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Amber M. Paul

Universities Space Research Association, paula6@erau.edu

Brooke D. Shepard

Space Life Sciences Training Program

Sharmila Bhattacharya

NASA Ames Research Center

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Stress-Induced Heat Shock Protein 40 and Immune Function in Altered Gravity

Brooke D. Shepard¹, Amber M. Paul², Sharmila Bhattacharya³

¹Space Life Sciences Training Program, KBRWyle, Moffett Field, CA

²Universities Space Research Association, Columbia, MD

³Space Biosciences Division, NASA Ames Research Center, Moffett Field, CA

Why *Drosophila melanogaster*?

- Rapid propagation, large sample size
- Short lifespan for multigenerational studies
- Simple genetic modification
- ~75% of disease-related *Drosophila* immune genes function similarly in humans.



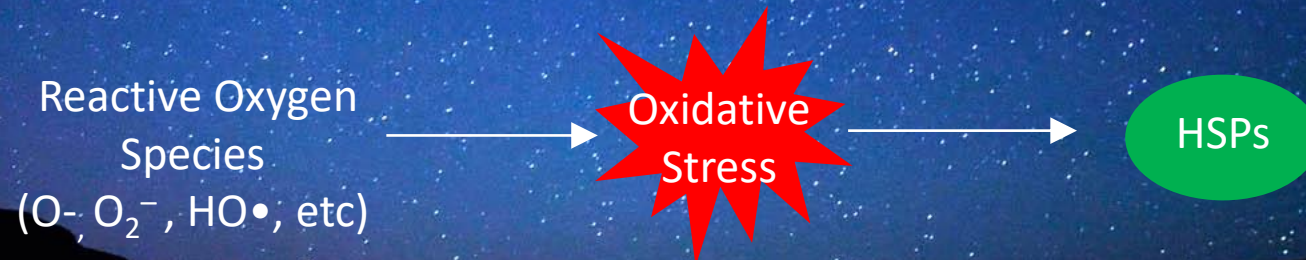
Yourgenome.org

Why hypergravity?

Hypergravity (>1g) or zero gravity (0g)

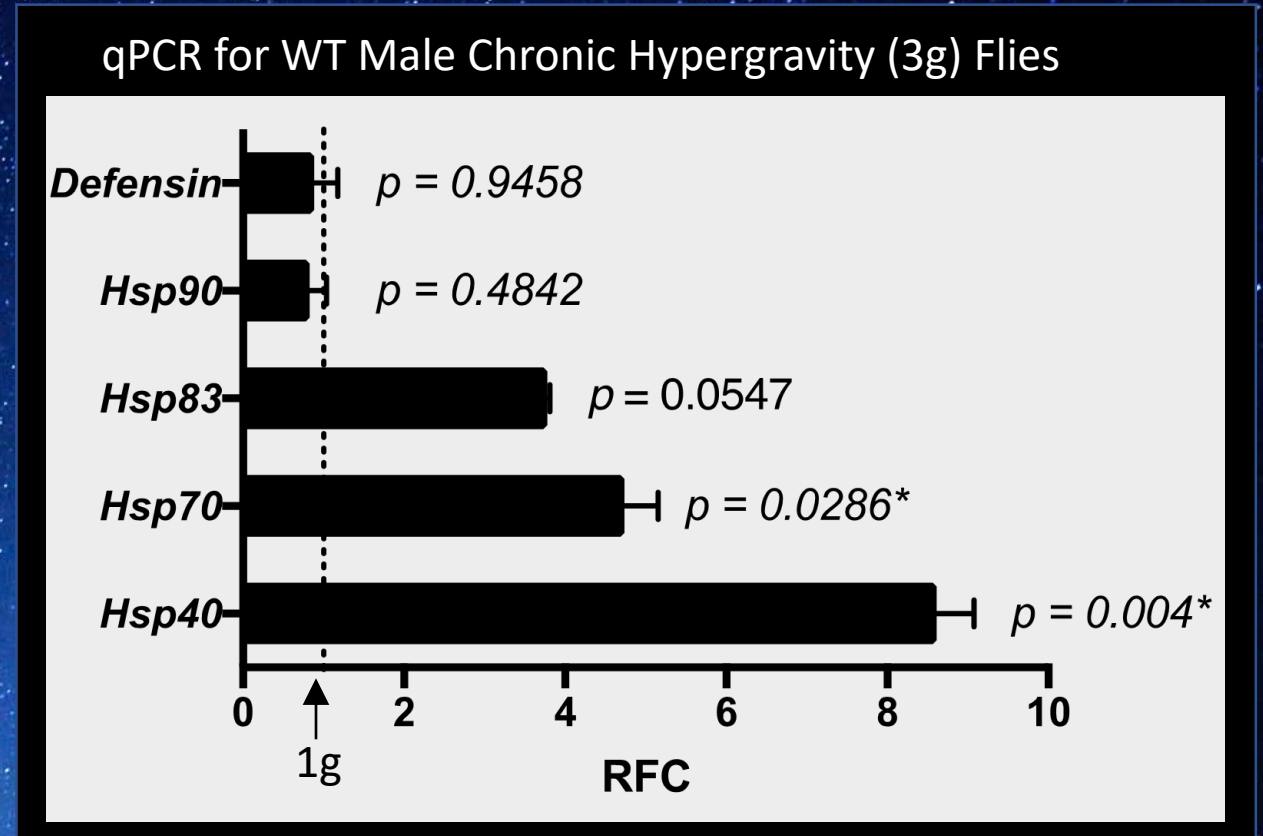
Chronic hypergravity can induce oxidative stress

- Process of interest



Preliminary Data

- *Hsp40* was significantly induced following our model of chronic hypergravity
- *Hsp40* research suggests complex relationship with immunity
 - Upregulates inflammatory cytokine expression (Pockley *et. al.*, 2008)
 - Associated with immune suppression (Binder, 2015)

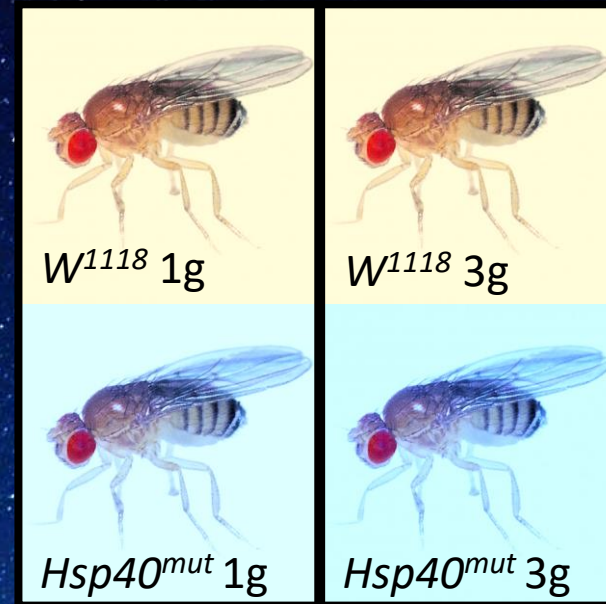


AM Paul, unpublished

We hypothesize that a loss of *Hsp40* would result in changes in immune function as a result of stress.

Methods

Expose *Drosophila* to chronic hypergravity (3g, 95 rpm) for 9 days

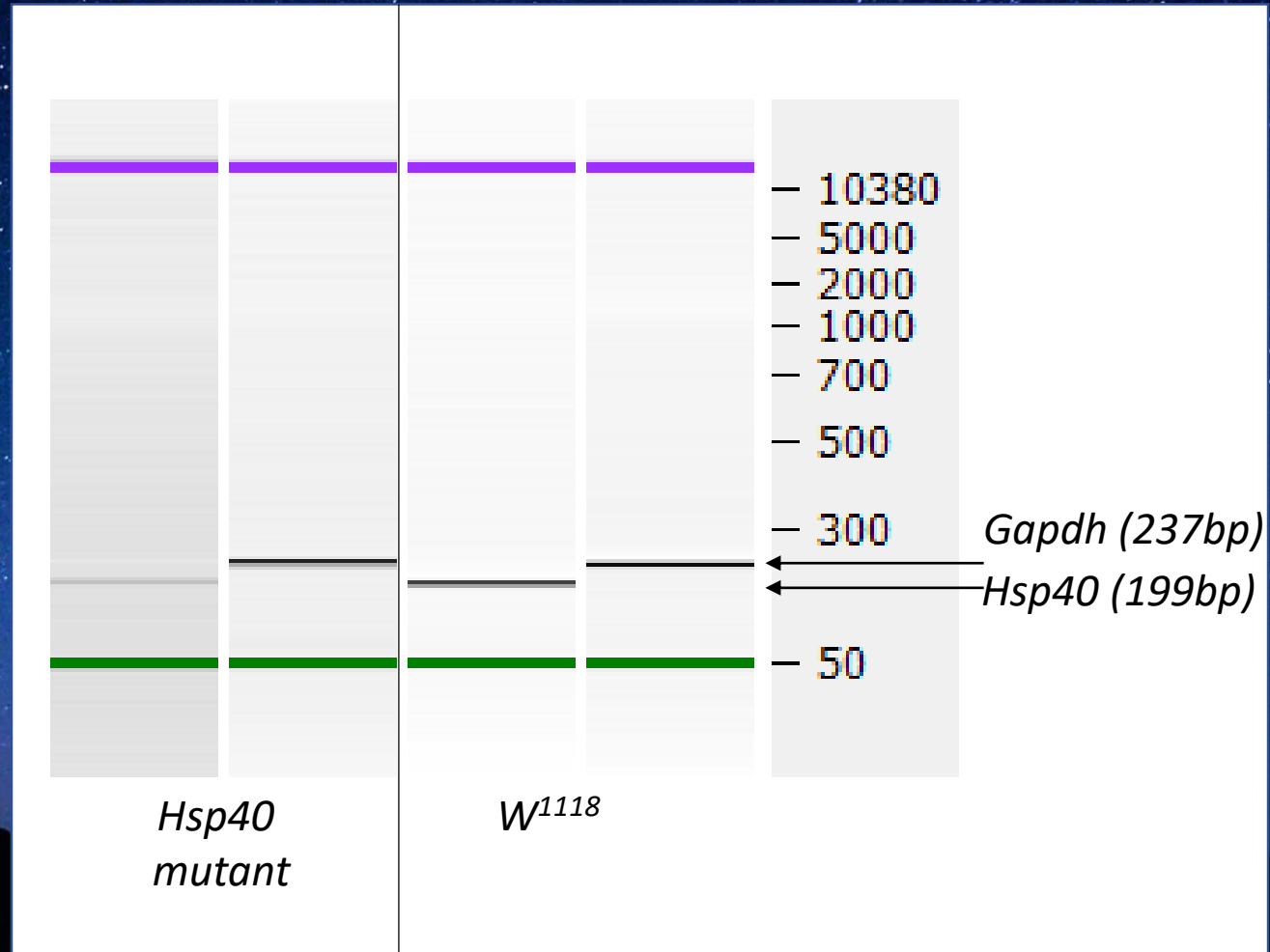


Quantitative real-time PCR → measure expression of genes involving immunity and oxidative stress response

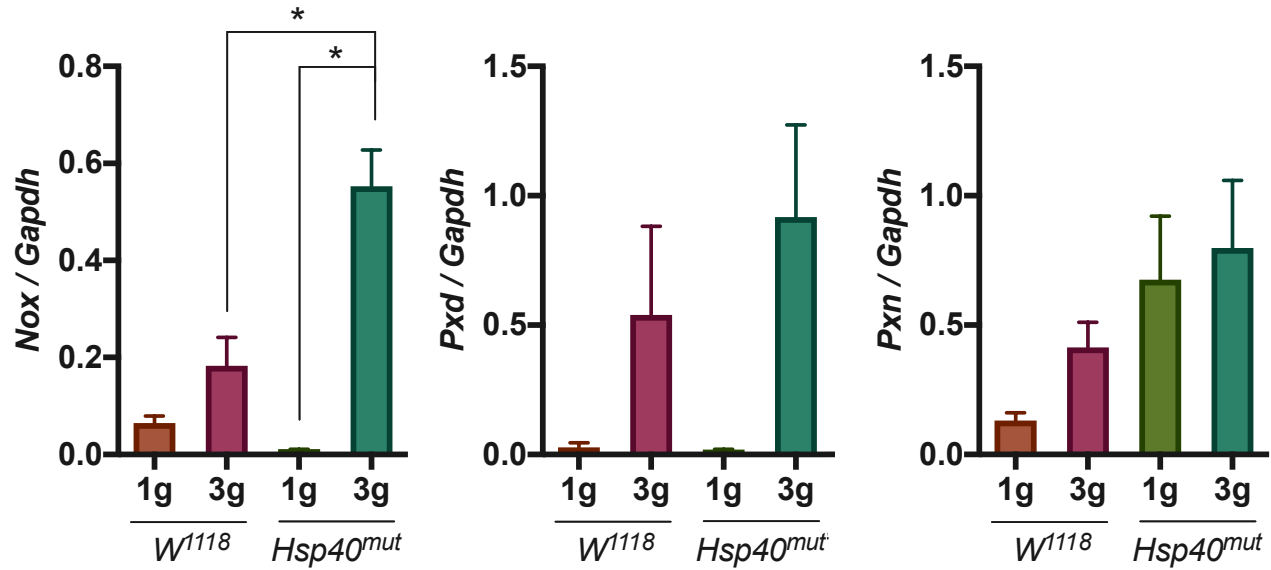
Housekeeping Gene	Innate Immunity Genes	Oxidative Stress Response Genes
<i>Gapdh</i>	<i>Defensin</i> <i>Imd</i> <i>Dif</i> <i>Met</i>	<i>Pxd</i> <i>Pxn</i> <i>Nox</i>

Results

Hsp40^{mut} flies have reduced *Hsp40* expression



Results

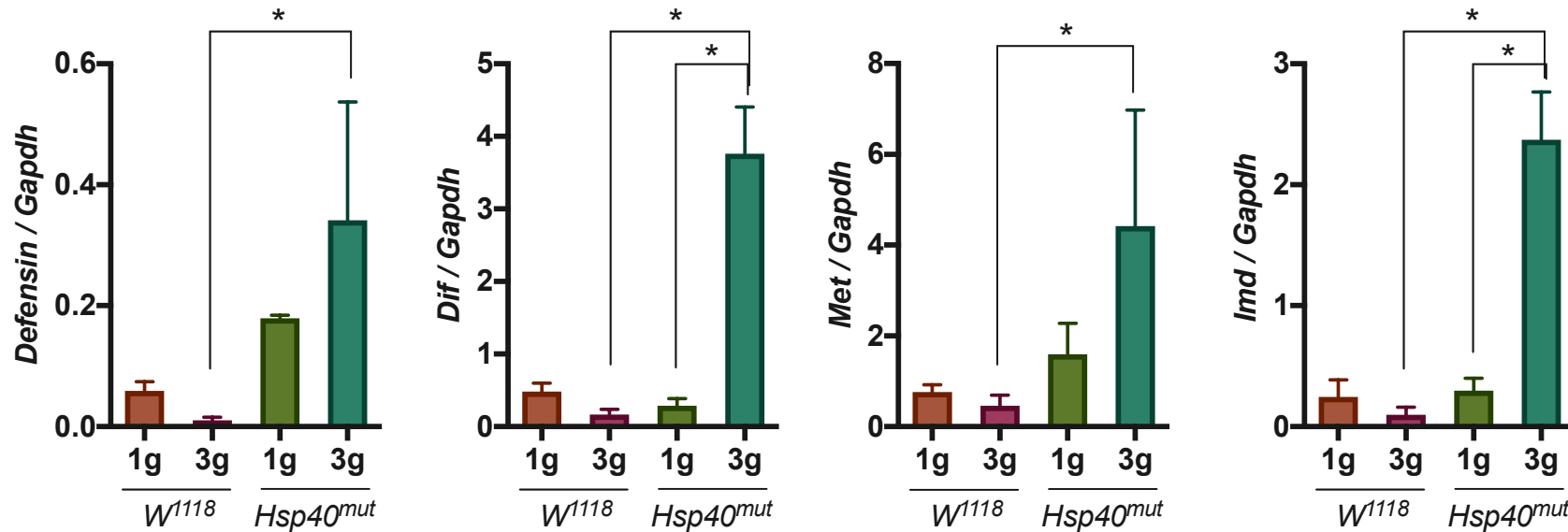


<i>Hsp40</i> 1g	n= 40 flies 4 samples
<i>Hsp40</i> 3g	n= 36 flies 4 samples
<i>W¹¹¹⁸</i> 1g	n= 88 flies 9 samples
<i>W¹¹¹⁸</i> 3g	n= 89 flies 7 samples

Oxidative Stress Response

Hsp40^{mut} ↑ expression of...

Nox* (NADPH oxidase)
Pxd (Peroxidase)
Pxn (Peroxidasin)

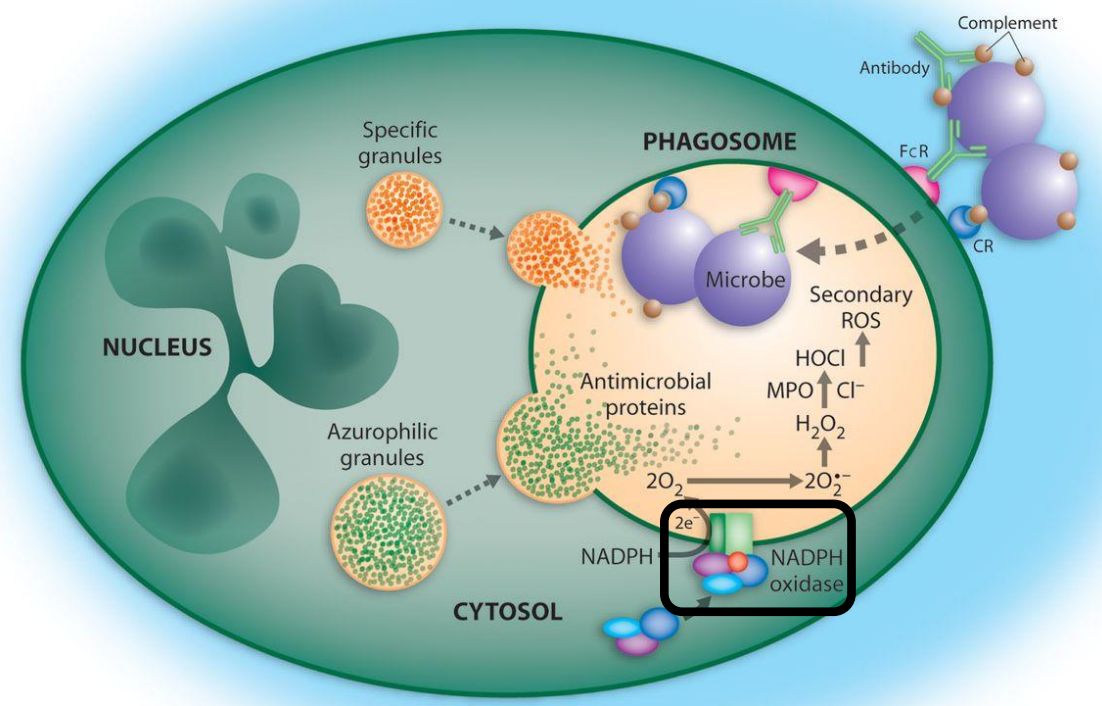
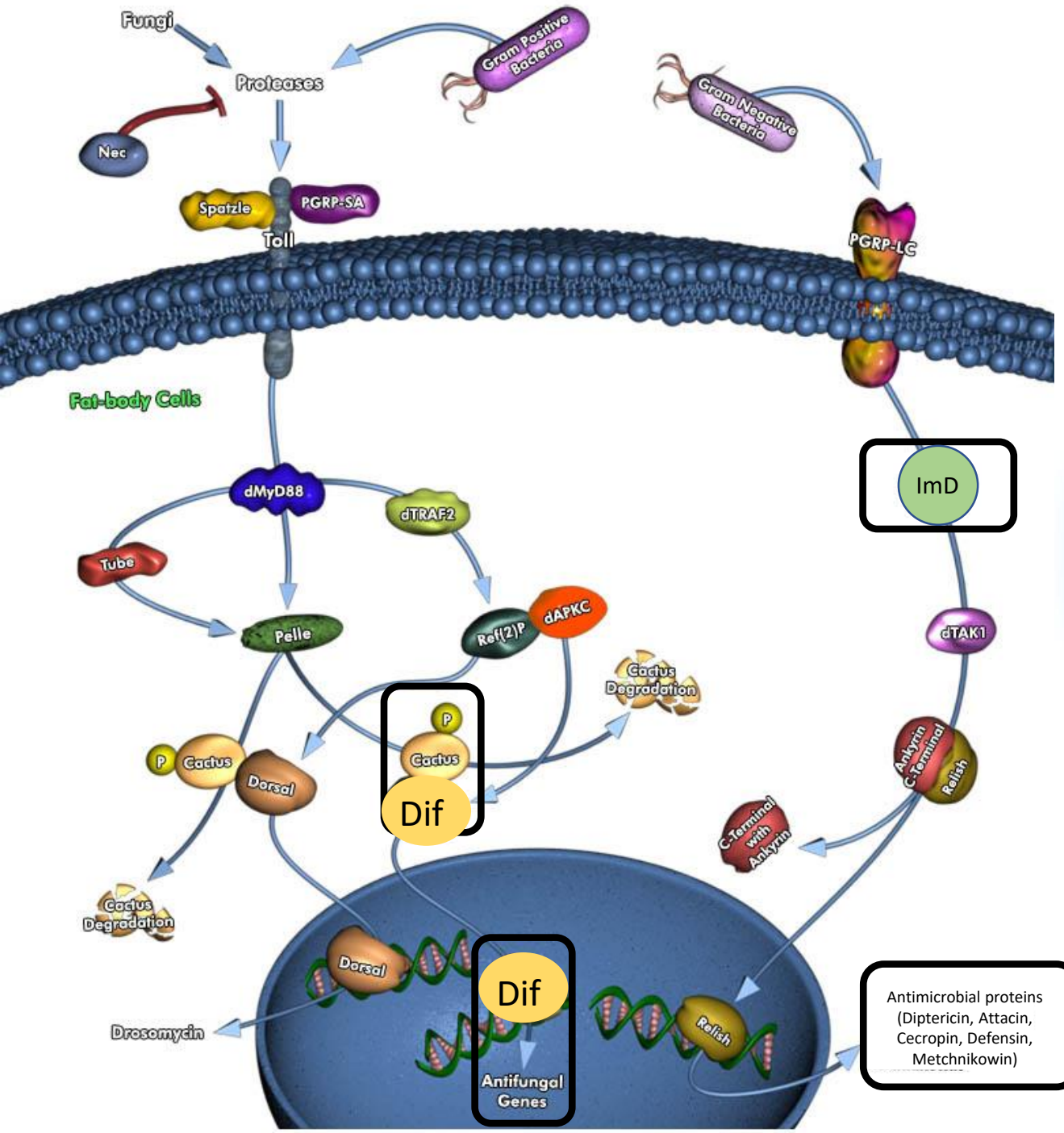


Immune Response

Hsp40^{mut} ↑ expression of...

Defensin*
Dif*
Met*
Imd*

One-way ANOVA with post hoc Sidak's multiple comparisons test
Data represented as standard error of the mean (SEM), $p \leq 0.05$ * SEM



Qiagen Sample to Insight
Quinn et al., 2006

Summary

Stress



Immune genes
Oxidative stress genes

Stress



Immune genes
Oxidative stress genes

Future Directions

- Nox signaling and immune regulation
 - Continue to study trends in oxidative stress genes
 - Diversify gene repertoire
-
- Space science and health science applications (rheumatoid arthritis (Tukaj *et. al.*, 2010), lung cancer (Oka *et. al.*, 2001))

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