



Published in final edited form as:

*Circ Res.* 2018 March 02; 122(5): 649–651. doi:10.1161/CIRCRESAHA.118.312655.

## Regulating Renewable Energy: Connecting AMPK $\alpha$ 2 to PINK1/ Parkin-Mediated Mitophagy in the Heart

Sarah Shires and Åsa B. Gustafsson

Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, La Jolla, California

### Keywords

Mitophagy; AMPK; PINK1; Parkin

Heart failure is a multifaceted disease in which mitochondrial dysfunction plays a large contributing role. Mitochondria are essential for maintaining cellular ATP levels sufficient to sustain the beating cardiac myocytes. Their function, however, extends beyond providing myocytes with energy, as they also regulate metabolism, heme synthesis, calcium homeostasis, ROS production, and programmed cell death<sup>1</sup>. To ensure a network of operational and efficient mitochondria, myocytes use mitochondrial autophagy or mitophagy to eliminate damaged or unwanted mitochondria. The most well-known and studied mitophagy pathway to date is mediated by two proteins, PINK1 and Parkin. The PINK1/Parkin pathway was initially identified as important regulators of mitochondrial integrity and function in muscle cells and dopaminergic neurons in *Drosophila*<sup>2, 3</sup>, but recent studies have additionally confirmed its importance in maintaining cardiac homeostasis and adaptation to stress<sup>4–6</sup>. Most mechanistic studies on the PINK1/Parkin pathway have been conducted *in vitro* using harsh mitochondrial toxins to activate mitophagy. However, these conditions do not appropriately mimic conditions *in vivo*. Thus, physiologic mechanisms of upstream PINK1 and Parkin activation are still under intense investigation. In this issue of *Circulation Research*, Wang et al. sheds light on the regulation of PINK1/Parkin-mediated mitophagy in the heart<sup>7</sup>. Specifically, the authors have identified a role for AMPK in activating mitophagy via PINK1 phosphorylation to prevent development of heart failure.

The selective degradation of mitochondria is an essential process for mitochondrial quality control, both under physiologic conditions and in response to pathologic stress. When mitochondria are healthy, the serine/threonine kinase PTEN-inducible kinase 1 (PINK1) is rapidly imported into the mitochondria via the TOM/TIM complex where it is cleaved by the mitochondrial protease PARL (Fig. 1A). Cleaved PINK1 is then retro-translocated into the cytosol and degraded by the proteasome<sup>8</sup>. However, upon mitochondrial damage, import is terminated and PINK1 accumulates on the outer mitochondrial membrane. PINK1 then

Corresponding author: Åsa Gustafsson, University of California, San Diego, 9500 Gilman Drive, La Jolla CA 92093-0758, (858) 822-5569 (phone), asag@ucsd.edu.

### Disclosures

None.

recruits the E3 ubiquitin ligase Parkin to the mitochondria to ubiquitinate mitochondrial substrates<sup>9</sup>. The buildup of ubiquitin flags the mitochondrion for selective elimination by autophagosomes. Thus, PINK1 accumulation acts as the initial mitochondrial stress sensor that activates mitophagy.

AMP-activated protein kinase (AMPK) senses changes in cellular energy levels and regulates a number of cellular processes that influence cardiac myocyte survival<sup>10</sup>. When cellular energy levels are compromised, AMPK reduces energy-consuming pathways while increasing ATP-generating catabolic pathways. In their study, Wang et al. identified an additional function for AMPK $\alpha$ 2 in myocytes: activating PINK1/Parkin-mediated mitophagy through PINK1 phosphorylation to protect against the development of heart failure (Fig. 1B).

### **Switching Sides: AMPK $\alpha$ isoforms switch during development of heart failure**

AMPK is a heterotrimer composed of a catalytic  $\alpha$  subunit and two regulatory  $\beta$ - and  $\gamma$ -subunits. Each subunit has multiple isoforms encoded by distinct genes which allow for the expression of 12 different AMPK complexes. An essential and largely unresolved question is what biological function do distinct AMPK isoforms and complexes serve. In the current study, Wang et al. compared expression of AMPK in normal and failing human hearts and noted that AMPK $\alpha$ 2 is the predominant form in the healthy human heart<sup>7</sup>. In contrast, AMPK $\alpha$ 1 is elevated and AMPK $\alpha$ 2 reduced in the failing heart. This isoform switch was confirmed using mice subjected to trans-aortic constriction (TAC) as a model of heart failure. In these experiments, the authors performed an extensive time course to distinguish physiologic changes that occur in the heart during early versus late phases of pressure overload. Intriguingly, they observed an increase in AMPK $\alpha$ 2 in the early, compensatory phase (3-5 days post TAC) followed by a decrease in the late phase. In contrast, they found a steady increase in AMPK $\alpha$ 1 levels up to heart failure development. Consistent with previous reports<sup>11</sup>, the authors found that both autophagy and mitophagy increase during the early phase of TAC, and subsequently decrease in late-phase. Interestingly, levels of autophagy and mitophagy rose and fell concurrently with levels of AMPK $\alpha$ 2. The decline in AMPK $\alpha$ 2 also correlates with an accumulation of dysfunctional mitochondria, implicating AMPK $\alpha$ 2 as a necessary factor for mitophagy in the heart.

### **The Mitophagy Connection: AMPK $\alpha$ 2 activates PINK1/Parkin-mediated mitophagy**

The specific role of AMPK $\alpha$ 2 in regulating PINK1/Parkin-mediated mitophagy *in vivo* was investigated through gain-of-function and loss-of-function experiments<sup>7</sup>. Overexpression of AMPK $\alpha$ 2 in the heart using AAV9-mediated gene delivery led to increased PINK1/Parkin-mediated mitophagy, preserved mitochondrial function and improved cardiac function in the chronic, late phase of TAC compared with control mice. In contrast, AMPK $\alpha$ 2-deficiency caused reduced mitophagy and impaired mitochondrial function in hearts and accelerated the

progression of TAC-induced heart failure. These findings establish a clear link between AMPK $\alpha$ 2, mitophagy, and mitochondrial function in heart failure.

The authors confirm their findings *in vitro* using cultured myocytes treated with phenylephrine (PE) to mimic the TAC-induced cardiomyocyte hypertrophy<sup>7</sup>. Although PE is effective at inducing hypertrophy, it only modestly induces mitophagy. Therefore, treatment with the potent mitochondrial uncoupler CCCP was also used to promote mitophagy. In line with the results of the TAC studies, the *in vitro* data demonstrated that treatment with PE for 24h reduces AMPK $\alpha$ 2 levels and PINK1/Parkin-mediated mitophagy, and increases the number of damaged mitochondria. As expected, these effects were diminished with AMPK $\alpha$ 2 overexpression. Taken together, these experiments further confirm the link between AMPK $\alpha$ 2 and the PINK1/Parkin pathway in myocytes.

### The Phosphorylation Game: AMPK $\alpha$ 2 phosphorylates PINK1 at Ser495

To determine how AMPK $\alpha$ 2 activates PINK1/Parkin-mediated mitophagy, the authors conducted co-immunoprecipitation experiments that confirmed that AMPK $\alpha$ 2, but not AMPK $\alpha$ 1, interacts with PINK1<sup>7</sup>. To investigate if PINK1 is an AMPK $\alpha$ 2 substrate, the authors treated myocytes with PE and ran a phos-tag gels. AMPK $\alpha$ 2 overexpression increases phosphorylation-induced mobility shift in PINK1 after PE treatment for 24h. Conversely, si-AMPK $\alpha$ 2 depleted the phosphorylated PINK1 in myocytes treated with PE for 6h. They also used software to predict potential phosphorylation sites and LC MS/MS to identify phosphorylated residues in PINK1 in the presence of AMPK $\alpha$ 2. Two serine residues were found to be consistently phosphorylated: Ser284 and Ser495. After analyses using site directed mutagenesis, Ser495 emerged as the candidate phosphorylation site. Specifically, the authors found that mutating Ser495 to alanine led to reduced PINK1/Parkin-mediated mitophagy, whereas mutating Ser495 to Asp to mimic phosphorylation enhanced mitophagy. The authors solidify these findings by demonstrating that recombinant AMPK $\alpha$ 2 $\beta$ 2 $\gamma$ 1 interacts with and phosphorylates PINK1 at Ser495 in a cell-free system, and that PINK1 Ser495 is phosphorylated *in vivo* after TAC surgery with AMPK $\alpha$ 2 overexpression, but not in AMPK $\alpha$ 2-deficient hearts. PINK1 activation in response to mitochondrial damage has previously been ascribed to inhibition of its import<sup>8</sup>; however, the study by Wang et al. suggests that an additional layer of regulation exists in myocytes.

### Going Forward: Insights into PINK1 Regulation, Mitophagy, and Heart Failure

This study has several major strengths. Because heart failure development is very complex process, it is especially crucial to study both the early and late phases in pressure overload induced heart failure. Additionally, the authors very convincingly demonstrate that PINK1 is phosphorylated by AMPK $\alpha$ 2 in a cell-free system, *in vitro*, and *in vivo*. However, limitations must also be considered when analyzing the findings of this study. The main limitation of this study is the lack of a physiologically relevant *in vitro* model. The authors use PE to simulate heart failure and treat the cells for 6h and 24h to mimic the early and late phases, respectively. While PE is able to faithfully activate cardiomyocyte hypertrophy, it has not been reported to replicate many other signaling cascades involved in the

development of heart failure including inflammation, fibrosis, and mitophagy. The absence of robust mitophagy activation prompts the authors to use CCCP in addition to PE. CCCP, however, is notoriously un-physiologic and completely depolarizes the entire pool of mitochondria. Whereas during the development of early and late phase heart failure in mice, the time frame is set at days and weeks and involves the activation of a myriad of signaling cascades, these un-physiologic *in vitro* conditions use a much shorter time period and stimulate only a couple of these pathways.

Nevertheless, the findings in this study raise many thought-provoking questions. For instance, how AMPK $\alpha$ 2-mediated phosphorylation affects the kinase activity of PINK1 needs to be investigated in further detail. The enhanced Parkin-mediated mitophagy could be due to either enhanced PINK1 kinase activity or increased total PINK1 levels. Another important unanswered question is under what conditions AMPK $\alpha$ 2 regulates mitophagy. Is it specific for pressure overload or does it play a role in mitophagy activated by a myocardial infarction or ischemia/reperfusion injury? These are just some of the questions that arise from the current study.

Overall, the findings presented by Wang et al. demonstrate that AMPK $\alpha$ 2 is an important regulator of PINK1/Parkin-mediated mitophagy in the heart. Also, the suppression of mitophagy during the chronic phase of TAC is, in part, due to a decrease in AMPK $\alpha$ 2. The data also reveal distinct functions for AMPK $\alpha$ 1 and AMPK $\alpha$ 2 catalytic subunits in the heart, which highlights the importance of developing isoform-specific drug targets for the treatment of cardiovascular disease.

## Acknowledgments

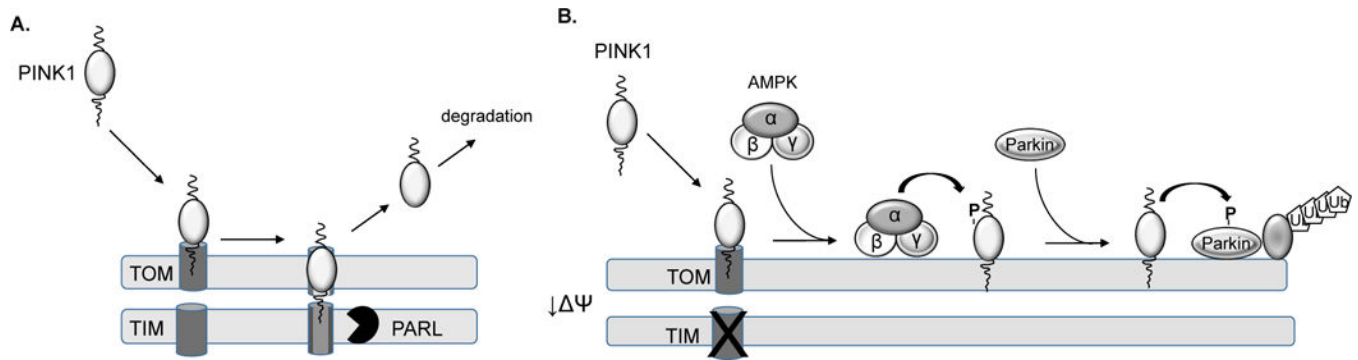
### Sources of Funding

ÅB Gustafsson is supported by an AHA Established Investigator Award, and by NIH R21AG052280, R01HL132300, R01HL138560 and P01HL085577. S Shires is supported by an AHA Pre-doctoral fellowship.

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**Figure 1.**

Activation of PINK1 by AMPK $\alpha$ 2 Regulation of PINK1. **A.** Import of PINK1 into healthy mitochondria via the TOM/TIM import complex. PINK1 undergoes proteolytic cleavage by PARL and cleaved PINK1 retro-translocates to the cytosol where it is degraded by the proteasome. **B.** Upon mitochondrial damage, import of PINK1 is abrogated and it accumulates on the outer mitochondrial membrane which leads to its phosphorylation by AMPK $\alpha$ 2.