Student Research Program
Project Description

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PROJECT TITLE (200 Characters max):
Regulation of longevity by Imd and Loco-Rpd3 signaling pathways

HYPOTHESIS:
The reduction of Imd and Loco-Rpd3 signaling enhance stress resistance and longevity through the downstream effectors.

PROJECT DESCRIPTION **(Include design, methodology, data collection, techniques, data analysis to be employed and evaluation and interpretation methodology)**

Investigating the mechanisms of the aging process is becoming more important and indispensable to biomedical research due to aging-related human diseases and concerns for public health. In particular, the fruit fly model system, using *Drosophila melanogaster*, is extremely useful to studying several aspects of the aging process, because both forward and reverse genetic approaches have been shown to be efficient and successful for elucidating the primary physiological aspects of the aging process. Recent investigations of the aging process have shown that several conserved signaling pathways are related to regulation of longevity in various organisms including yeast, worms, flies and mammals. Using *D. melanogaster* as an aging model system, we will study longevity mechanisms with Imd and Loco signaling pathways that are known to function in immune response and blood-barrier formation, respectively, and evolutionarily conserved between insect and human.

1. Fly strain and aging test. As a wild type, the lab stock strain y^1w^1 (Bloomington stock center) will be used for all the experiments including stress response, aging and nutritional content studies. To equalize genetic background, the flies obtained from the outside (stock centers and other labs) will be backcrossed to the y^1w^1 flies six times and then homogenized for six generations. Virgin flies will be collected from the bottles in which larval density is controlled in a standard cornmeal medium without live yeast. For the aging test, 200 virgin flies (20 flies per vial) will be counted and transferred to fresh standard cornmeal vials every 2 to 3 days (Fig. 1).

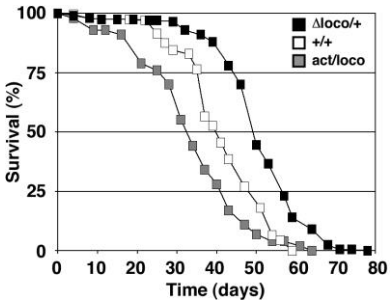

Figure 1. The aging test of male adult flies, showing that *Drosophila*'s lifespan is significantly changed by the extent of *loco* expression ($P < 0.0001$, log-rank test). Δloco/+: $loco^{P283}$ heterozygote; +/+: wild-type; act/loco: *loco* overexpression with *act*-Gal4 driver and UAS-genomic *loco*.

2. Stress response and nutrient assay. For the starvation test, a group of 100 flies (20 flies per vial) will be maintained in the vials containing two filters wetted with 300 ul of water at 25°C. For the oxidation test, adult flies, starved for the initial 6 hrs, will be maintained in the vials containing two filters wetted with 300 ul of 20 mM paraquat in 5% sucrose solution at 25°C. For the heat test, adult flies will be maintained in standard cornmeal vials at 37°C with 30% humidity. For nutrient, cAMP and MnSOD assays, all 20 flies will be weighed and homogenized with a specific buffer for each assay.

3. RT-PCR and iTRAQ. For RT-PCR, oligo dT-primed cDNAs will be made from 5 ug total RNA purified from adult flies, and they will be used as templates for quantitative real-time PCR.

To measure phospho-peptides, iTRAQ analyses will be performed using total protein lysates purified from adult flies in CAPR proteomics facility (http://njms.rutgers.edu/proweb/).

SPONSOR'S MOST RECENT PUBLICATIONS RELEVANT TO THIS RESEARCH:

1. K. Kim, Y-R. Lin, and **Y. Park** (2010) Enhancement of stress resistances and downregulation of Imd pathway by lower developmental temperature in *Drosophila melanogaster*. ***Exp. Gerontol.***, 45, 984-987. (http://www.ncbi.nlm.nih.gov/pubmed/20849943)

2. Y-R. Lin, K. Kim, Y. Yang, A. Ivessa, J. Sadoshima, and **Y. Park** (2011) Regulation of longevity by Regulator of G-protein Signaling (RGS) protein, Loco. ***Aging Cell***, 10, 438-447. (http://www.ncbi.nlm.nih.gov/pubmed/21255223)

3. Y-R. Lin, H. Parikh*****, and **Y. Park** (2011) Loco signaling pathway in longevity. ***Small GTPases***, 2, 158-161. (http://www.ncbi.nlm.nih.gov/pubmed/21776417)

4. Z. Kopp, J. Hsieh, A. Li, W. Wang, D. Bhatt, A. Lee, S. Kim, D. Fan, V. Shah, E. Siddiqui, R. Ragam, K. Park, D. Ardeshna, K. Park, R. Wu, H. Parikh, A. Parikh, Y. Lin, and **Y. Park** (2015) Heart-specific Rpd3 downregulation enhances cardiac function and longevity. ***Aging***, 7(9), 648-663. (http://www.ncbi.nlm.nih.gov/pubmed/26399365)

IS THIS PROJECT SUPPORTED BY EXTRAMURAL FUNDS?

Yes X or No ☐

(IF YES, PLEASE SUPPLY THE GRANTING AGENCY'S NAME)

Agency: National Institute on Aging (NIA), 1R21AG042021-01A1 (06/01/13-05/31/17)
Title: Loco/RGS14 signaling pathway in longevity

THIS PROJECT IS: ☐ Clinical X Laboratory ☐ Behavioral ☐ Other

THIS PROJECT IS CANCER-RELATED: No

THIS PROJECT IS HEART, LUNG & BLOOD- RELATED: Yes

The insect (*Drosophila*) and mammalian innate immune responses exhibit a great deal of evolutionary conservation, one of which is the Imd pathway that cascades NF-κB signaling for the control of immune-induced gene expression. *Drosophila* uses several distinct effector mechanisms in blood for immune protection including clotting, melanization, encapsulation, cell-based phagocytosis, and the inducible production of a battery of antimicrobial peptides. This antimicrobial peptide response is critical for protection against many microbial pathogens. The Imd signaling pathway regulates the production of these antimicrobial peptides in *Drosophila*. Recent work has shown that the intensity and duration of immune response is tightly regulated in *Drosophila*. As in mammals, over-exuberant immune responses are detrimental, and the proper down modulation of immunity is critical for health. In this project, we focus on new insight that the Imd pathway regulates the aging process.

THIS PROJECT EMPLOYS RADIOISOTOPES: No

THIS PROJECT INVOLVES THE USE OF ANIMALS: No

THIS PROJECT INVOLVES THE USE OF HUMAN SUBJECTS: No

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THIS PROJECT IS SUITABLE FOR:

UNDERGRADUATE STUDENTS	☐	ENTERING FRESHMAN	☐
SOPHMORES	☐	ALL STUDENTS	**X**

THIS PROJECT IS WORK-STUDY: Yes ☐ or No X

THIS PROJECT WILL BE POSTED DURING ACADEMIC YEAR FOR INTERESTED VOLUNTEERS?: Yes X or No ☐

WHAT WILL THE STUDENT LEARN FROM THIS EXPERIENCE?

Before starting the experiments of this project, a student will study the previous knowledge of aging research related to the Imd and Loco signaling pathways. Then, PI will discuss the ideas how to design and perform this research with the student. Under PI's advice, the student will learn 1) purpose of the experiment, 2) procedural design, 3) data collection, 4) processing/analysis of data, and 5) drawing conclusions. Finally, the student will understand how the information obtained from our studies will advance general knowledge of the eukaryotic aging process and help us find ways to cure or delay aging-related diseases such as Alzheimer's disease. The previous volunteer student, Hardik Parikh, had performed this project and published a paper as co-author (* in reference 3). Since 2012 the summer students had successfully worked on this project as the medical student of NJMS and the college student of BS/MD program. Recently, we have published a paper (reference 4) with the summer students and data resulted from the Summer Student Research Program.