

Calcium regulates cellular life and death

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BACKGROUND

- ❖ **Autophagy**, derived from the Greek word meaning self-eating, is a process by which cells can get rid of old constituents and generate energy.
- ❖ **Calcium** in our cells is thought to control autophagy. Its role in this process is unclear.
- ❖ Calcium is found in many cell entities such as the **endoplasmic reticulum** (protein production sites) and **mitochondria** (powerstations of the cell).

WHY STUDY AUTOPHAGY?

- ❖ Dysregulation of autophagy is involved in conditions such as **Alzheimer's disease**, many **cancers** and **diabetes**.
- ❖ Understanding how calcium controls autophagy may provide insight into these disorders.
- ❖ This could lead to new ways of treating some presently incurable diseases.

THE PROCESS OF AUTOPHAGY

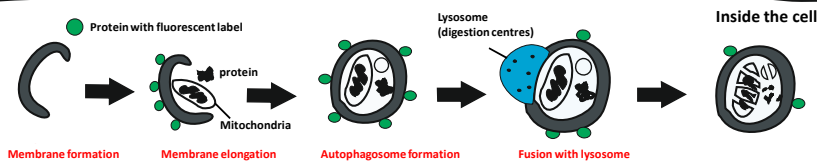


Figure 1: Autophagy is a disposal system for unwanted cellular contents. The sacrifice of cellular components in this way provides energy during starvation. A key feature of autophagy is the formation of cellular structures called autophagosomes. These are small vesicles that engulf the cellular material to be degraded. Autophagosomes fuse with lysosomes. Lysosomes contain degradative enzymes.

Fluorescent labels can be incorporated into the autophagosomes so that they can be visualized as green **puncta** within cells (Figure 2).

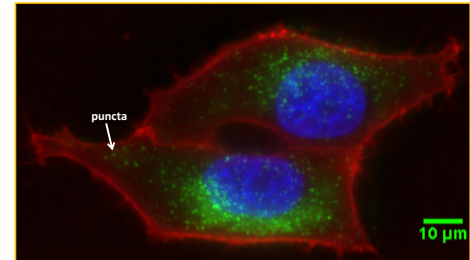


Figure 2: Human cancer cells (HeLa) with many green puncta (autophagosomes) that are indicators of autophagy. The blue colour indicates the cell nucleus, and red shows the cell membrane.

HOW DOES CALCIUM REGULATE AUTOPHAGY?

- ❖ What cellular sources of calcium are involved in the regulation of autophagy?
- ❖ Is the effect of calcium on autophagy positive or negative?

MATERIALS & METHODS

- ❖ HeLa cells
- ❖ Drugs such as 2-APB
- ❖ Fluorescence microscopy
- ❖ Molecular biology approaches (Figure 3)

Blocking calcium release from the endoplasmic reticulum genetically

- ❖ DNA encoding a protein of interest is introduced into cells using a lipid reagent and the protein of interest is produced in the cell.

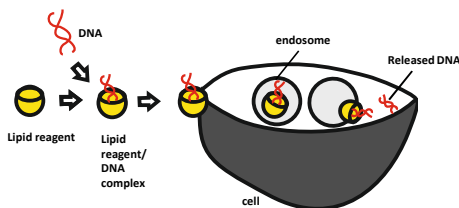


Figure 3: Schematic illustrating the introduction of DNA into cells.

The protein of interest **IP₃ 5'-phosphatase** was used to block calcium release from the calcium channel (IP₃R) on the endoplasmic reticulum.

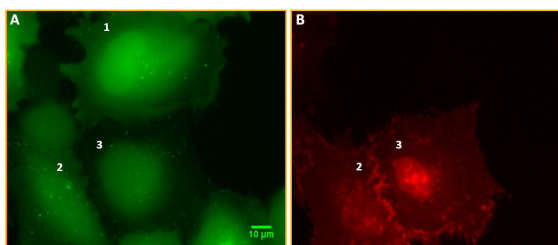


Figure 4: (A) HeLa cells with different levels of autophagosomes. Cell 1 calcium release is normal, has fewer puncta than cells 2 and 3, where calcium release is blocked (shown in red in B). (B) Cells 2 and 3 in red have calcium release blocked.

Blocking calcium release from the endoplasmic reticulum pharmacologically

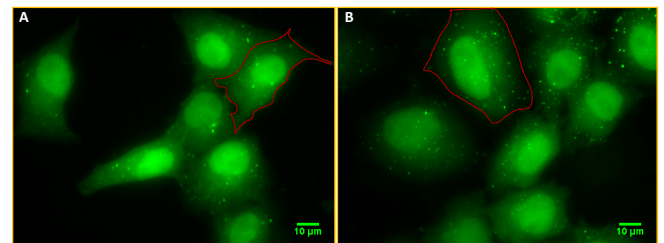


Figure 5: (A) HeLa cells with normal calcium transfer (i.e. without calcium channel blocker). (B) HeLa cells treated with calcium channel blocker (2-APB) showing a higher level of autophagy (increase in the number of autophagosomes).

CONCLUSIONS AND FUTURE WORK

- ❖ Blocking calcium release from IP₃R on the endoplasmic reticulum resulted in a high level autophagy.
- ❖ Calcium release from IP₃R is important for maintaining cellular health.
- ❖ To investigate other sources of calcium inside the cell such as the **lysosomes** and their link to autophagy.

REFERENCES

1. Kondrasky A. et al (2013) "Calcium-permeable ion channels in control of autophagy and cancer" *Frontiers in Physiology* (4):272
2. Maycotte, P., Thorburn, A., (2011) "Autophagy and cancer therapy" *Cancer Biology & Therapy* (11):127- 137

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