
dGALK	11.7	19.62	19.30
dGALT	17.25	38.49	24.31
dGALE	48.26	78.09	81.42

Generating a GALT-null fruit fly

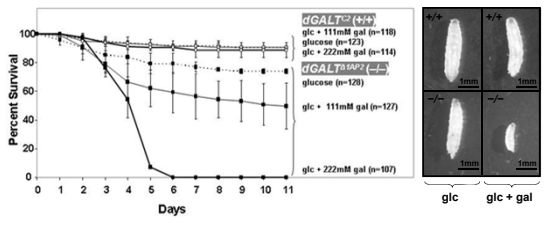


Imprecise excision (*dGALT*$^{\Delta 1AP2}$ and *dGALT*$^{\Delta 1V2}$) junction sequences:

...GTGCCAAACTCAAACTAAACcatgatgaaataa GGAAATAGTCTAATACGACT...

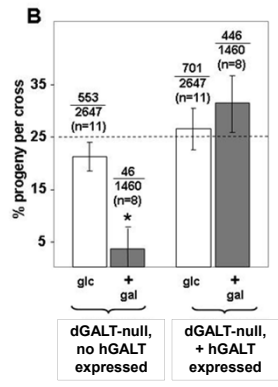
Kushner et al (2010) DMM

GALT-null fruit flies show acute galactose-sensitivity as larvae



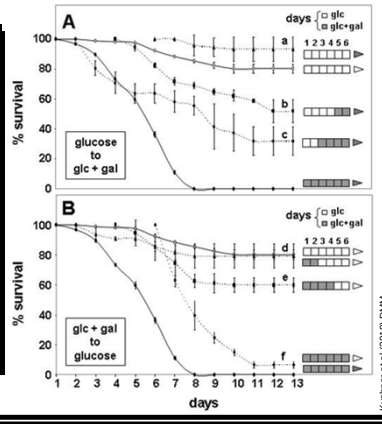
Kushner et al (2010) DMM

GALT-null fruit flies are rescued by expression of a human GALT transgene

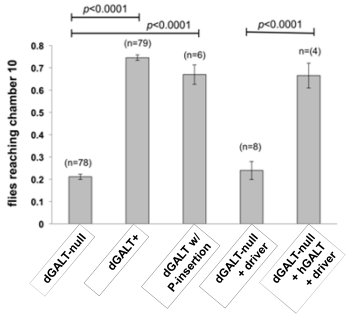


Kushner et al (2010) DMM

GALT-null fruit flies are also rescued by dietary restriction of galactose

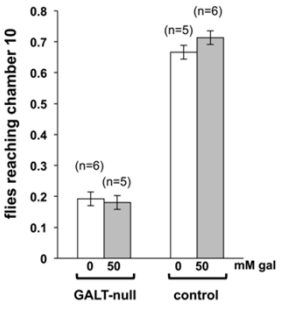


a long-term climbing defect in GALT-null fruit flies on galactose-restriction



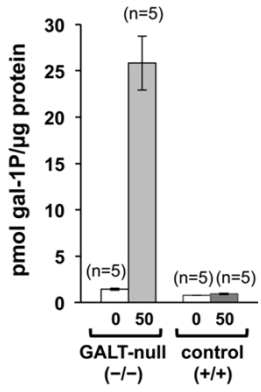
So, now that we have an animal model of GALT-deficiency that demonstrates both acute and long-term complications, what questions can we ask?

Does low-level galactose exposure of larvae worsen their adult movement defect? no

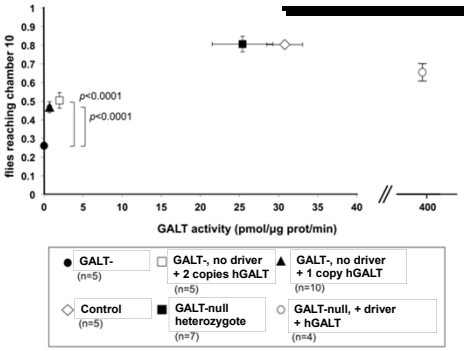


Does low-level galactose exposure of GALT-null larvae cause gal-1P levels to rise?

yes

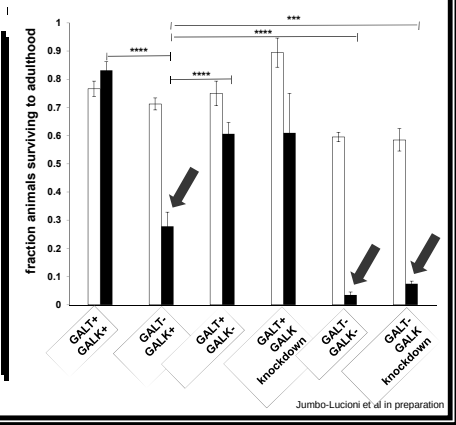


Does cryptic GALT activity in fruit flies impact adult outcome? yes



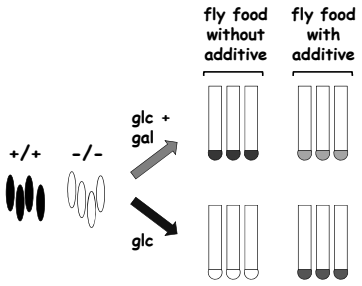
Does loss of GALK rescue acute outcome in GALT-null fruit flies?

no

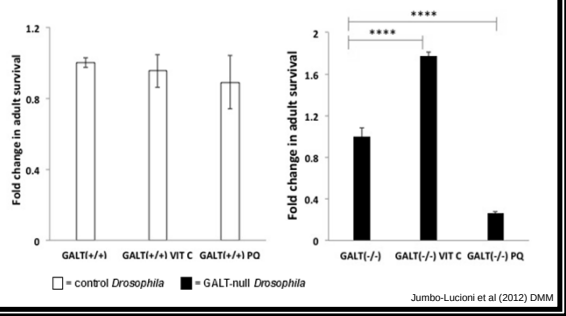


Jumbo-Lucioni et al in preparation

testing the impact of environmental additives on acute outcome



Oxidants lower and anti-oxidants raise survival rates of GALT-null larvae exposed to galactose



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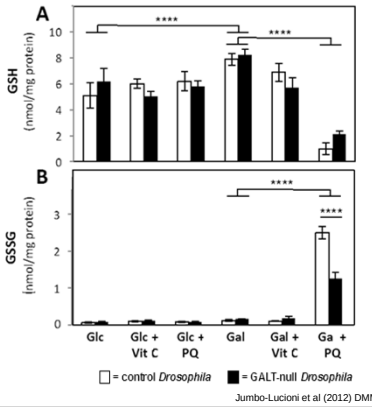
Galactose exposure contributes to oxidative stress in GALT-null larvae



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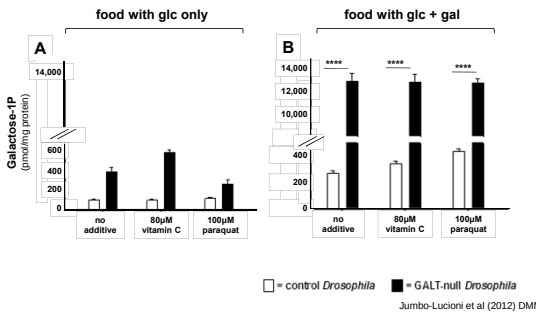
Evidence of oxidative stress from altered gene expression

GST genes induced in larvae exposed to galactose

	expression level relative to *ACT5C*	
	GSTD6	*GSTE7*
control larvae (no galactose exposure)	0.344 ± 0.052	0.591 ± 0.114
control larvae (12 hrs galactose exposure)	14.794 ± 0.456 (>40-fold)	3.122 ± 0.195 (>5-fold)
GALT-null larvae (no galactose exposure)	0.354 ± 0.058	0.456 ± 0.131
GALT-null larvae (12 hrs galactose exposure)	29.467 ± 1.189 (>80-fold)	3.885 ± 1.004 (>8-fold)

Impact of oxidative stress on survival of GALT-null fruit flies acts downstream or independent of gal-1P



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What's next?

- Characterization of other long-term outcomes in GALT-null flies (e.g. cognitive)
- Test impact of current candidate modifiers on long-term outcomes
- Genetic screens for novel modifiers
- Pharmacologic studies testing drugs that impact relevant pathways
- Secure funding to continue the work (please encourage your leaders in Washington to raise the NIH budget!)

Thank you for your attention, and thank you to:

- amazing people who did the work ➔
- NIH, who funded the work.