# Mitochondrial Stress Signaling During Ischemia Reperfusion Injury: The Roles of Mitochondrial Ca2+ Uptake, Dynamics, and Cell Death

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# Outline

- Presentación de Miembros
- Intro/Background
- Objective
- MCU
- Fusion
- Fission
- Apoptosis
- Discussion
- Conclusion

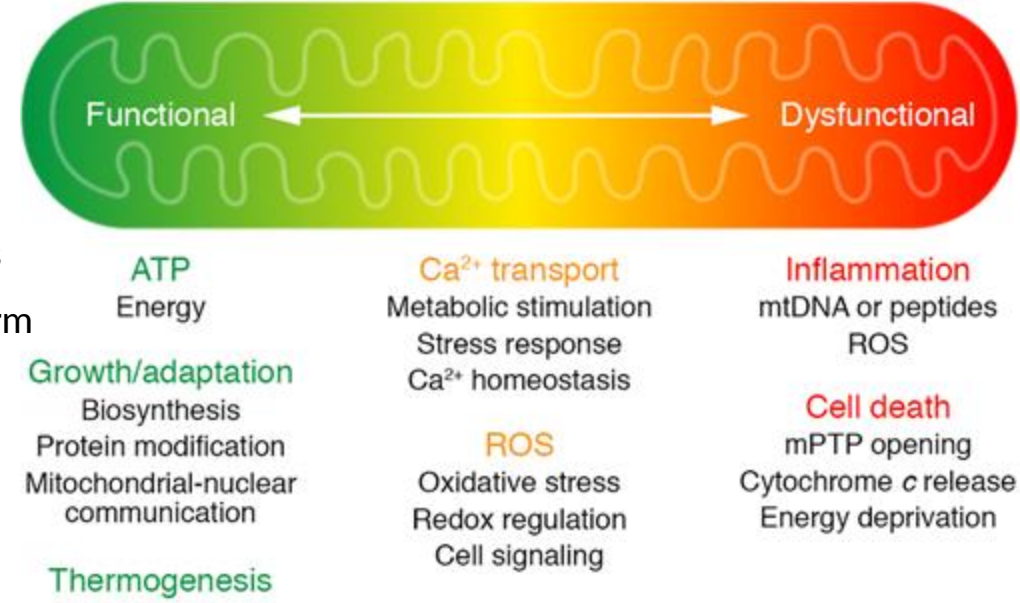
# Introduction- The Mitochondria

Mitochondrial structure:

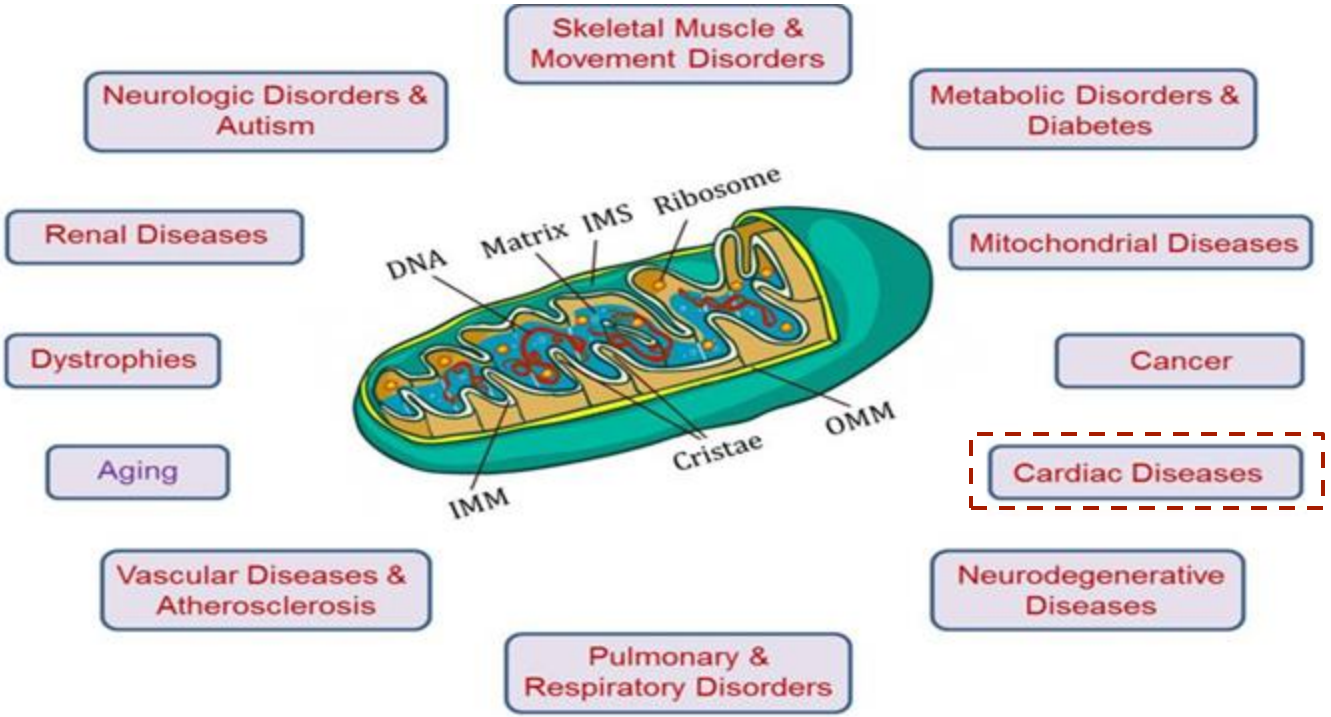
- Double membrane organelle

Mitochondrial function:

- The “powerhouse” of the cell, produces ~90% of cellular energy in the form of ATP.
- Ion homeostasis
- ROS production
- Cell growth
- Cell death signaling



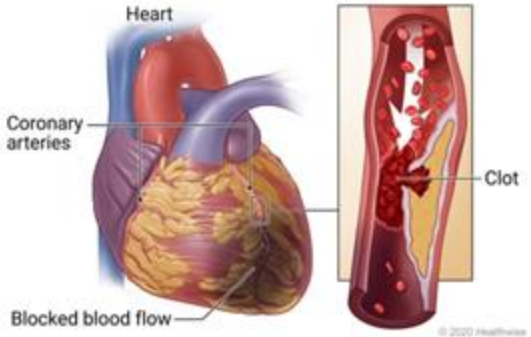
# Introduction- Mitochondrial Pathologies



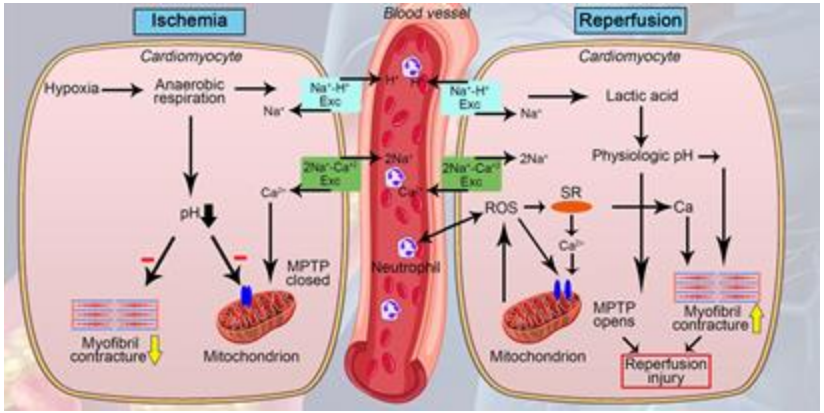
# Introduction- Ischemia and Reperfusion

**Cardiac IR injury is divided into two phases:**

- *Ischemic phase*: reduction in blood flow resulting in decreased oxygen and nutrient supplies to a tissue.
- *Reperfusion phase*: the restoration of oxygen and nutrient supplies to a tissue.




Healthwise staff.Riverside County.(2020)




Gunata et al.Cell Biochemistry and Function.(2020)

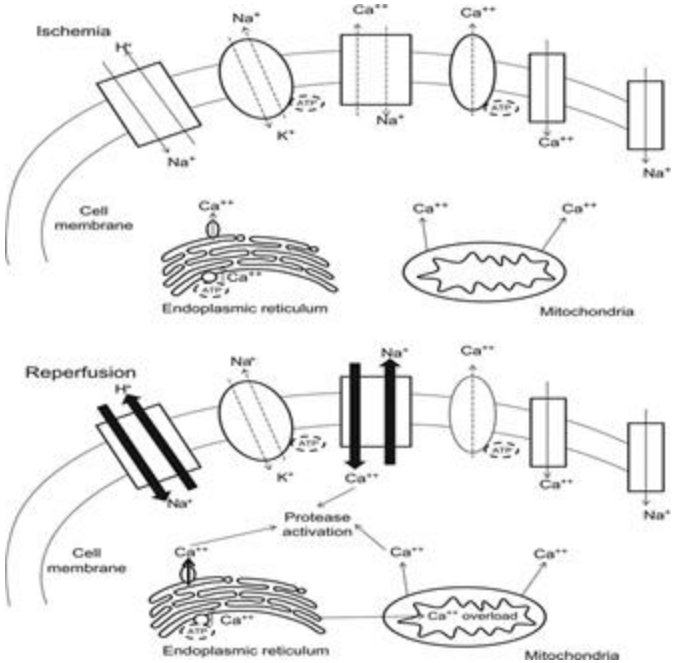
# Introduction- Ischemia and Reperfusion

**Impacts of Ischemia:**

- Creates a more acidic environment
- Imbalance of ionic homeostasis (specifically $Ca^{2+}$)

**Impacts of Reperfusion:**

- Production of ROS
- Alteration of mitochondrial dynamics
- Initiation of cell death signaling

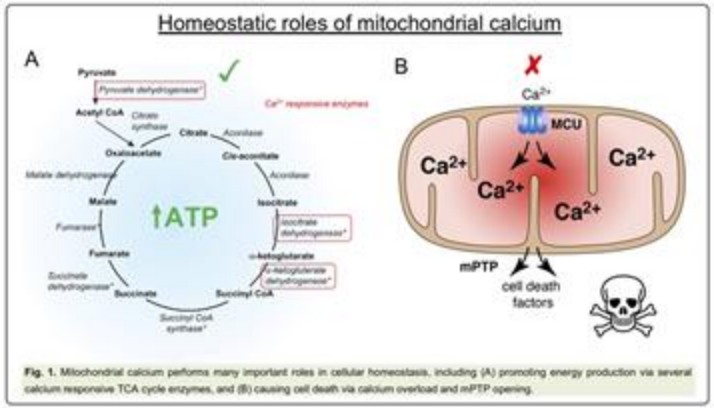


# Objective

- We will be focusing on the relationship between acute IR injury and its role in Ca2+ overload, the effects of IR injury on mitochondrial dynamics (fusion and fission), and the regulation of cell death once the mitochondria becomes compromised due to IR injury.

# Role of Mitochondrial Calcium Uptake via the MCU

- Intracellular Ca2+ are some of the most versatile signaling molecules, their functions range from promoting muscle contraction to participating cell migration.
- Excess Ca2+ may function as a mediator of cell death mechanisms, specifically apoptosis, thus maintaining regulating intracellular Ca2+ levels is vital.
- The mitochondria serve a critical role in maintaining intracellular Ca2+ levels by buffering excess Ca2+ via the mitochondrial Ca2+ uniporter (MCU).



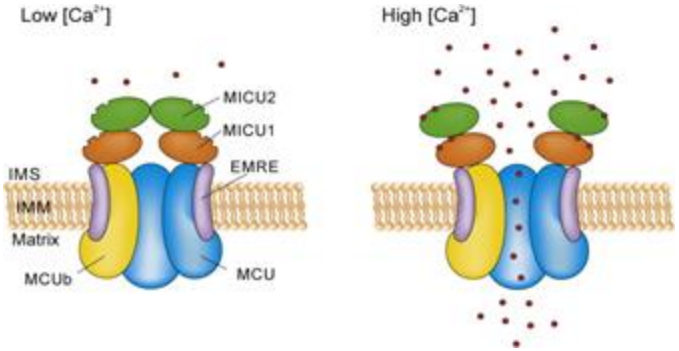

physiological | pathological

# The Mitochondrial Calcium Uniporter (MCU)

- The MCU is a is a ~700 kD multi-subunit channel located in the inner mitochondrial membrane.
- The MCU is a one directional channel that regulates Ca2+ into the mitochondrial matrix.
- Regulation of the MCU is key to conserving calcium homeostasis in the mitochondria, heightening the importance and demand for MCU regulators.

**Regulation of MCU:**

- The MCU is regulated by two central regulators called MICU1 and MICU2.
- Ruthenium red/Ru360 can been used to prevent Ca2+ entry.



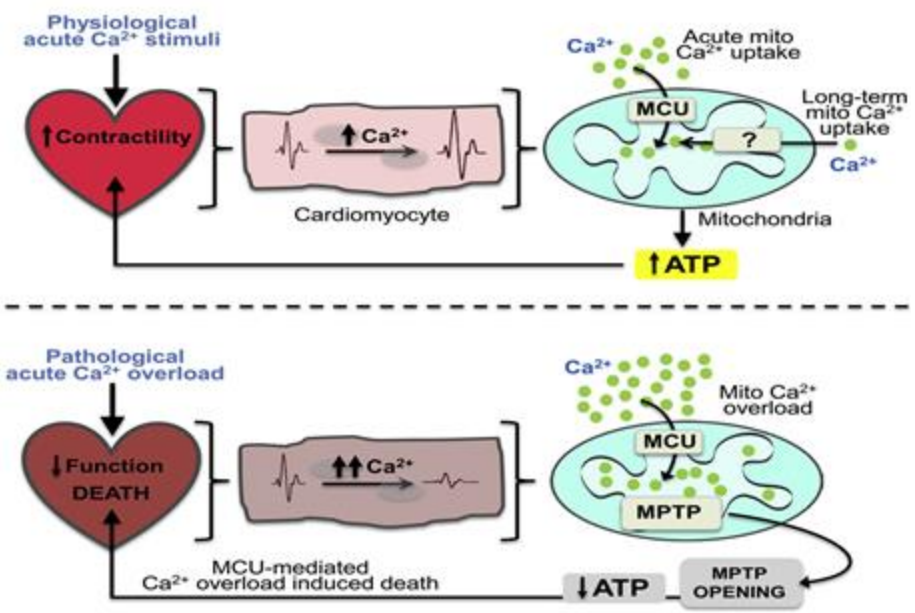

Mammucari et al. BBA. (2016)

# MCU in the Context of IR

- The MCU has been demonstrated to play a **physiological role** in mitochondrial Ca2+ loading to promote ATP production during high cardiac workload conditions.
- After IR Ca2+ loading effect tends to be the main driver of mitochondrial dysfunction.

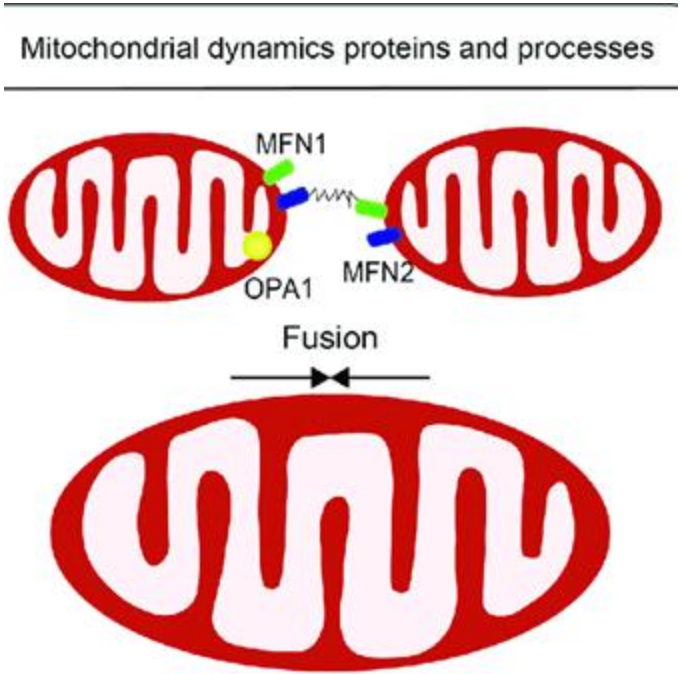
**The effect of pathological Ca2+ overload in the mitochondria include**:

- The production of ROS, the opening of the mitochondrial permeability pore
- The release of pro-apoptotic factor cytochrome C
- <u>Inhibition of mitochondrial fusion</u>



# Mitochondrial Fusion

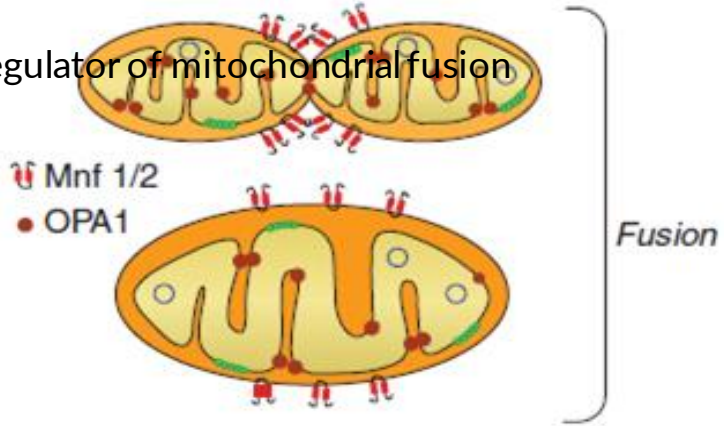
- The mitochondrion is a dynamic organelle that is constantly conducting repair of damaged mitochondria structures.
- Maintenance of important cellular procedures occurs via mitochondrial fusion.
- Mitochondrial fusion is regulated by the interactions between the proteins MFN 1/ 2 and Optic atrophy protein 1 (OPA-1).



# Role of Proteins in Mitochondrial Fusion

- MFN 1 and MFN 2 are responsible for the fusion of the outer

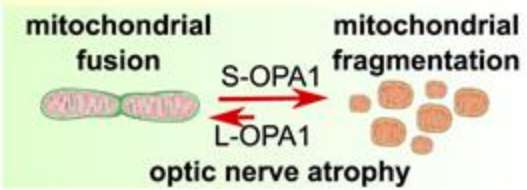
  mitochondrial membrane
- Inner mitochondrial membrane fusion is produced by OPA-1
- OPA-1 is the main regulator of mitochondrial fusion



# OPA-1 Role in IR Injury

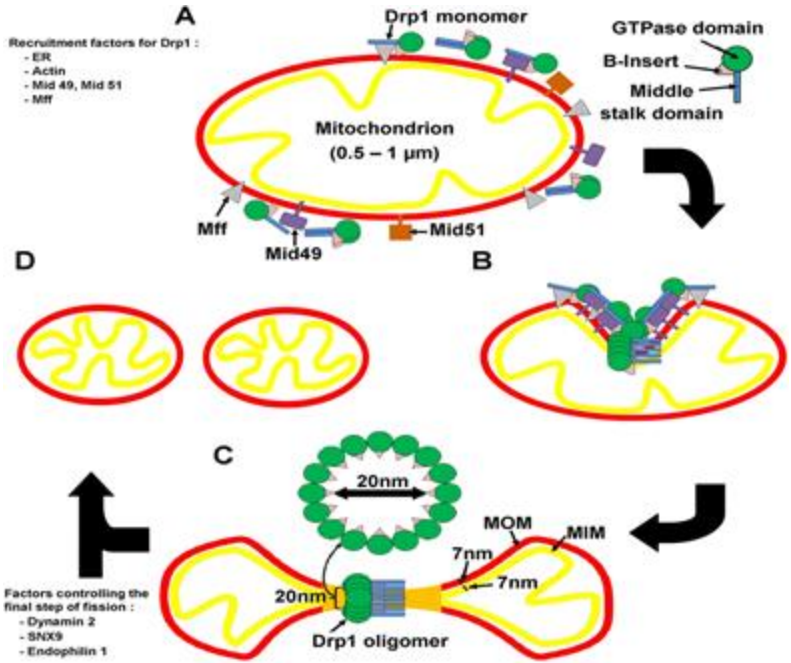
Optic atrophy protein 1 (OPA-1) degradation during IR.

- OPA-1 is important for maintaining mitochondrial cristae structure
- Deficiency of the OPA-1 protein induce mitochondrial fission and increases sensitivity to IR
- OPA-1 degradation may lead to pathological swelling and apoptosis signaling
- S-OPA-1 is the degraded form
  S for short
- L-OPA-1 is the complete form
  L for long



# Role of Mitochondrial Fission

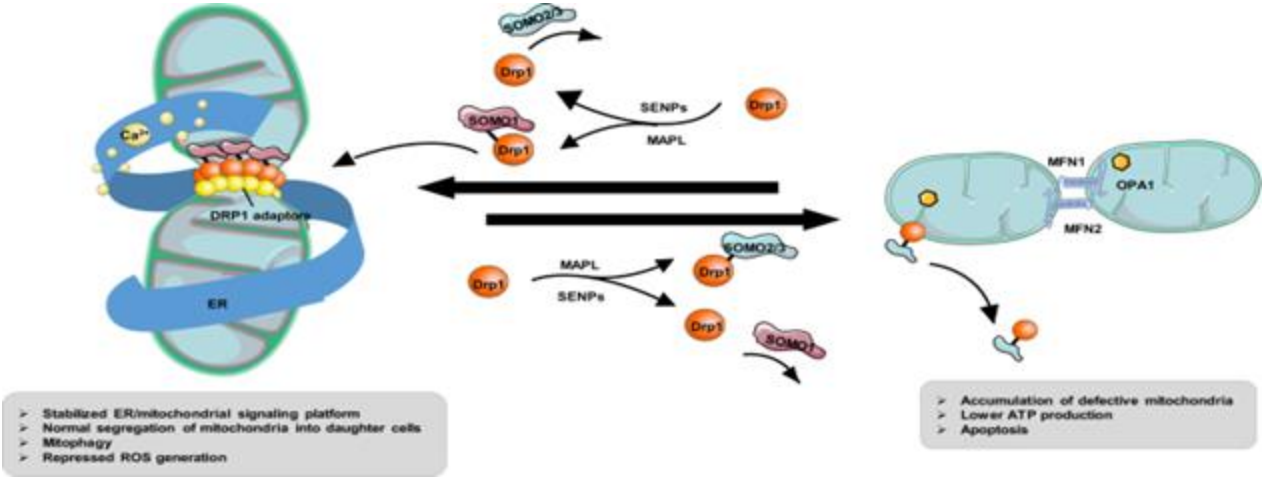
- These processes that are accompanied by fusion manage the mitochondrial distribution inside the cell and maintain quality control by regulating many cellular activities including the activation of apoptosis.
- Consists of dividing functional portions of the mitochondrial network to relocate them within the cell for maintaining energy production.
- Dynamin-related protein-1 (DRP-1) it is a dynamin related protein that regulates the fission process through the outer mitochondrial membrane




Basu et al.Plos One.(2017)

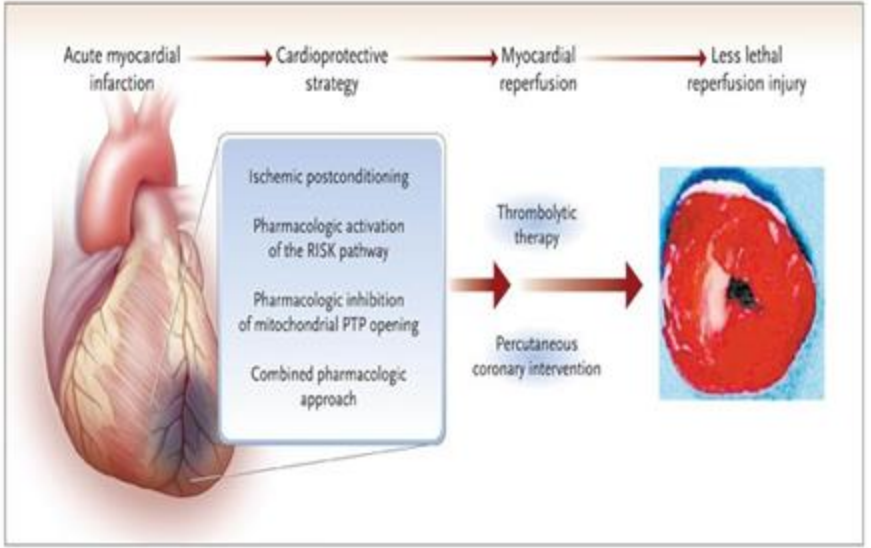
# How Does DRP-1 Work?

DRP-1 works by translocating to the outer mitochondrial membrane to eliminate mitochondria damaged through mitophagy, a mitochondrial degradation process. DRP-1 actively targets the outer mitochondrial membrane through non-GTPase receptor proteins, such as mitochondrial fission protein 1 (FIS1), mitochondrial fission factor (MFF), and mitochondrial elongation factor.



# Ischemia Reperfusion Injury

- This protein has been shown to contribute to the prevention of long-term heart failure as seen in the KO model.
- Another KO model in mice found that DRP-1 plays a protective role against cardiac pressure overload during heart failure by preventing mitochondrial dysfunction.
- DRP-1 protein inhibition  and the relationship between cell death facilitate the production of mitochondrial directed interventions to prevent IR injury.



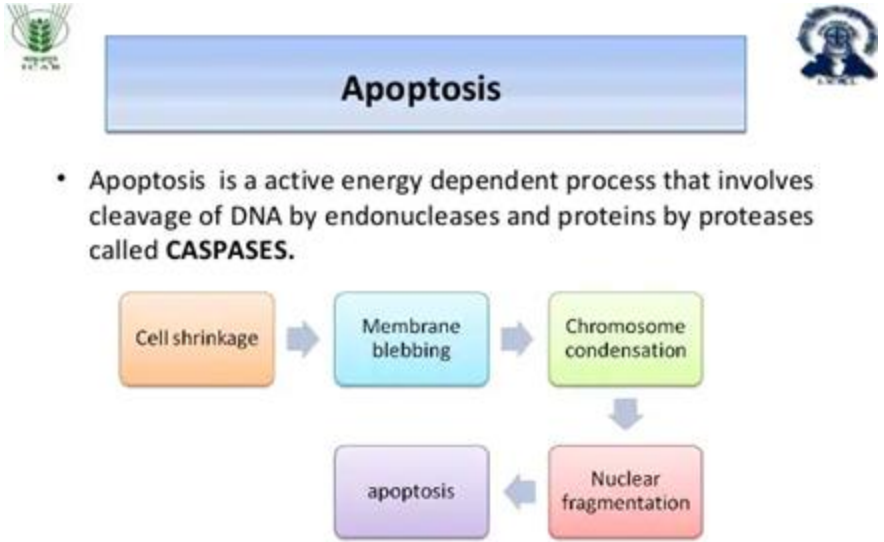
# Mitochondrial Apoptosis Signaling in the Heart

**What is apoptosis?**

- Apoptosis is known as **programmed cell death**.
- This is because apoptosis removes cells from the body without damaging the surrounding tissues.
- Apoptosis is necessary for the removal of damaged cells avoiding the accumulation of these cells in the body.

**What are common characteristics of apoptosis?**

- Cell contraction
- Chromatin marginalization
- Protein cleavage
- DNA break




Low e et al. *J Cell Biol* (2000)

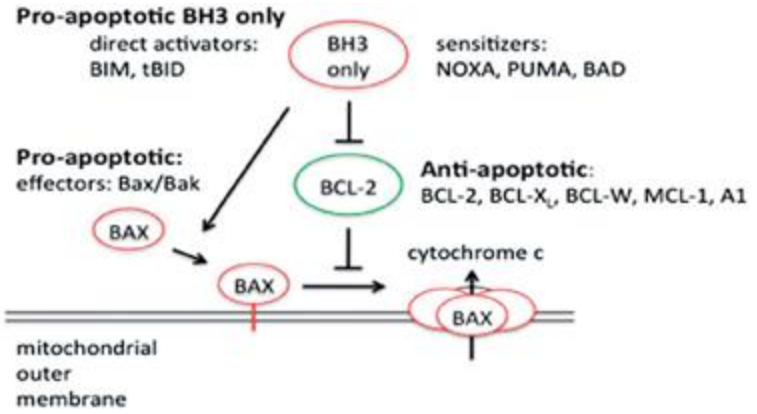
# Mitochondrial Apoptosis Signaling in the Heart

**What are the factors that inhibit, activate, regulate and carry out apoptosis?**

- Apoptosis is activated by proteins of the **BH3** subfamily.
- Apoptosis is regulated by the proteins of the **Bcl-2** family, which some are pro-apoptotic and others are anti-apoptotic.
- Apoptosis is carried out by **Bak** and **Bax** proteins. They create a pore in the mitochondrial outer membrane, which releases **cyt C** and the mechanism of apoptosis.
- Anti apoptotic proteins reside in the outer mitochondrial membrane and inhibit the activation of the pro apoptotic proteins Bax and Bax.

**What other factors are involved in apoptosis?**

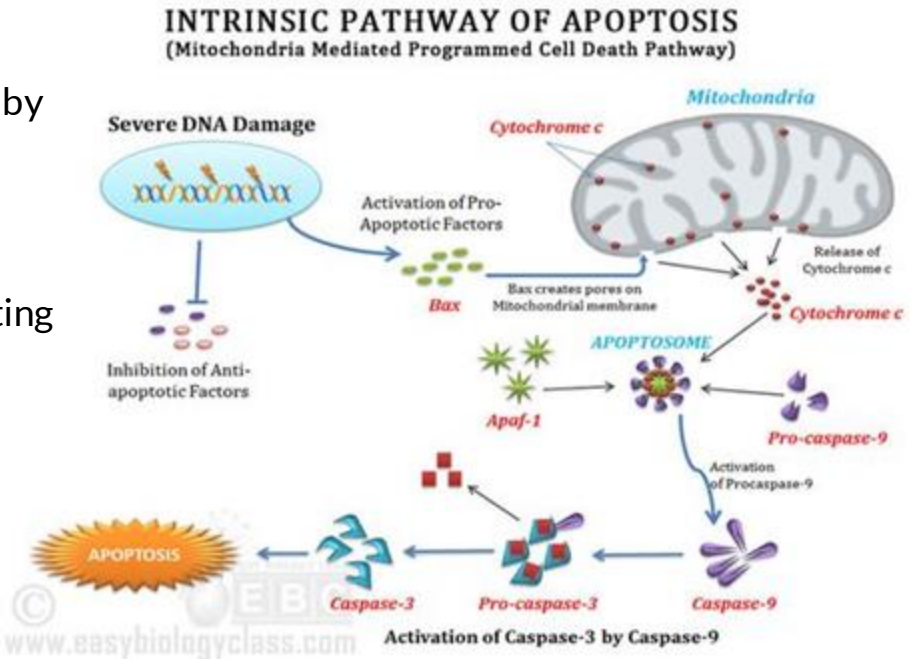
- Other Bcl-2 proteins
- Heat shock proteins as they prevent cell death
- Caspases because they coordinate cell death
- The P53 gene because it stops cell growth



# Mitochondrial Apoptosis Signaling in the Heart

**What is the role of mitochondria in apoptosis?**

- Mitochondria induces cell death through apoptosis by releasing pro-apoptotic factors such as **cyt C**.
- Under intracellular stress, mitochondria release the pro apoptotic factor cyt C which interacts with the apoptotic protease activating factor 1 (**Apaf1**) creating a signal that activates **caspases 9 and 3** initiating apoptosis.

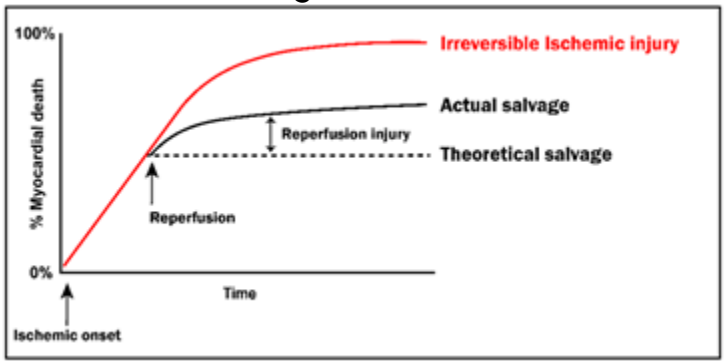



Easy Biology Class (2018)

# Mitochondrial Death Signaling in the Heart

**How are ischemia and reperfusion related to apoptosis?**

- There are studies with rats showing that ischemia alone can cause apoptosis.
- However, studies with dogs and rabbits found that apoptosis is initiated only by reperfusion.
- Prolonged periods of myocardial ischemia are associated with increased rates of necrosis, while reperfusion leads to an increase in apoptosis.
- Additionally, it was found that the activity of caspase depends on the time of ischemia, since the time of ischemia affects the damage to the mitochondria which will then activate the caspase.



# Discussion - Ca2+ Uptake

Mitochondrial Ca+ Uniporter **(MCU)** has repeatedly been considered to be responsible for Ca homeostasis in cardiac cell which is important for maintaining cell viability and sustaining cardiac activity. Additional points that we'd like to mention:

- The absence of the MCU makes little to no difference in the viability of the mice with genetic MCU KO, meaning that the role of other Ca2+ buffering mechanisms may play a larger role.
- A limitation in this field is that the relationship between mitochondria and endoplasmic reticulum (ER), which is known to play a prominent role in calcium buffering, remains poorly understood.
- Further research is needed to explain the relationship between mitochondrial and ER during Ca2+ buffering after cardiac IR injury.

# Discussion - Mitochondrial fusion

In recent studies, it has been seen that long membrane-bound forms of OPA-1 are required for mitochondrial fusion but, their processing to short soluble forms limits fusion and can facilitate mitochondrial fission. However we may also consider:

- Isomorphic variants of OPA-1 are not understood with certainty.
- The way in which OMA1 is regulated under IR remains unknown.

# Discussion - Mitochondrial fission

The phosphorylation of DRP-1 is considered the facilitator protein for mitochondrial fission.

- However, more work needs to be done in understanding the regulatory role of post-translational modifications on DRP-1 and its relationship to mitochondrial fission.
- Nytrolysation, SUMOylation, ubiquitation, and O-GlucNAcylation are post-translarion modification that have been reported on DRP-1, but remain poorly understood.

# Discussion - Cell death

A lot has been uncovered about mitochondrial outer membrane permeabilization (MOMP), however more clarification needs to be made regarding the mechanism of Bak/Bax and the initiation of MOMP.

- Bak/Bax proteins are responsible for the MOMP, however the mechanism is still under evaluation.
- Some researchers have found that proteins such as voltage-dependent anion channel (VDAC) in the OMM may be involved in the process.

# Discussion - Cell death

Myocardial tissue has been repeatedly studied under IR injury, but few studies have been dedicated to the contribution of the different cell deaths that occur during the ischemic phase or the reperfusion phase. Additionally, it is unknown whether apoptosis is caused by ischemia or by reperfusion, as well as the initiation of other types of cell death process.

- More research should be performed to evaluate the contribution of different cell death pathways during IR injury and when they ar initiated (during ischemia or reperfusion).

# Conclusion

- Mitochondria plays  a key regulatory role in cell survival during ischemia reperfusion injury.
- It's  role in regulating Ca2+ level was demonstrated to play a vital role in both cell death signaling and mitochondrial dynamics.
- Maintaining a balance between fusion and fission is essential for sustaining healthy cardiac activity.
- Understanding how these mechanisms work could help develop therapeutics or manufacture new drugs that help mediate cardiac recovery after IR injury.

# Reference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