The Role of TGF-Beta/activin and mTORC2 Signaling in Cardiac Homeostasis

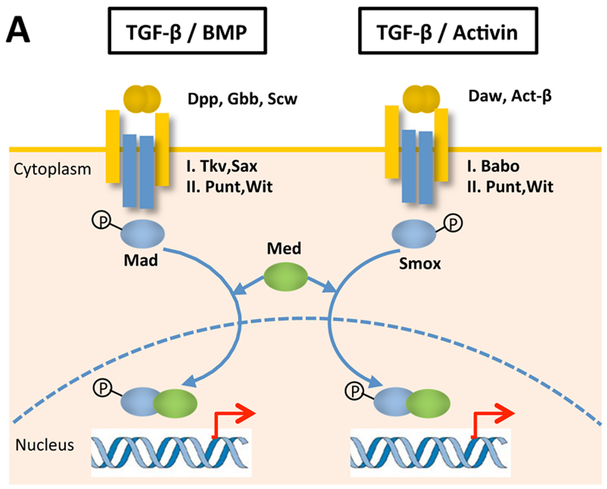
Kai Chang[1], Hua Bai[1]

[1] Department of Genetics, Development, and Cell Biology, Iowa State University, Ames, IA 50011

Abstract

Cardiovascular disease is the worldwide leading cause of death, especially in elder population. Understanding the mechanisms underlying cardiac aging is crucial for developing effective therapeutic interventions to promote cardiac health. Impaired autophagy and mitochondrial quality control have been previously linked to age-related declines of cardiac function. Mitophagy, the cargo-specific autophagy, is essential for removal of damaged mitochondria. Mitochondrial fusion/fission regulates mitochondrial morphology and quantity under stress and aging. Yet, how those quality control mechanisms are altered during cardiac aging still remains elusive. Here using *Drosophila* heart as a model system, we show that activin signaling, a member of TGF-beta superfamily, negatively regulates cardiac autophagy and cardiac health during aging. We found that cardiac-specific knockdown of Daw, an activin-like protein in *Drosophila*, increased cardiac autophagy and prevented age-related cardiac dysfunction, including arrhythmia and bradycardia (slow heart rate). Inhibition of autophagy blocked Daw knockdown-mediated cardio-protection. Intriguingly, the key autophagy regulator, mechanistic target of rapamycin complex 1 (mTORC1), was not involved in activin-mediated autophagy. Instead, activin signaling genetically interacted with Rictor, the key subunit of mTORC2, to regulate autophagy and cardiac aging. Knockdown of Daw increased the mRNA expression of Rictor and the phosphorylation of AKT in fly hearts. Additionally, mTORC2/Rictor has been implicated in mitochondrial quality control previously and in our studies even though the precise mechanism is unknown. Thus, our findings highlight an emerging role of activin signaling and mTORC2 in the regulation of cardiac homeostasis during aging.

Introduction



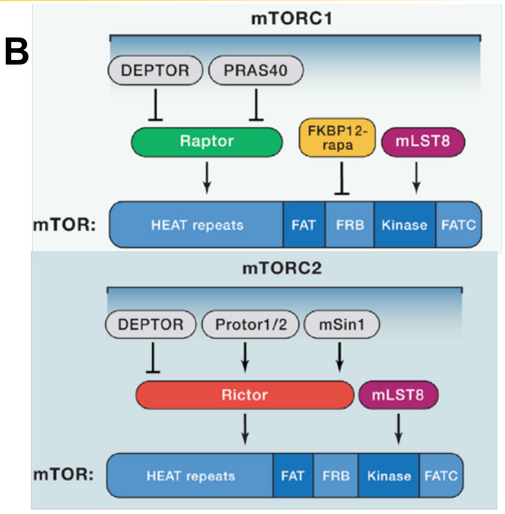


Figure A: Schematic showing distinct TGF-β pathways in Drosophila: BMP and Activin [1]. **Figure B:** mTORC1 and mTORC2 subunits and representative binding sites on mTOR [2].

Results

Activin signaling increases in aged heart, reduced activin/Daw slows cardiac aging

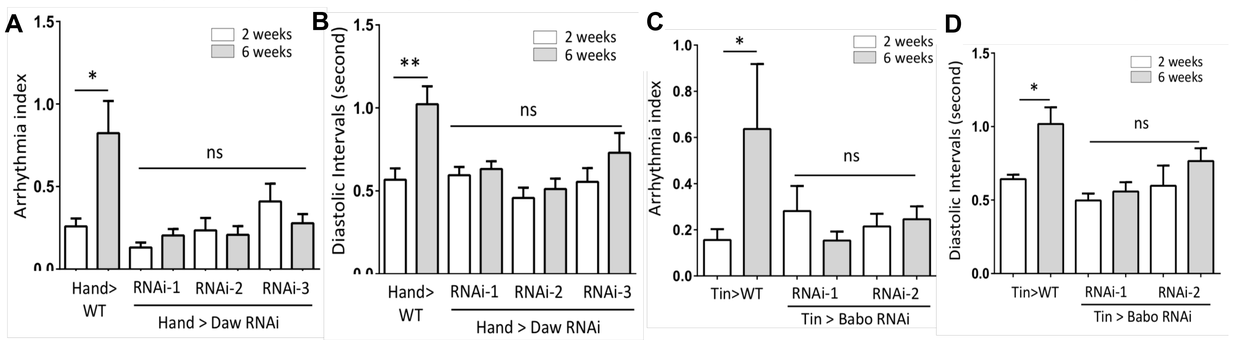


Figure A-B: Knockdown of *Daw* using *Hand-gal4* (heart-specific tissue driver) prevents age-induced cardiac arrhythmia and diastolic interval. **Figure C-D:** Age-induced arrhythmia index, diastolic interval are delayed by cardiomyocyte-specific (*Tin-gal4*) knockdown of *Babo*. One-way ANOVA (**p<0.01, * p<0.05, ns = not significant). N=15-31 [3].

Activin signaling negatively regulates cardiac autophagy

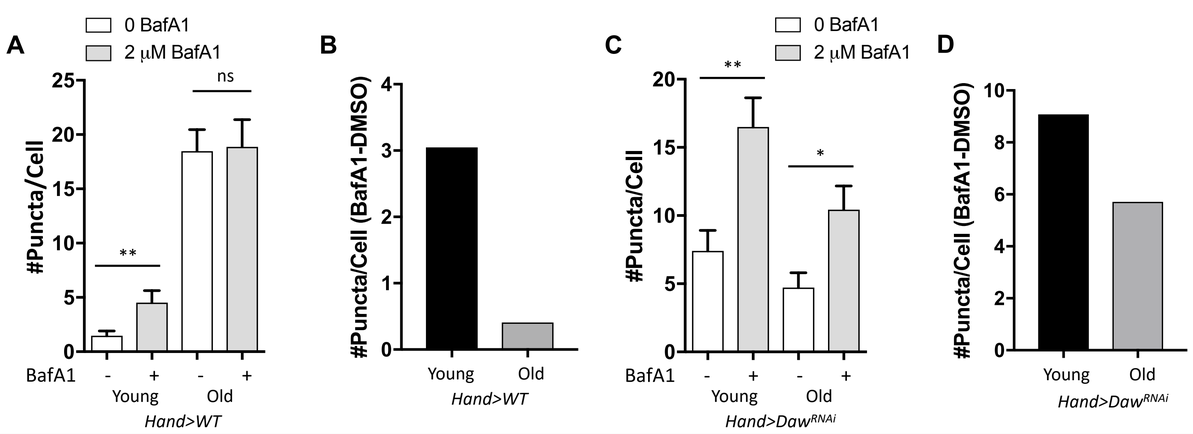


Figure A: The autophagosome number labeled by GABARAP antibodies in young and old *wildtype* heart (*Hand-gal4*) before and after adding BafA1. The quantification for autophagic flux by autophagosome number induction after adding BafA1 shown in **Figure B**. **Figure C**: The autophagosome number labeled by GABARAP antibodies in young and old *Daw^RNAi* heart (*Hand-gal4*) before and after adding BafA1. The quantification for autophagic flux by autophagosome number induction after adding BafA1 shown in **Figure D**. BafA1: Bafilomycin A1, ANOVA (ns: not significant, *: p<0.05, **: p<0.01), N=15 [3]. Young: 2-week-old, Old: 7-week-old.

Activin signaling regulates cardiac autophagy and aging through mTORC2/Rictor

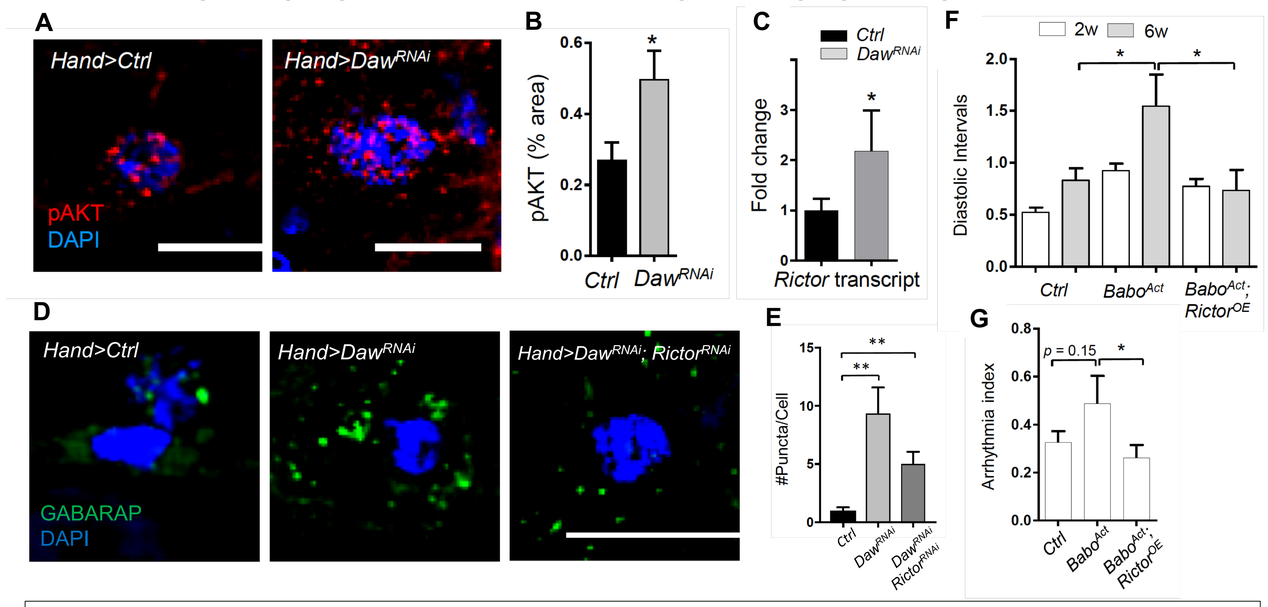


Figure A: Representative images of phospho-AKT staining in cardiomyocytes of *control* and *Daw* knockdown flies (*Hand-gal4*). Scale bar: 10μm. Quantification shown in **Figure B**. N=5. Student t-test (*p<0.05). **Figure C**: qRT-PCR analysis of Rictor expression in fly hearts with *Daw* and *Babo* knockdown (*Hand-gal4*). N=3. Student t-test (*p<0.05). **Figure D:** Representative images of GABARAP immunostaining in cardiomyocytes of *control*, *Daw^RNAi*, and *Daw^RNAi; Rictor^RNAi*. Scale bar: 20μm. Quantification shown in **Figure E**. **Figure F-G:** Diastolic intervals and arrhythmia in flies with heart-specific expression of *Babo^Act*, or both *Babo^Act* and *Rictor* (*Hand-gal4*). N=11-27. One-way ANOVA (**p<0.01, *p<0.05) [3].

mTORC2/Rictor regulates cardiac mitochondrial morphology

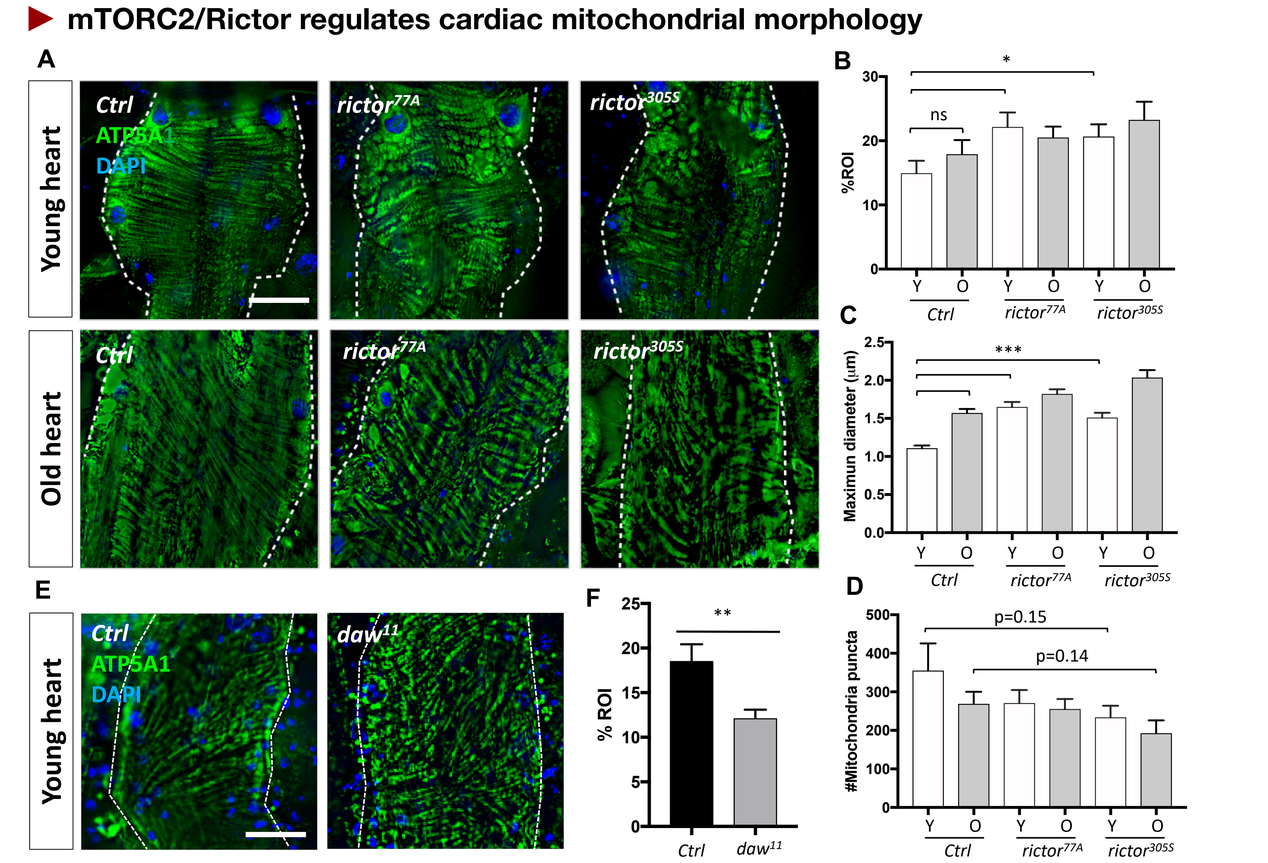
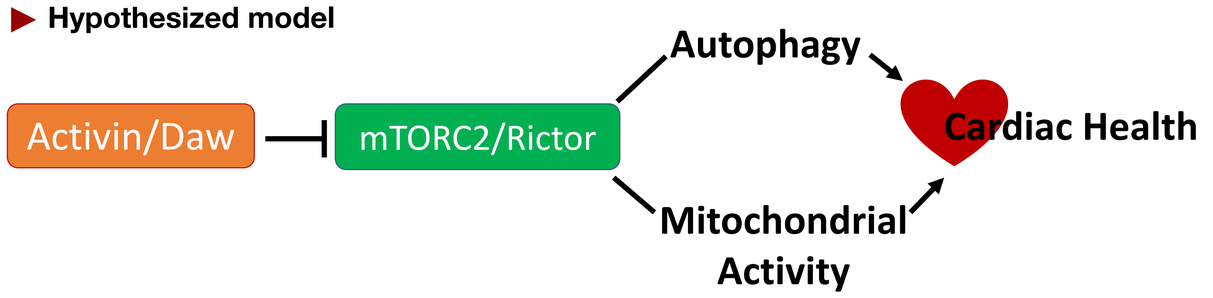


Figure A: Representative images of ATP5A1 immunostaining in cardiomyocytes of *control*, *rictor^77A and rictor^305S* flies. Quantification for percentage area per region of interest (ROI) shown in **Figure B**, maximum diameter shown in **Figure C**, and puncta number shown in **Figure D**. One-way ANOVA (***p<0.05, **p<0.01, *p<0.05, ns: not significant), N=5 (for Figure B), N=129-348 (for Figure C, D). **Figure E:** Representative images of ATP5A1 immunostaining in cardiomyocytes of *control* and *daw^11*. Quantification shown in **Figure F**. student t-test (**p<0.01), N=5. Y: Young (2-week-old), O: Old (6-week-old). Scale Bar: 20μm.

Hypothesized model



Acknowledgement and Reference

We thank Bloomington Stock Center, Michael O'Connor (University of Minnesota, US), Ernst Hafen (Institute of Molecular System Biology, Switzerland) for fly lines. This work is supported by NIH Research grant to Hua Bai and AHA Predoctoral Fellowship to Kai Chang.

References:

[1] Bai et al, 2013, PLOS Genetics.
[2] Saxton and Sabatini, 2017, Cell.
[3] Chang et al, 2018, BioRxiv.

NIH

American Heart Association.