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ABSTRACT

Mitochondria are crucial organelles that generate cellular energy and metabolites. Recent studies indicate that mitochondria also regulate immunity. In this review, we discuss key roles of mitochondria in immunity against pathogen infection and underlying mechanisms, focusing on discoveries using *Caenorhabditis elegans*. Various mitochondrial processes, including mitochondrial surveillance mechanisms, mitochondrial unfolded protein response (UPR^mt), mitophagy, and reactive oxygen species (ROS) production, contribute to immune responses and resistance of *C. elegans* against pathogens. Biological processes of *C. elegans* are usually conserved across phyla. Thus, understanding the mechanisms of mitochondria-mediated defense responses in *C. elegans* may provide insights into similar mechanisms in complex organisms, including mammals.

INTRODUCTION

Mitochondria are regarded as subcellular organelles with highly dynamic properties. Mitochondria participate in various essential cellular processes, including energy production, metabolite synthesis, and calcium homeostasis. Interestingly, recent studies indicate that mitochondria also function in unconventional processes, including immune and defense responses against pathogens and other stressors. For example, mitochondrial reactive oxygen species (mtROS) and damage-associated molecular patterns (DAMPs) can activate anti-bacterial and anti-viral innate immune responses in mammalian cells (1, 2). However, most studies on the role of mitochondria in immunity have been performed using cultured cells but rarely using live organisms.

    Caenorhabditis elegans is an outstanding model organism to study innate

immunity for several reasons (3, 4). First, diverse bacteria such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* that infect *C. elegans* are also human pathogens. Second, *C. elegans* is genetically tractable for identifying key immune factors. Third, assays that measure the death of *C. elegans* on pathogens, the ultimate outcome of infection and impaired immunity, are rapid and inexpensive. Fourth, changes in both host and pathogen can be simultaneously monitored. Fifth, and most importantly, several key immune response pathways such as p38 mitogen-activated protein kinase (MAPK) pathway are conserved between *C. elegans* and mammals. Using these advantages of *C. elegans*, many important discoveries in the research field of innate immunity, including roles of mitochondria in organismal immunity, have been made over the last two decades.

In this review, we will discuss how mitochondrial processes, including mitochondrial surveillance mechanisms, mitochondrial unfolded protein response (UPR^{mt}), mitophagy, and generation of reactive oxygen species (ROS), participate in *C. elegans* immunity. We further describe mechanisms, pathways, and types of innate immune response genes regulated by these mitochondrial processes to protect animals against pathogens and stressful environments. Information gained using *C. elegans* is generally and widely applicable to other organisms. Therefore, understanding the role of mitochondria in *C. elegans* immunity will provide important clues regarding mitochondrial function in immunity in mammals, including humans.

2. Mitochondrial surveillance

Mitochondrial surveillance is important for survival upon pathogen infection

Mitochondria are frequent targets of virulence factors and microbial toxins as they are

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rich in hemes and iron-sulfur proteins (5), which are scarce in the environment but crucial for the survival of pathogens (6, 7). Early warning signals of mitochondrial dysfunction are used as one of the first lines of defense to initiate immune responses. Mitochondrial surveillance mechanisms constantly monitor the disruption of mitochondrial homeostasis and repair damaged mitochondria, thus protecting the mitochondria from various environmental stresses (8). Up-regulation of several defense-related genes, including those involved in drug-detoxification, mitochondrial repair, and response to pathogen infection, is an important part of the mitochondrial surveillance pathway. This restores mitochondrial homeostasis under environmental stresses and increases host survival (9). Overall, mitochondrial surveillance mechanisms can sense mitochondrial dysfunction and activate genes involved in mitochondrial damage repair, thus improving host immunity and its chance of survival.

Conserved abiotic stress mechanism contributes to mitochondrial surveillance-mediated immunity

Ethanol and stress response element (ESRE) network is a conserved defense mechanism in response to various abiotic stresses. Activation of ESRE gene network contributes to the immunity of *C. elegans* against *P. aeruginosa* infection through mitochondrial surveillance mechanisms (Figure 1) (10). *P. aeruginosa* can utilize a variety of virulence factors to colonize and infect diverse hosts, including *C. elegans* and humans. ESRE is a conserved 11-nt sequence motif that is enriched in regulatory regions of genes specifically up-regulated in *C. elegans* in response to liquid culture-based infection of *P. aeruginosa*. Importantly, the induction of ESRE-containing genes during *P. aeruginosa* infection is required for innate immune responses in *C. elegans*.

The ESRE gene network is activated in response to pyoverdine, a virulent factor toxin and high-affinity iron chelator secreted by *P. aeruginosa* for iron uptake (11). Pyoverdine targets mitochondria and causes a hypoxia-like response in *C. elegans*, which is different from the classical innate immune response against intestinal *P. aeruginosa* colonization (10). *C. elegans* appear to monitor iron level and mitochondrial damage, both of which can act as signals to activate the ESRE network against *P. aeruginosa*. The induction of ESRE gene network appears to be mediated by several bZIP transcription factors whose functions are crucial for innate immune responses against *P. aeruginosa*. Overall, these studies suggest that the ESRE defense network contributes to mitochondrial surveillance which in turn promotes immunity against *P. aeruginosa*.

3. Mitophagy

Mitophagy is an autophagic process that degrades damaged mitochondria for recycling (12). One of the first processes of mitophagy is the interaction between PTEN-induced mitochondrial kinase 1 (PINK1) and cytosolic E3 ubiquitin ligase Parkin (13). The accumulation of PINK1 on damaged mitochondria is both necessary and sufficient for the recruitment of Parkin (14-17). This event also enables the autophagic machinery to distinguish between dysfunctional and healthy mitochondria. Damaged mitochondria are subsequently engulfed by autophagosomes and delivered to lysosomes for degradation (18). Mitophagy is critical for maintaining energy and calcium homeostasis and for metabolite synthesis. Recent studies have shown that the interplay between the orchestrated mitochondrial complex and mitophagy machinery is critical for immune response (19). *P. aeruginosa* infection disrupts mitochondrial homeostasis of *C. elegans*

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because of iron chelation, which triggers mitochondrial turnover (Figure 1). This leads to mitophagy in *C. elegans* to cope with the loss of iron and bring the electron potential across the mitochondrial membrane back to normal. Increase in the expression of PINK1 and autophagy machinery proteins following *P. aeruginosa* infection activates mitophagy in *C. elegans*. This appears to be a key event for immunity because the disruption of genes required for mitophagic regulation (*pink-1*/PINK1 and *pdr-1*/Parkin) or general autophagy (*bec-1*/Beclin 1, *lgg-1*/microtubule-associated protein light chain 3 (LC3) and *mboa-7*/membrane-bound O-acyltransferase domain-containing protein 7) significantly increases the mortality of *C. elegans* upon infection with *P. aeruginosa* (19). Thus, the induction of mitophagy is essential for enhancing resistance against pathogen infection.

4. Mitochondrial unfolded protein response

Unfolded protein response (UPR) maintains protein homeostasis important for cellular function and organismal survival

UPR is an evolutionarily conserved proteostasis (protein homeostasis) system in various organisms (20-26). Multiple proteostasis pathways exist in the cytosol, endoplasmic reticulum, and mitochondria. These pathways are coordinated by and/or communicate with the nucleus. Mechanisms of heat-shock response in the cytoplasm and of UPR in the endoplasmic reticulum (UPR^{ER}) have been extensively studied (21, 22, 24). UPR^{mt} has also been recently discovered and functionally characterized (23, 26).

The accumulation of misfolded or unfolded proteins in the mitochondrial matrix beyond the capacity of mitochondrial chaperones triggers UPR^{mt} (27). This

event drives ATFS-1, a bZip transcription factor that possesses both mitochondrial and nuclear localization sequences, to translocate from mitochondria to the nucleus (27, 28). Subsequently, a mitochondrial matrix protease, CLPP-1, cleaves excessive proteins into short peptides (29). HAF-1, an ATP-binding cassette (ABC) transporter, then exports these small peptides from mitochondria to the cytosol and slows mitochondrial import of ATFS-1, further assisting its nuclear localization (27, 28). Nuclear localized ATFS-1 then transactivates mitochondrial protective genes, including those encoding mitochondrial chaperones that help mitochondrial proteostasis, and anti-microbial genes (27, 30, 31).

Exposure to *P. aeruginosa* is one of the stimuli that cause mitochondrial stress and activate UPR^{mt} in *C. elegans (30)*. *P. aeruginosa* accumulates in the intestine of worms upon infection and causes mitochondrial stress in intestinal cells (Figure 2). Increased expression of mitochondrial chaperone genes and ATFS-1 translocation to nuclei are detected during *P. aeruginosa* infection. Subsequently, nuclear localized ATFS-1 induces the expression of innate immunity genes in addition to mitochondrial protective genes to cope with the infection. Thus, utilizing UPR^{mt} through ATFS-1 is an important means to detect pathogen infection by sensing impaired mitochondria and initiating innate immune responses in *C. elegans*.

Mitochondrial chaperone HSP-60 enhances innate immunity against pathogenic bacteria

We have previously performed genetic screening of over 200 evolutionarily conserved mitochondrial components to identify novel mitochondrial factors that affect *C. elegans*

innate immunity against *P. aeruginosa* (32). Our subsequent functional analysis has shown that genetic inhibition of HSP-60, one of candidates identified from the screen, drastically reduces the chance of survival of *P. aeruginosa*-infected worms. HSP-60 is a nuclear encoded mitochondrial chaperone and a key component of UPR^{mt} (23, 26). Mitochondrial chaperones including HSP-60 can reduce the level of misfolded or unfolded proteins and help maintain mitochondrial proteostasis. We have shown that HSP-60 acts in intestinal cells and neurons to enhance the innate immunity of *C. elegans* against *P. aeruginosa* (32). Furthermore, HSP-60 has a small effect on the lifespan and pathogen avoidance behavior of *C. elegans*, suggesting that HSP-60 can specifically increase innate immunity against *P. aeruginosa*.

HSP-60 up-regulates p38 MAPK/PMK-1 signaling to enhance immunity

The main immune signaling pathways in *C. elegans* include PMK-1 (p38 MAPK), ZIP-2, SKN-1/Nrf, and DAF-16/FOXO pathways (3, 4, 33). HSP-60 increases innate immunity against *P. aeruginosa* in a PMK-1-dependent manner (Figure 2). However, HSP-60-mediated innate immunity is independent of other immune regulators such as ZIP-2, SKN-1/Nrf, and DAF-16/FOXO (32). PMK-1 is activated via phosphorylation by upstream kinases that are important for resistance against *P. aeruginosa*. HSP-60 appears to contribute to the maintenance of active phosphorylated PMK-1 by increasing the level of upstream MAPKK. Interestingly, a large fraction of HSP-60 is localized in the cytosol as well as the mitochondria. The cytosolic HSP-60 physically binds to MAPKK and increases resistance against *P. aeruginosa* via stabilizing MAPKK. Overall, our findings suggest that HSP-60, previously known as a mitochondrial chaperone, can act through cytosolic PMK-1 signaling to enhance innate immunity in *C.*

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elegans.

5. Mitochondrial ROS

ROS act as signaling molecules for physiology

ROS, such as superoxide, hydroxyl radical, hydrogen peroxide, and singlet oxygen, are highly reactive and toxic molecules (34, 35). Mitochondria generate high amounts of ROS as byproducts of the electron transport chain during oxidative phosphorylation. Various cellular stimuli including cytokines, hypoxia, and increased calcium concentration regulate levels of mtROS. mtROS are not only toxic byproducts but also play key roles in various cellular processes, including cell differentiation, autophagy, metabolic adaptation, longevity, and immunity.

A feedback mechanism regulates mtROS-mediated immunity

We have previously shown that mtROS can increase resistance against pathogenic bacteria via hypoxia-inducible factor 1 (HIF-1) and AMP-activated protein kinase (AMPK) (36) (Figure 3). Mild inhibition of mitochondrial respiration has been shown to increase lifespan and the level of mtROS (37, 38). We have shown that increased mtROS levels up-regulate AMPK and HIF-1, both of which are important mediators of longevity (36). HIF-1 has a conserved AMPK recognition motif that is directly phosphorylated by AMPK. Interestingly, AMPK and HIF-1 mutually down-regulate each other. This appears to be important for equilibrating signaling between these two factors.

AMPK and HIF-1 also regulate mtROS levels through feedback mechanisms. AMPK inhibits mtROS production in a negative feedback manner whereas HIF-1

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increases mtROS production through positive feedback regulation. Together, AMPK and HIF-1 maintain mtROS at an optimal level to increase innate immunity against various pathogenic bacteria, including *P. aeruginosa*, *Enterococcus faecalis*, and pathogenic *E. coli* (36). In contrast to HIF-1 and AMPK, other known factors regulating the longevity of mitochondrial respiration mutants that contain high ROS levels have small or no effects on innate immunity. Therefore, the regulation of appropriate mtROS levels through feedback mechanisms involving AMPK and HIF-1 enhances innate immunity.

Mitochondrial proline catabolism regulates innate immunity via ROS and SKN-1/Nrf in *C. elegans*

Treatment of *C. elegans* with proline increases its innate immunity against *P. aeruginosa* (39) (Figure 3). Proline dehydrogenase (PRODH) catabolizes proline to pyrroline-5-carboxylate (P5C). PRODH is required for proline-induced immunity by increasing ROS levels and for the activation of SKN-1/Nrf, an important transcription factor for innate immunity and detoxification. Increasing proline and PRODH levels leads to the accumulation of P5C, which acts as a signal for ROS production and SKN-1/Nrf activation in response to *P. aeruginosa* infection. Additionally, BLIS-3/DUOX, a plasma membrane-localized NADPH oxidase/dual-oxidase, is another major source of ROS production. BLIS-3/DUOX is required for proline-induced immune response. Cytosolic ROS produced by BLIS-3/DUOX subsequently attack pathogenic bacteria and increase protective SKN-1/Nrf activity in a p38 MAPK-dependent manner in the intestine (40-42). Thus, communication between mitochondria and plasma membrane via ROS contributes to resistance of *C. elegans* against pathogenic bacteria. In addition

to these findings, epidermal wounding generates mtROS in response to Ca^{2+} influx during wound closure (43). Increased mtROS play a role in wound healing via regulating actin ring closure. Together, these studies suggest that mtROS play critical roles in defense mechanisms such as anti-bacterial immunity and wound healing in *C. elegans*.

CONCLUSIONS

Because mitochondria have very important cellular functions, alterations or defects in mitochondrial activity will adversely affect energy metabolism, development, health span, and lifespan in various organisms (26, 44-46). However, the role of mitochondria in immunity has only been recently established. Molecular genetics studies in *C. elegans* have answered many important research questions. Mitochondrial surveillance mechanisms such as UPR^mt, mitophagy, and ROS production are recently discovered mitochondrial processes that participate in *C. elegans* immune response against pathogen infection. These mitochondrial processes utilize different types of established immune pathways, such as p38 MAPK/PMK-1, PINK1, HIF-1, AMPK, and SKN-1/Nrf.

The mitochondrial processes covered in this review are evolutionarily well conserved in diverse species. Therefore, findings in *C. elegans* immunity also have implications in other species, including mammals. Further studies on the network of mitochondrial processes and defense mechanisms are needed to assist the development of novel therapeutic interventions that can improve pathogen resistance and human health.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

FIGURE LEGENDS

Figure 1. Mitochondrial surveillance and mitophagy contribute to immunity.

Pyoverdine, a siderophore produced by *Pseudomonas aeruginosa*, binds and removes iron in hosts, triggering mitochondrial stress. Host mitochondrial surveillance mechanism detects mitochondrial stress and activates ESRE (Ethanol and Stress Response Element) gene network. The induction of ESRE-containing genes increases immunity. Reduced mitochondrial iron levels also induce mitophagy that enhances immunity, acting through autophagy machinery proteins, including PTEN-induced putative kinase 1 (PINK1), Beclin 1, and microtubule-associated protein light chain 3 (LC3).

Figure 2. Mitochondrial unfolded protein response mediates *C. elegans* immunity.

Pathogen infection causes mitochondrial stress and activates mitochondrial unfolded protein response (UPR^{mt}) in *C. elegans*. ATFS-1, a key UPR^{mt} regulatory transcription factor, is translocated to the nucleus to induce anti-microbial genes and mitochondrial chaperones. Mitochondrial chaperone HSP-60 directly binds to cytosolic mitogen-

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activated protein kinase kinase (MAPKK), which up-regulates p38 mitogen-activated protein kinase (PMK-1) immune signaling.

Figure 3. Mitochondrial reactive oxygen species promote immunity.

Upon pathogen infection, mitochondrial ROS (mtROS) increase immunity in *C. elegans*. AMP-activated protein kinase (AMPK) and hypoxia-inducible factor 1 (HIF-1) mediate mtROS-induced immunity through negative and positive feedback mechanisms, respectively. Mitochondrial proline catabolism also regulates ROS levels and immunity. The intermediate metabolite produces cytosolic ROS by dual oxidase (DUOX) and activates SKN-1/Nrf, an important immune regulator. Proper communication between mitochondrial and cytosolic ROS leads to enhanced immunity against *P. aeruginosa.*

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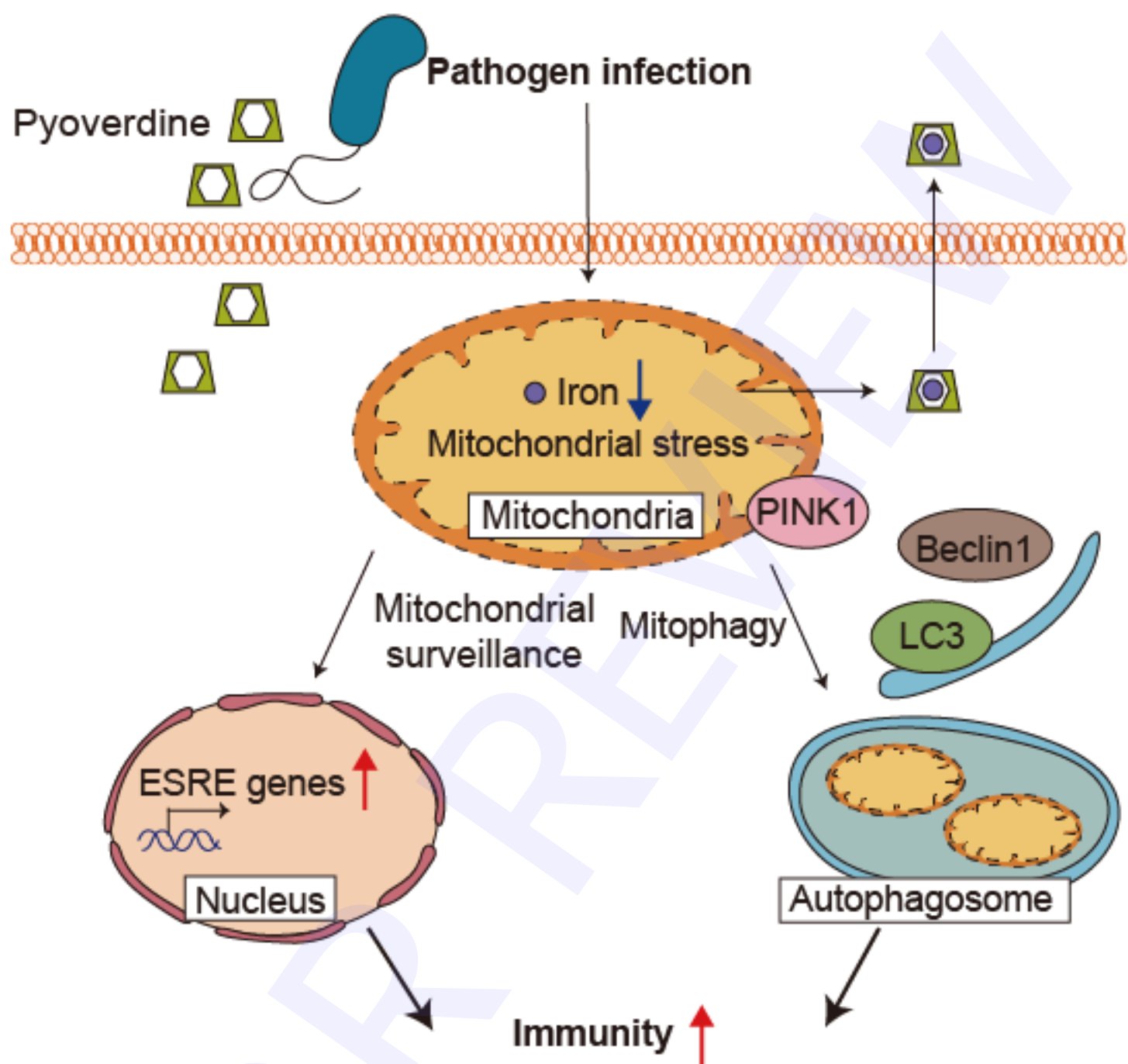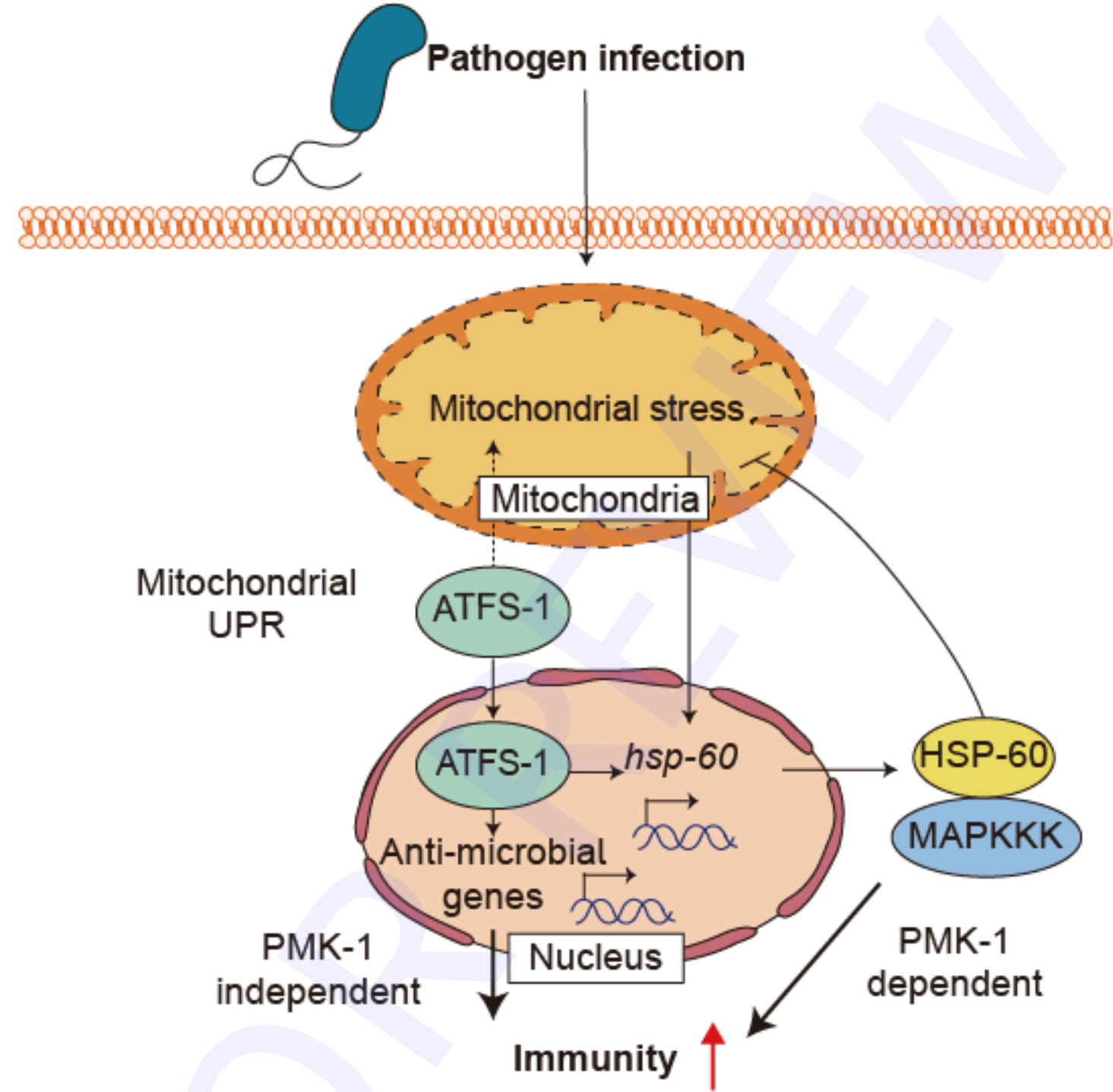
Figure 1

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