Pivotal Role of the Cyclin-dependent Kinase Inhibitor $p21^{WAF1/CIP1}$ in Apoptosis and Autophagy

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Eiji Iwado, Naoki Shinojima, Yasuko Kondo, and Seiji Kondo

Journal of Biological Chemistry 283: 388-397, 2008

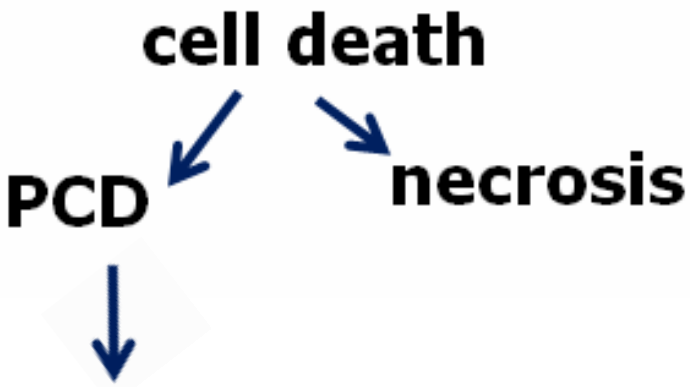
Speaker: Ya-Fei Li

Advisor: Dr. Chin-Tsan Huang

Date: 2008.3.25

Place: The 6th classroom

PROGRAMMED CELL DEATH

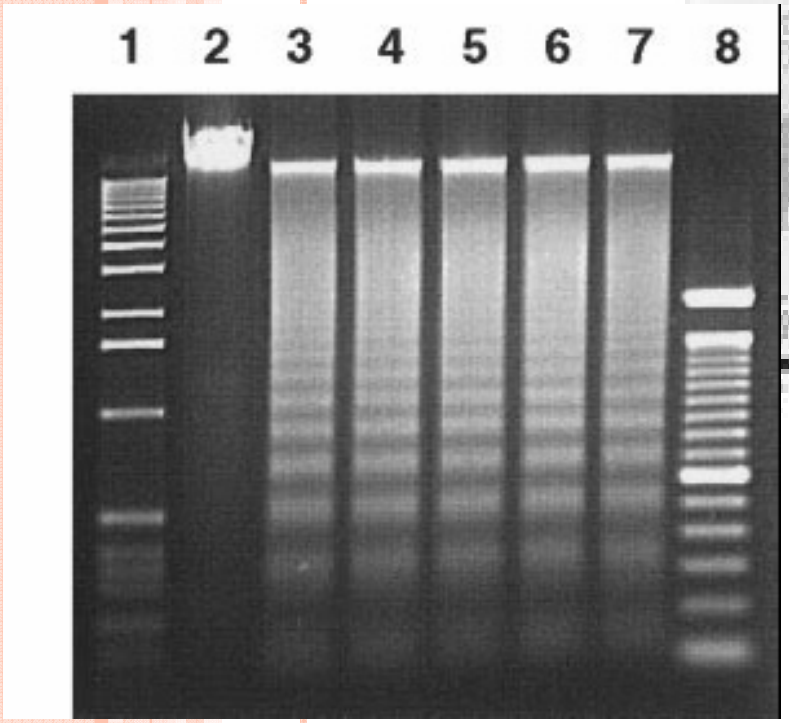


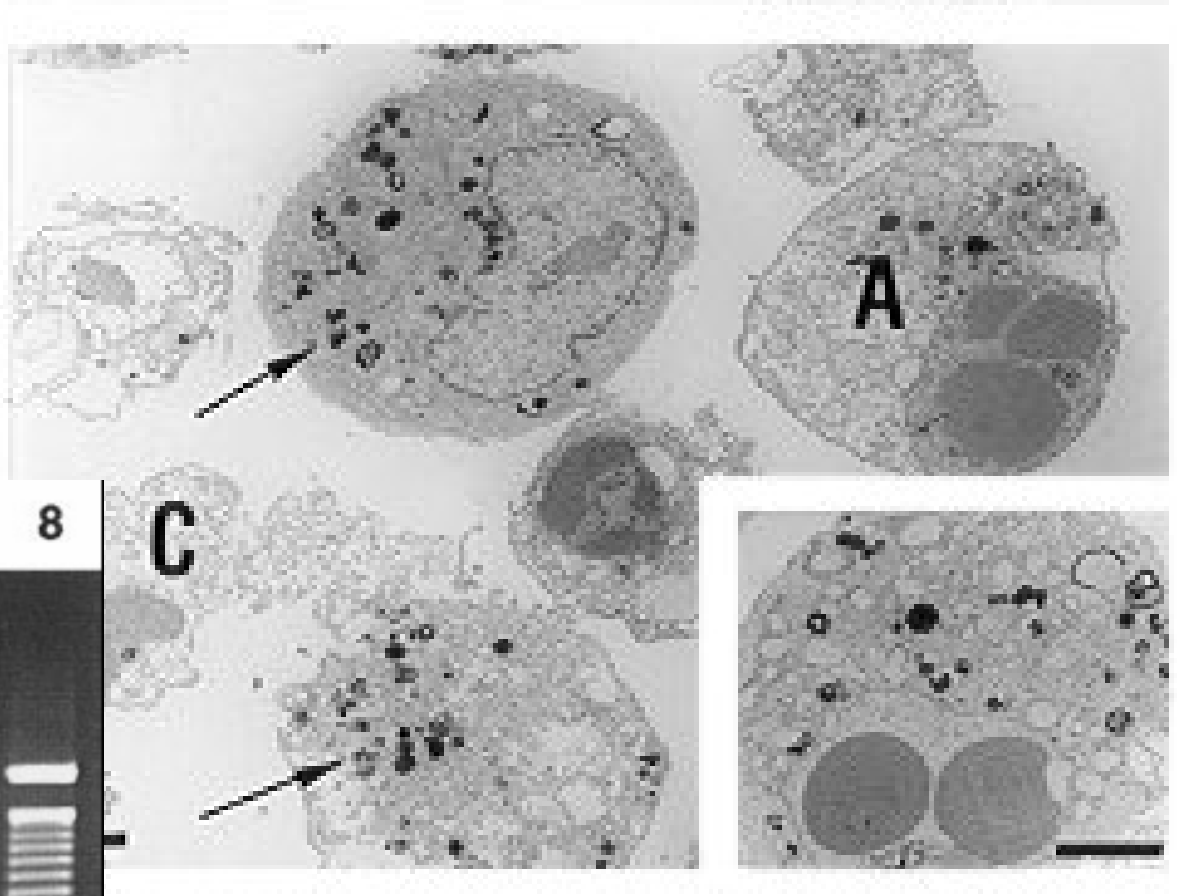
Type I : Apoptosis
Type II : Autophagy

Differerence:
- cell-surface morphology,
- nuclear ultrastructure,
- DNA fragmentation,
- and polyubiquitin gene expression.

APOPTOSIS

DNA ladder





Nuclear fragmentation ,
chromatin condensation
→**Apoptotic Body**

www.facm.ucl.ac.be/cellular_toxicity.htm
Analytical Biochemistry **266, 110–115 (1999)**

APOPTOSIS

Pros

- Cell number balance
- Development
- Cell cycle
- **Dangerous cells clean out**

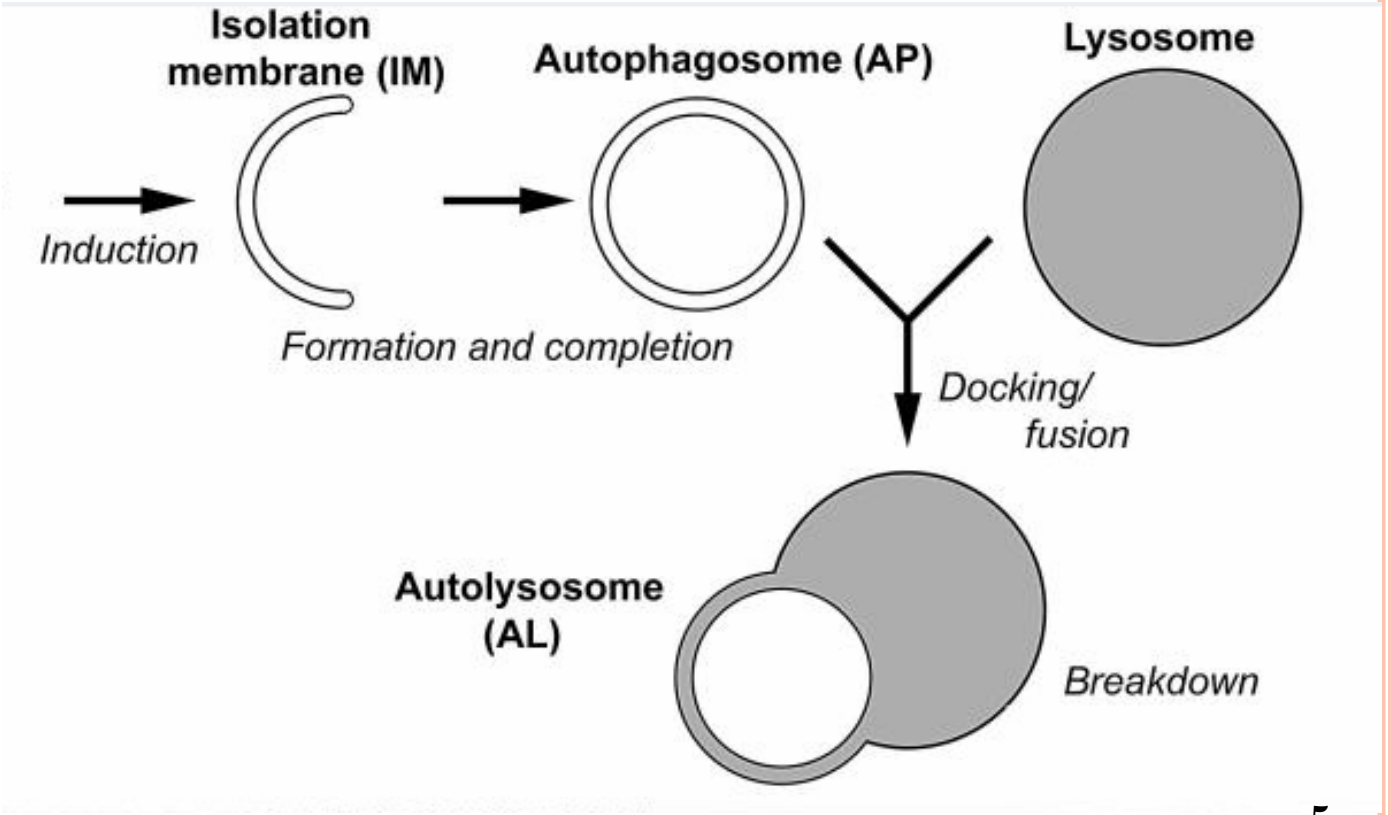
Cons

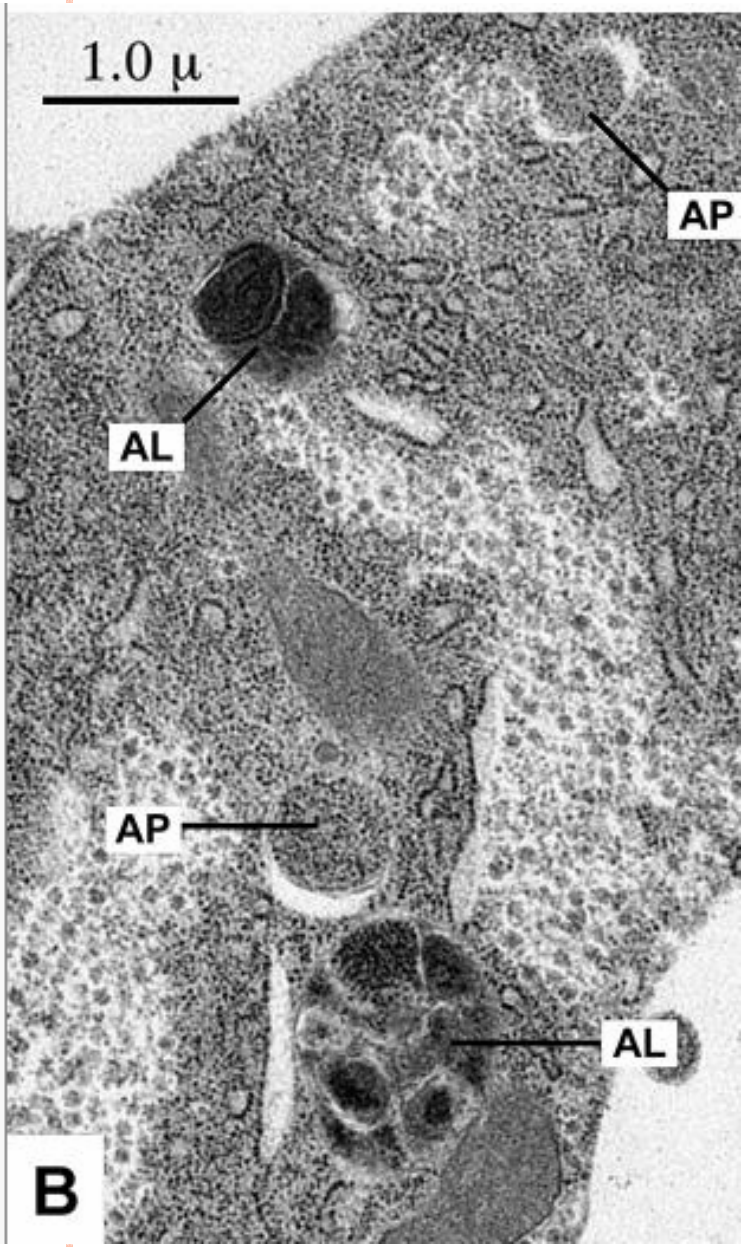
Uncontrolled condition—
contribute to disease

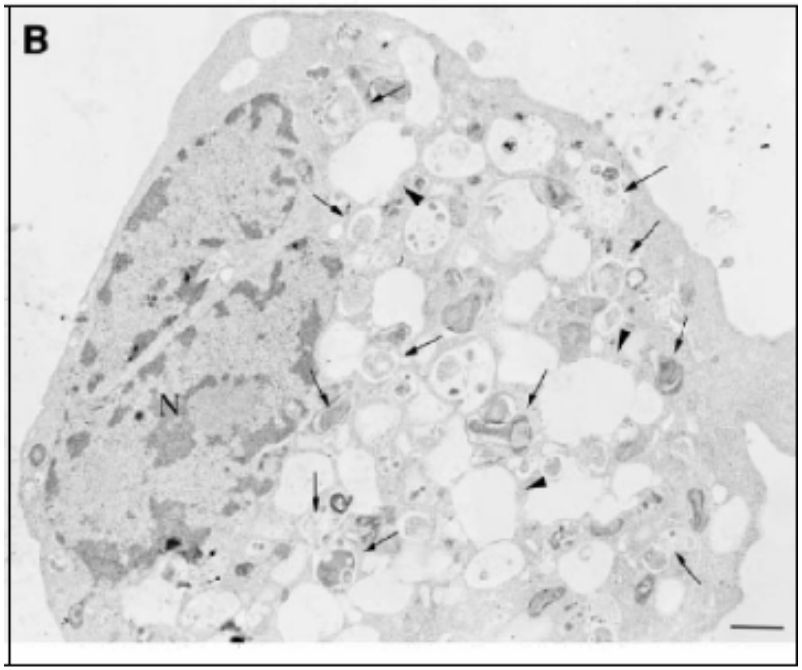
AUTOPHAGY

- Lysosomal degradation
- Starvation reaction

- Cell number balance
- Recycle







↑ in the fat body of a fruit fly larva

PLoS Biol. 2006 February; 4(2): e36.

AUTOPHAGY-RELATED GENE (Atg gene)

Atg5 : Atg5-Atg12

Biogenesis of autophagic vesicles (autophagosome)

Beclin 1 (Atg6)：a Bcl-2 interacting protein

Form complex with PI3K to regulate autophagy.

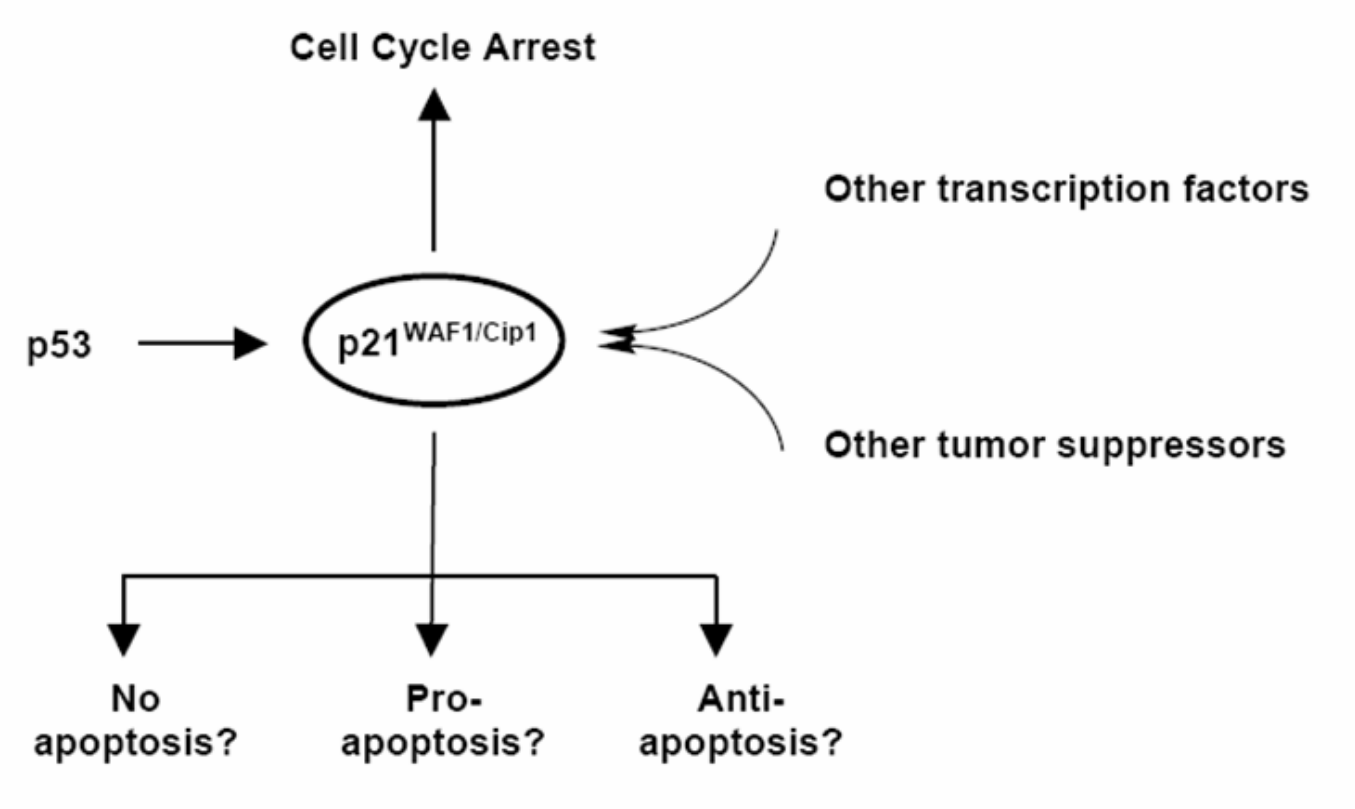
LC3 (Atg8)：

Normally spread throughout the cytoplasm.

Specifically concentrated on autophagosomes.

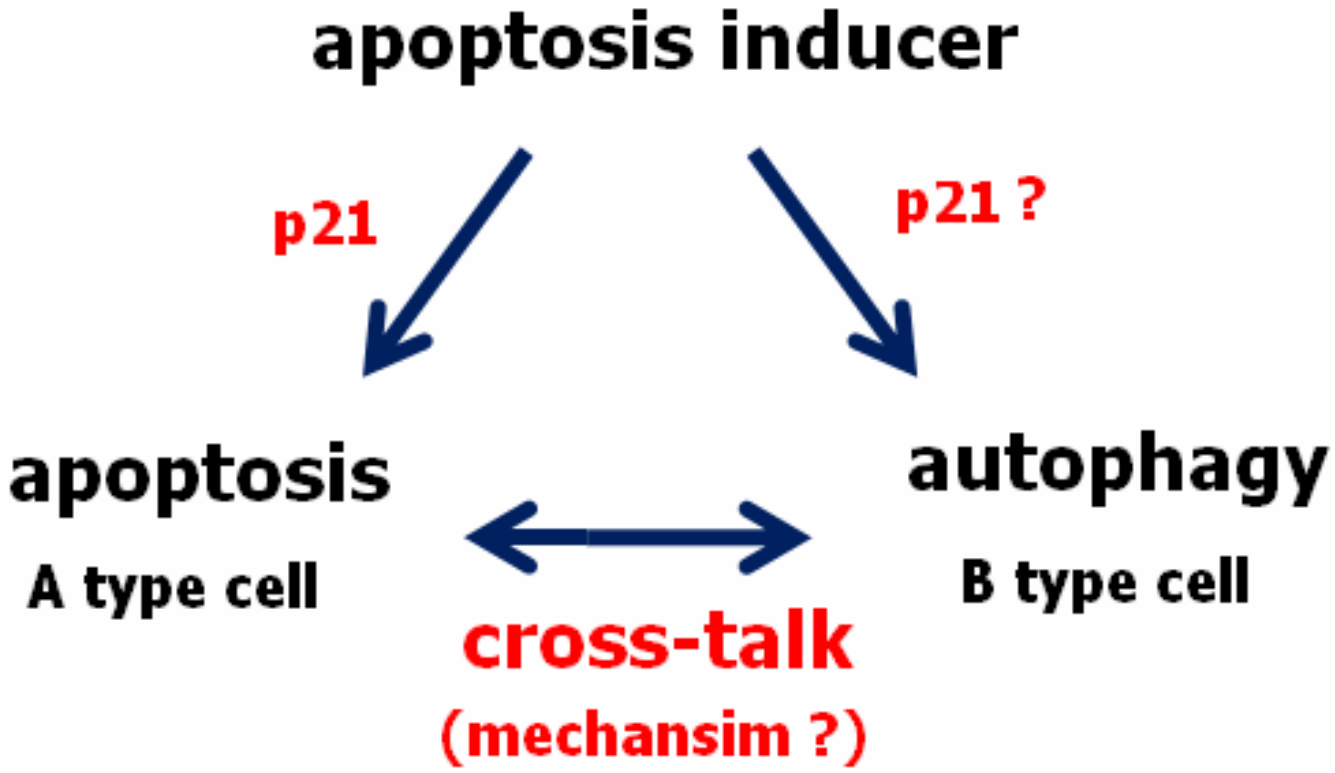
P21

- **A CDK inhibitor (CKI)**
- **p53 downstream apoptosis regulation**



Molecular Cancer Therapeutics *Vol. 1, 639–649, June 2002*

Previous Studies



Focus of this Research

To assess the role of p21 in the cross-talk of apoptosis and autophagy.

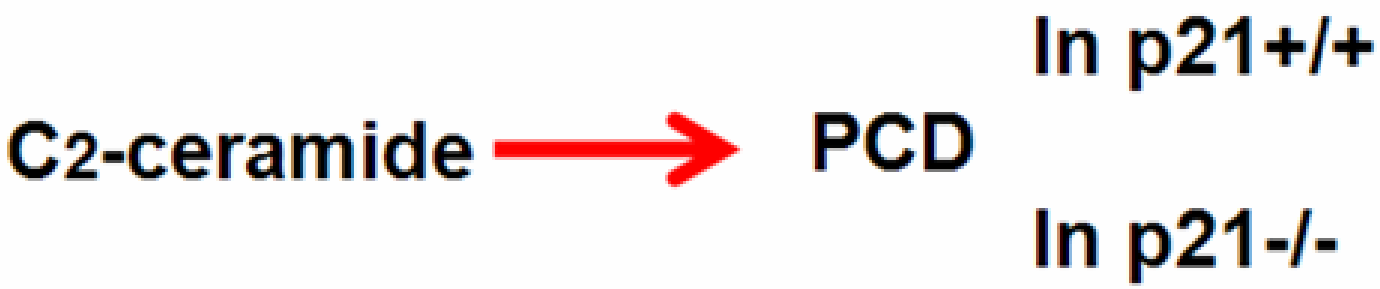
C2-ceramide is important in signaling pathway of PCD.

Nature 380, 75–79 (1996)

*J. Cell Sci. 119,*259–270 (2006)

First, to assure that

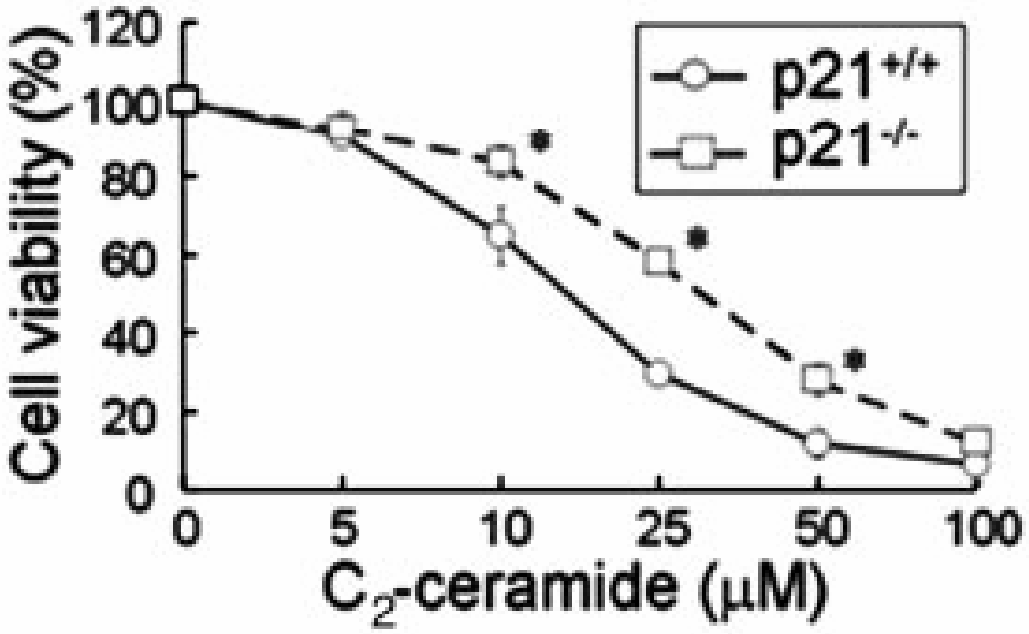
C2-ceramide can really reduce cell viability.



C_2-ceramide reduce cell viability

p21 promotes ceramide-induced apoptosis.

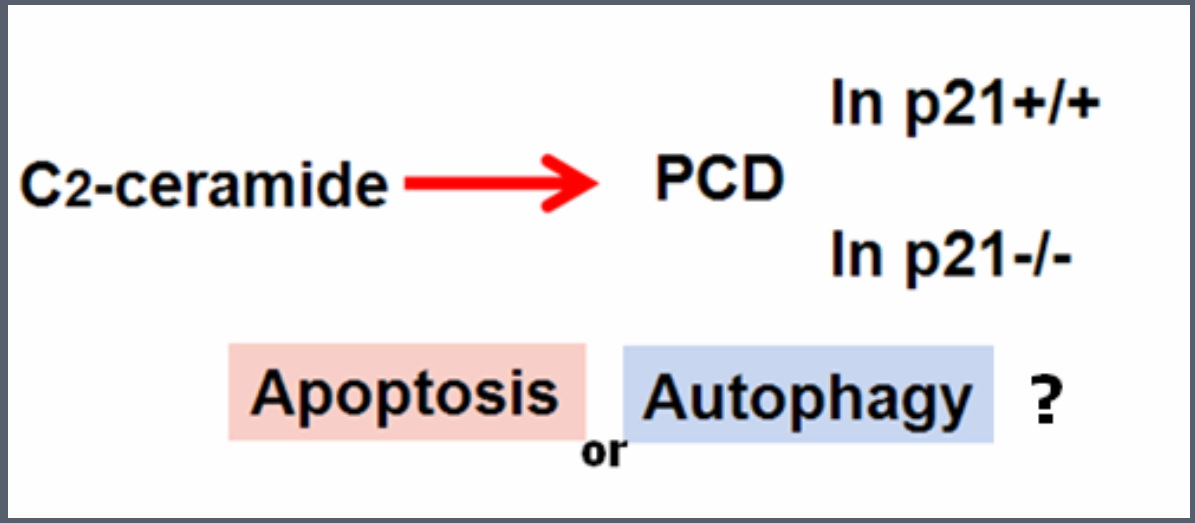
Exp. Cell Res. 253,403–412 (1999)



p21+/+ : cells with p21 wild type
p21-/- : cells lack p21

C_2-ceramide indeed reduces cell viability.

But by which PATHWAY ?

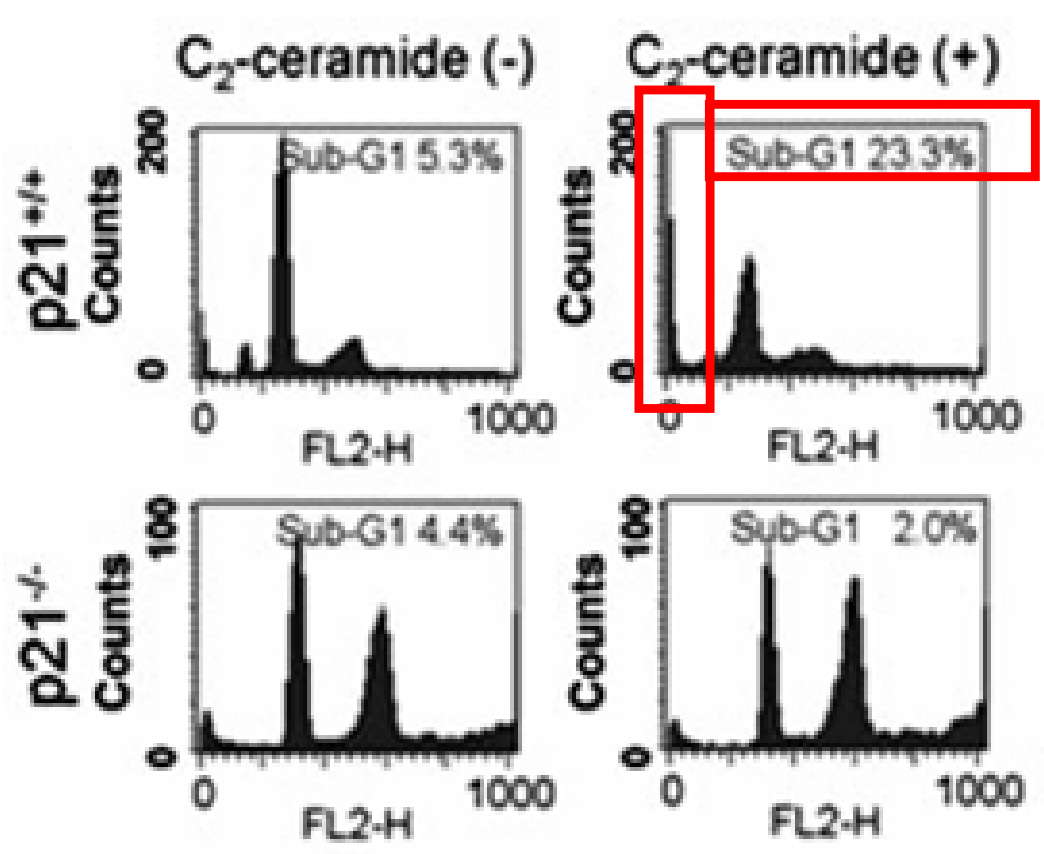


C_2-ceramide in p21+/+ cells ?

C_2-ceramide → PCD In p21+/+ ...?

C2-ceramide induce apoptosis in p21+/+

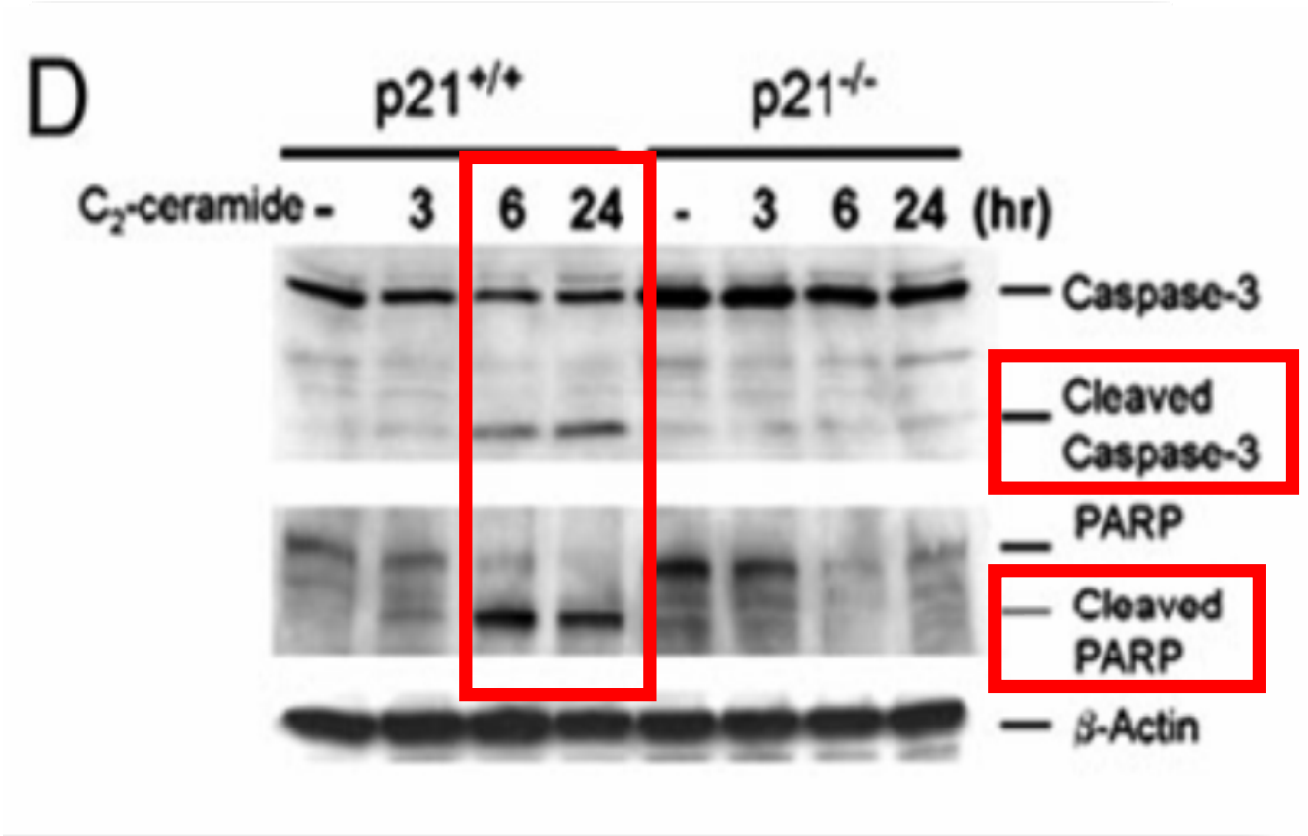
Apoptotic cell : sub-G1 phase



→ **more sub-G1, more apoptotic cell**

C2-ceramide induce apoptosis in p21+/+

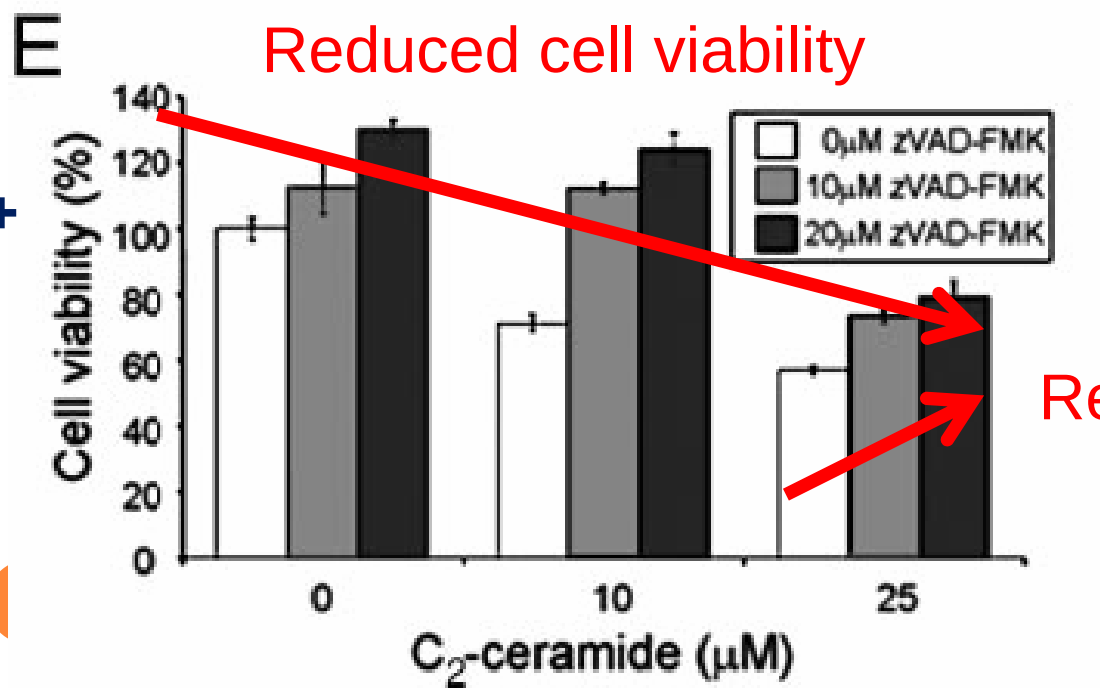
Cleaved caspase-3 & PARP :
exist during **apoptosis**



C2-ceramide induce apoptosis in p21+/+

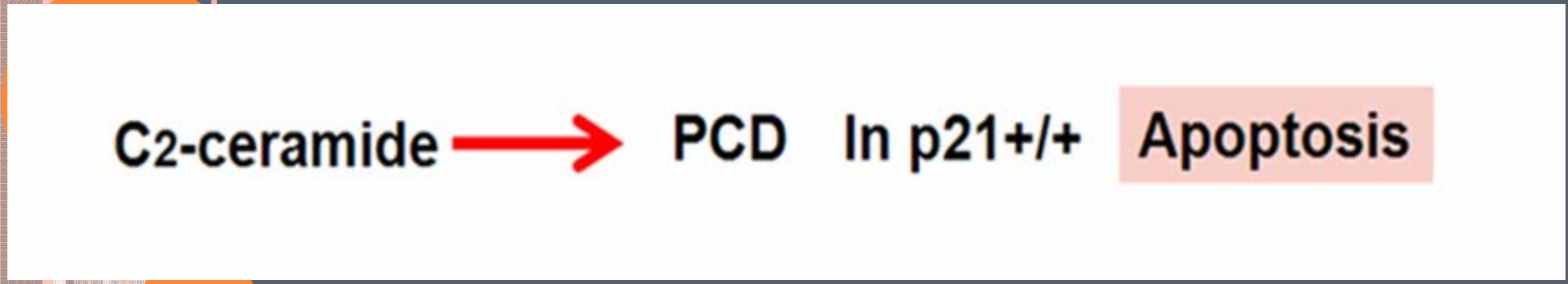
Z-VAD-FMK: a caspase inhibitor

p21+/+

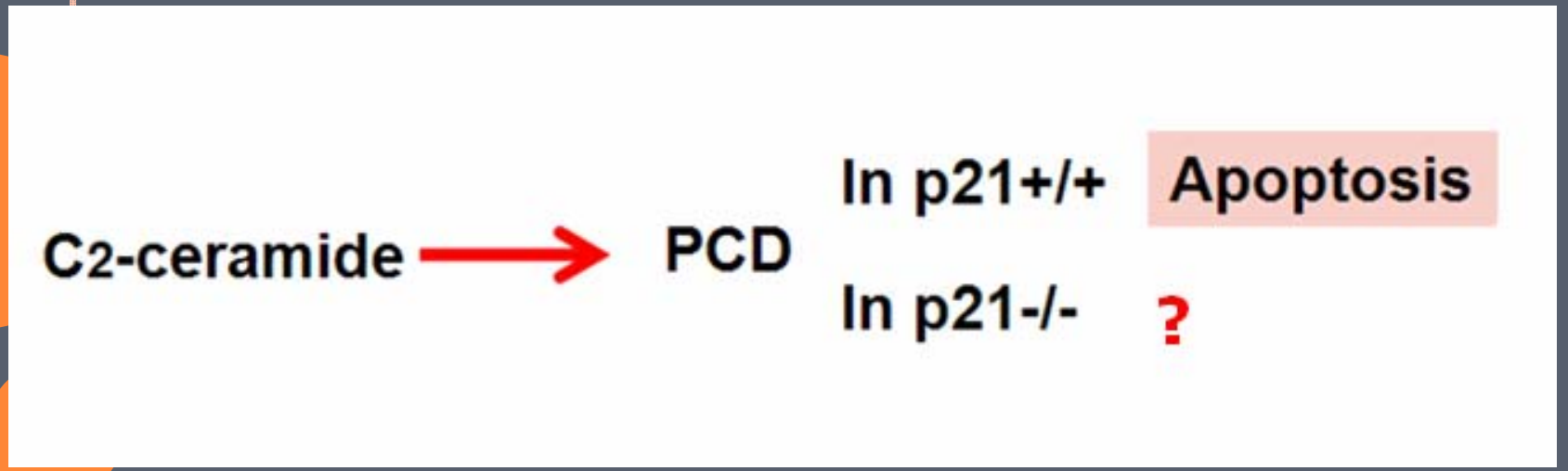


- Caspase inhibitor could restored the viability reduced by C2-ceramide.

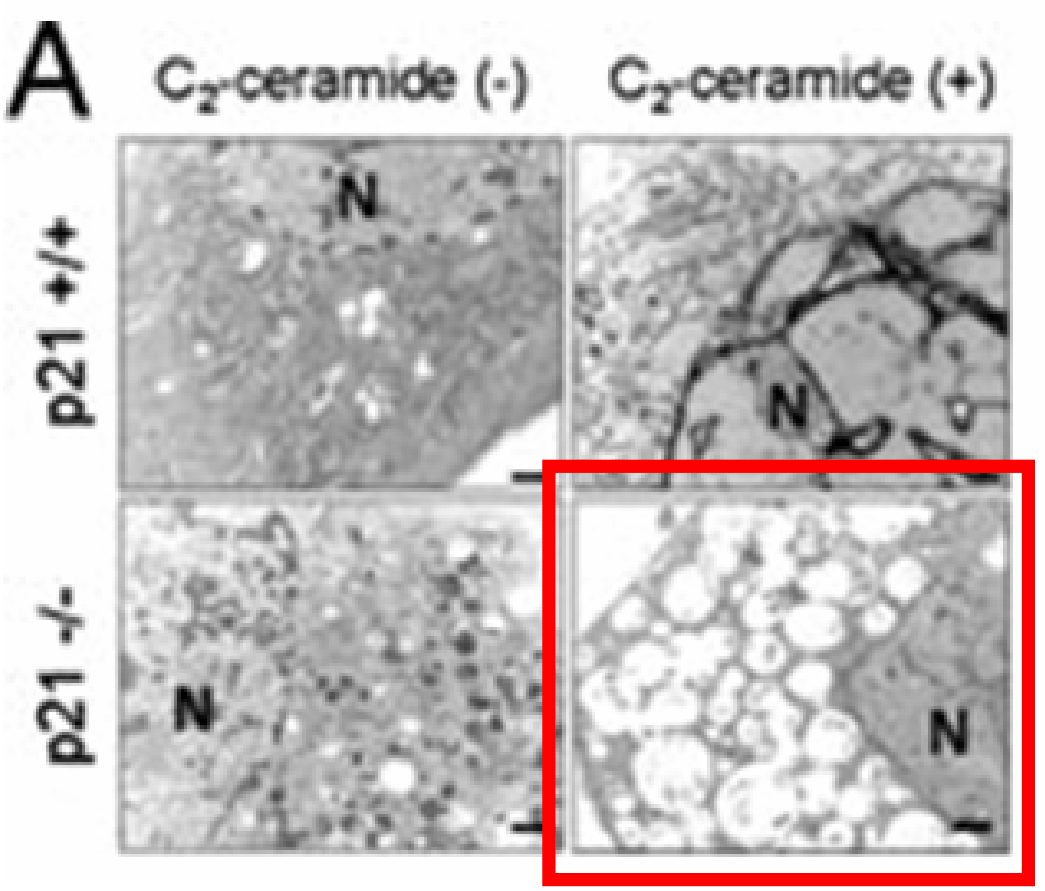
C_2-ceramide induces apoptosis in p21+/+ cells.



How about C_2-ceramide in p21-/- ?



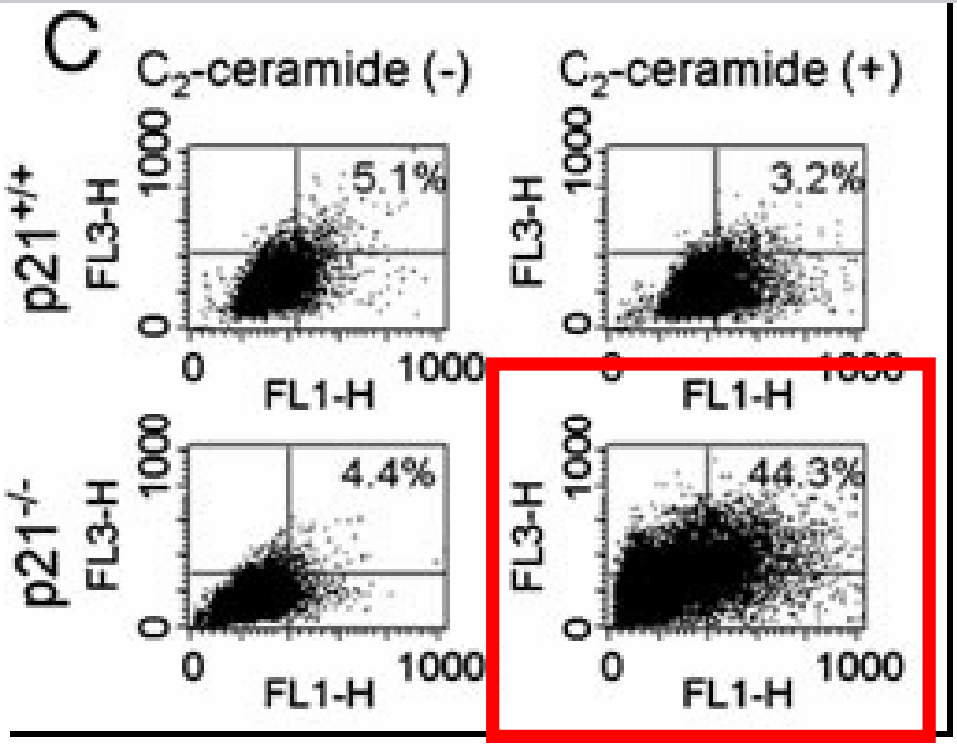
C2-ceramide induce autophagy in p21-/-



Autophagic vesicles

C2-ceramide induce autophagy in p21-/-

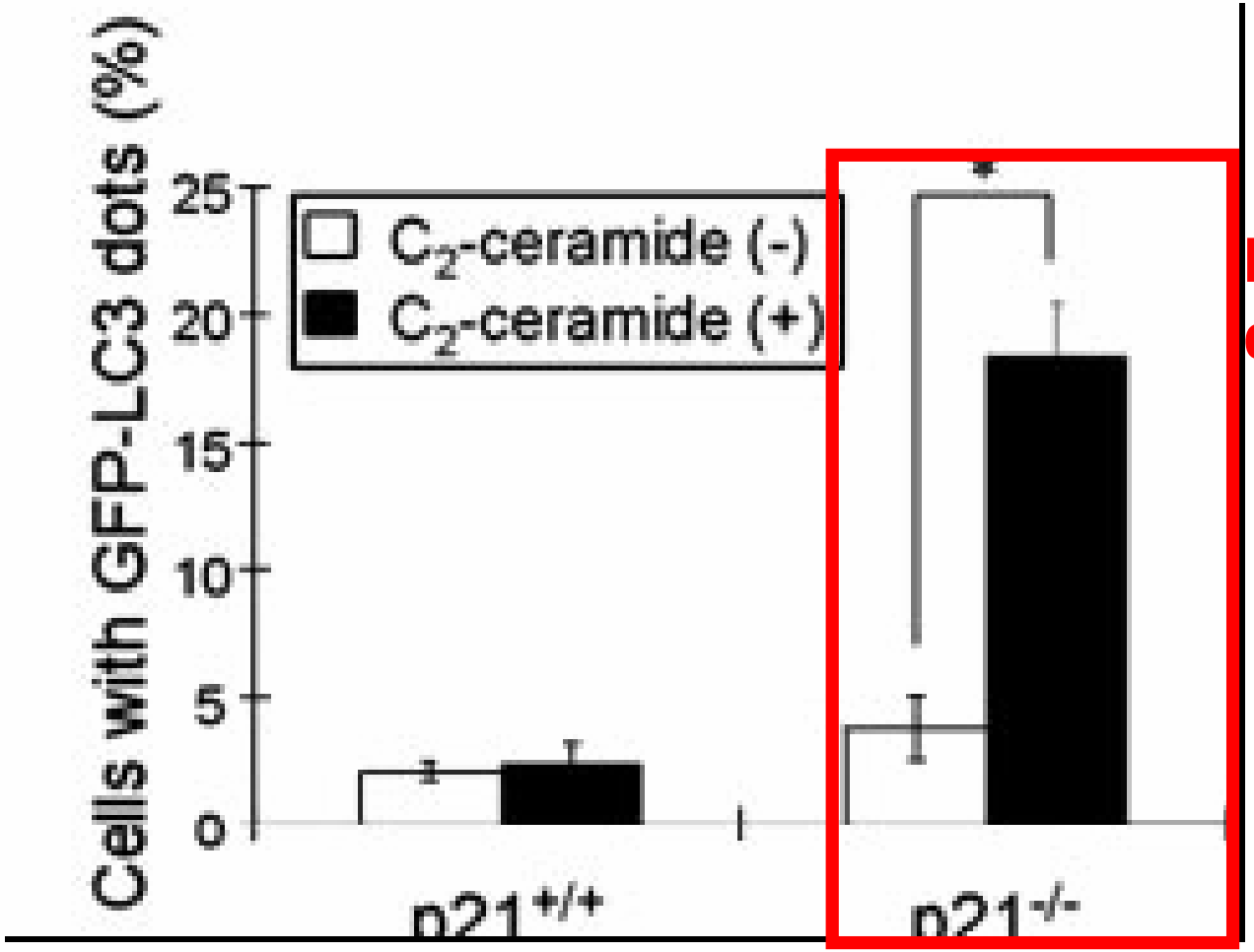
AVOs development: a marker of autophagy
(acidic vesicular organelles)



More dots, more cells with AVOs

C_2-ceramide induce autophagy in p21-/-

Dotted GFP-LC3: a marker of autophagy

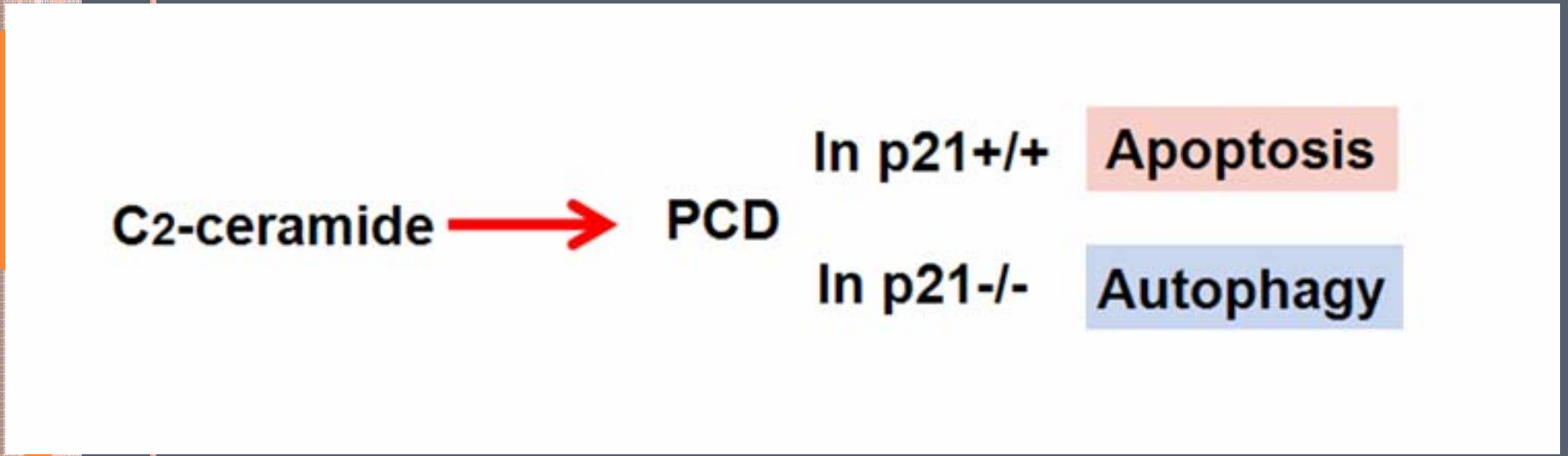


Diffused distribution

Dotted distribution

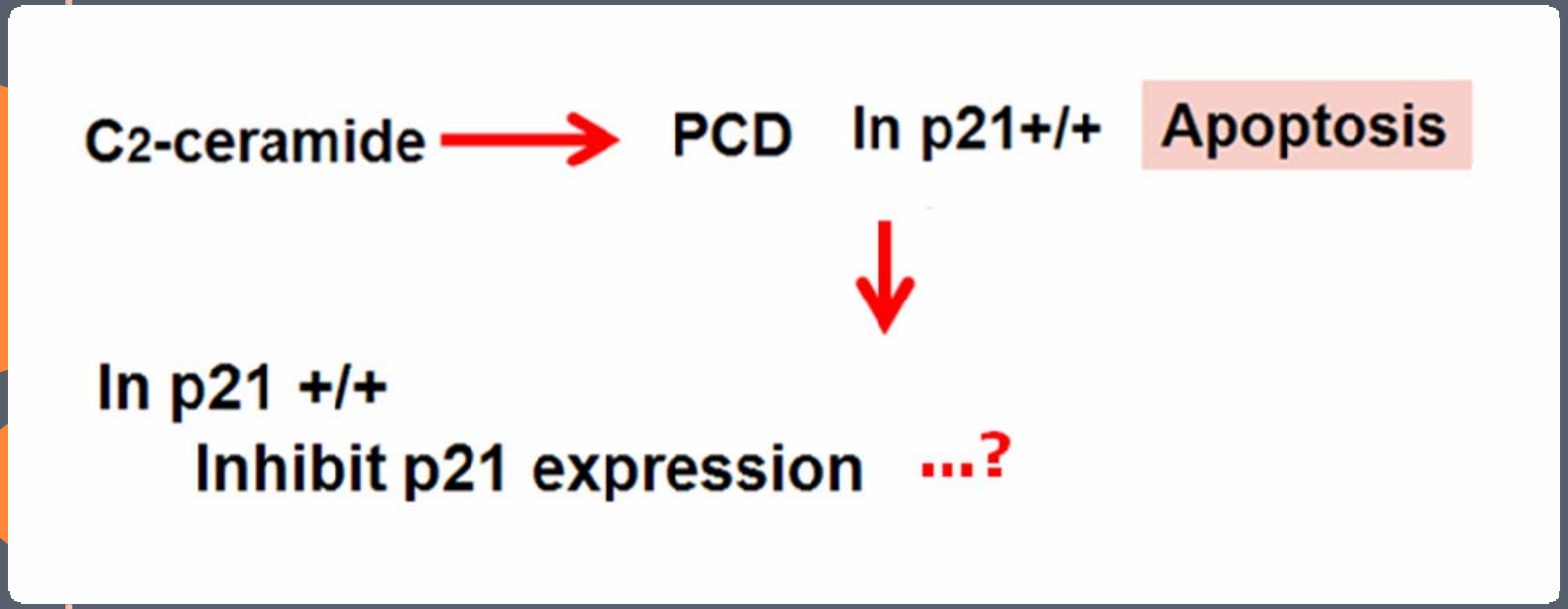
C_2-ceramide induces autophagy in p21-/-

C2-ceramide induces apoptosis in p21+/+ & autophagy in p21-/-



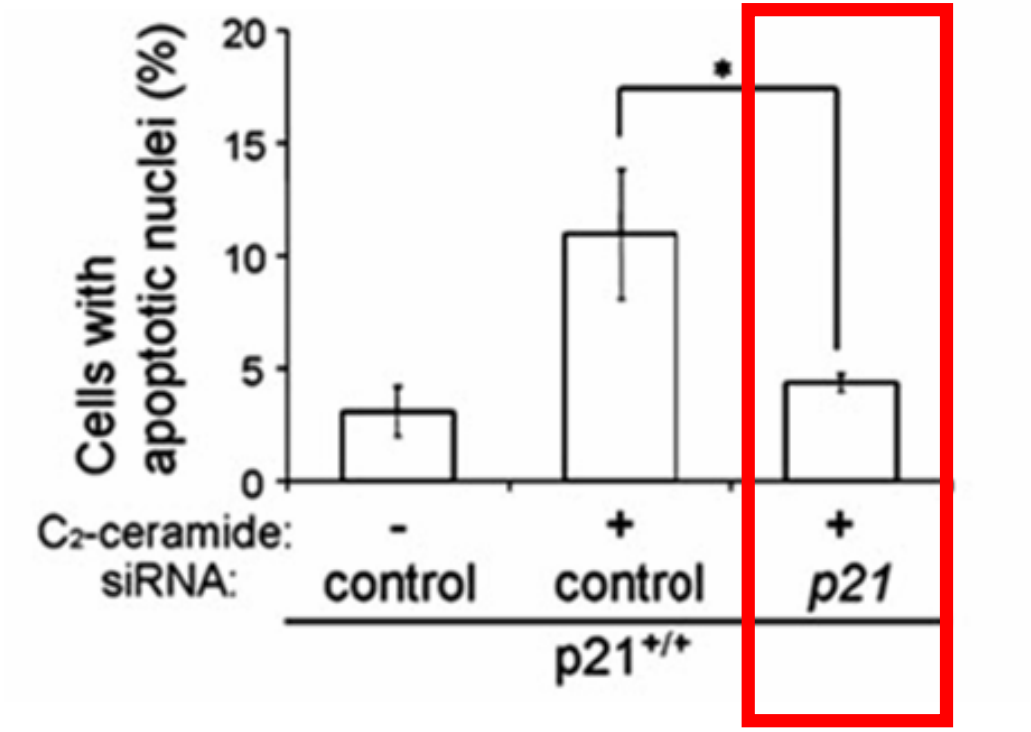
C2-ceramide induces apoptosis
in p21+/+…

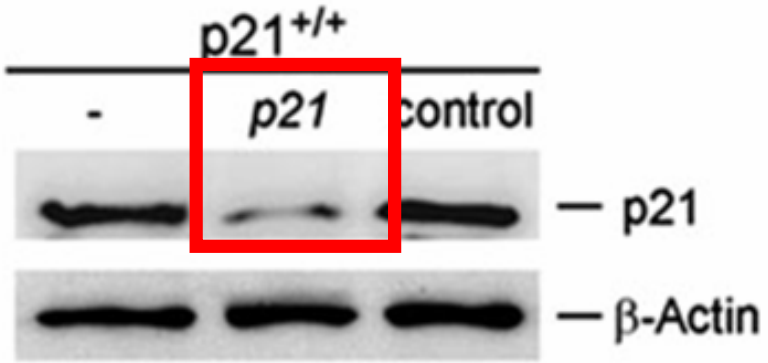
Then if we inhibit p21 expression…?



p21 silencing reduce apoptosis in p21+/+

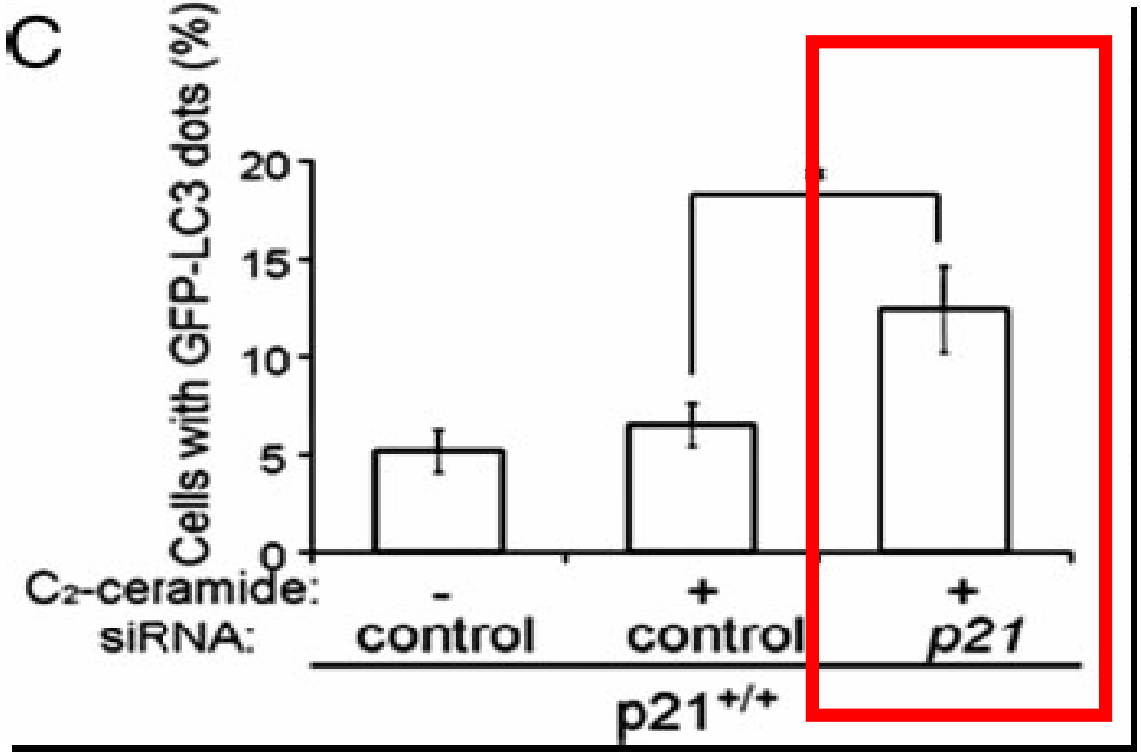
Reduced apoptosis ↓





inhibit p21 expression → reduced apoptosis

p21 silencing reduce apoptosis in p21+/+



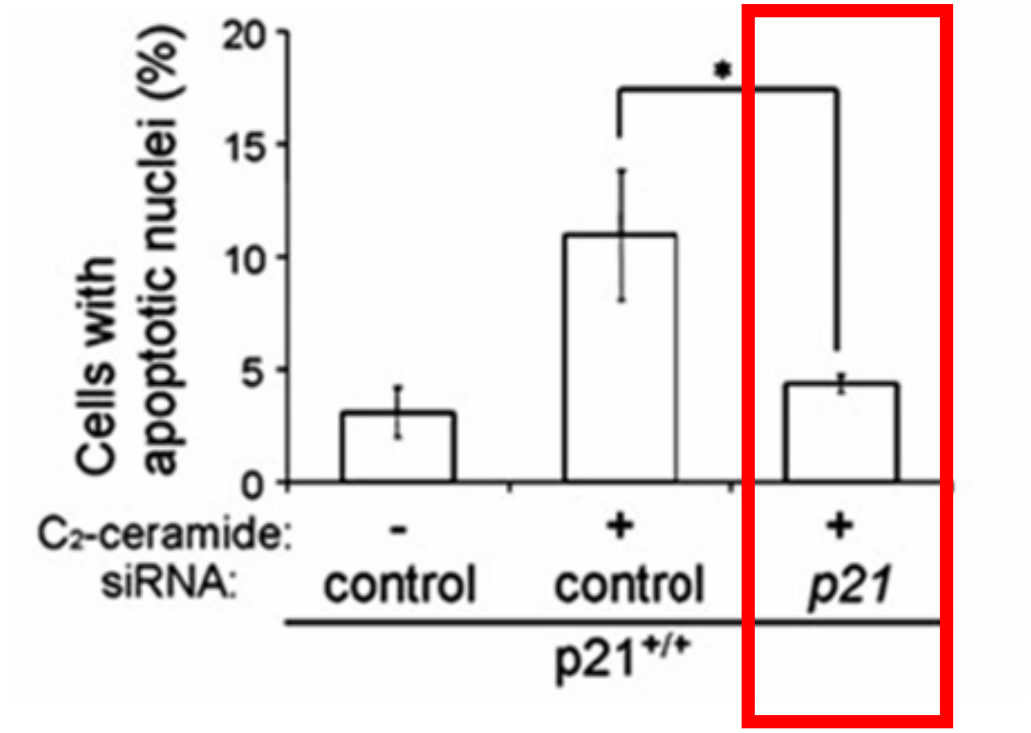
Apoptosis reduced, but viability still lower

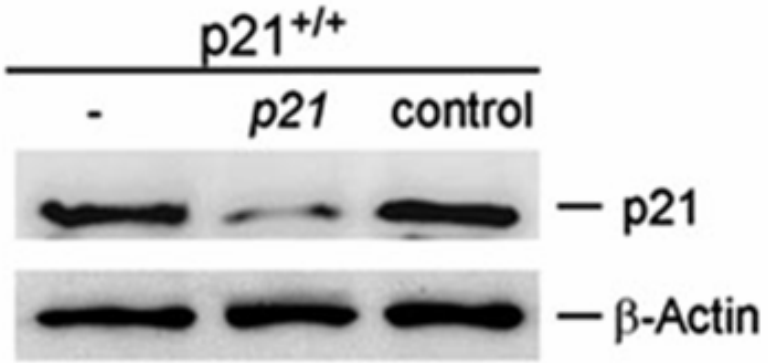
?

More cells with GFP-LC3 dots→ Autophagy

p21 silencing reduce apoptosis in p21+/+

Reduced apoptosis ↓

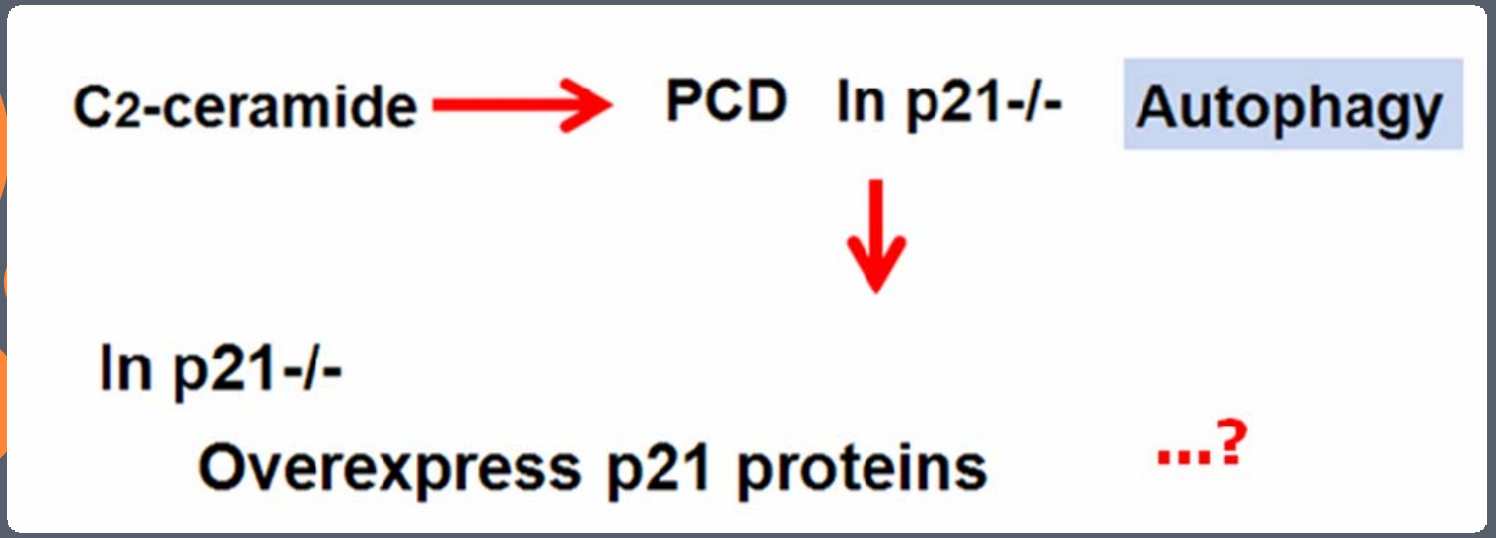




inhibit p21 expression → reduced apoptosis

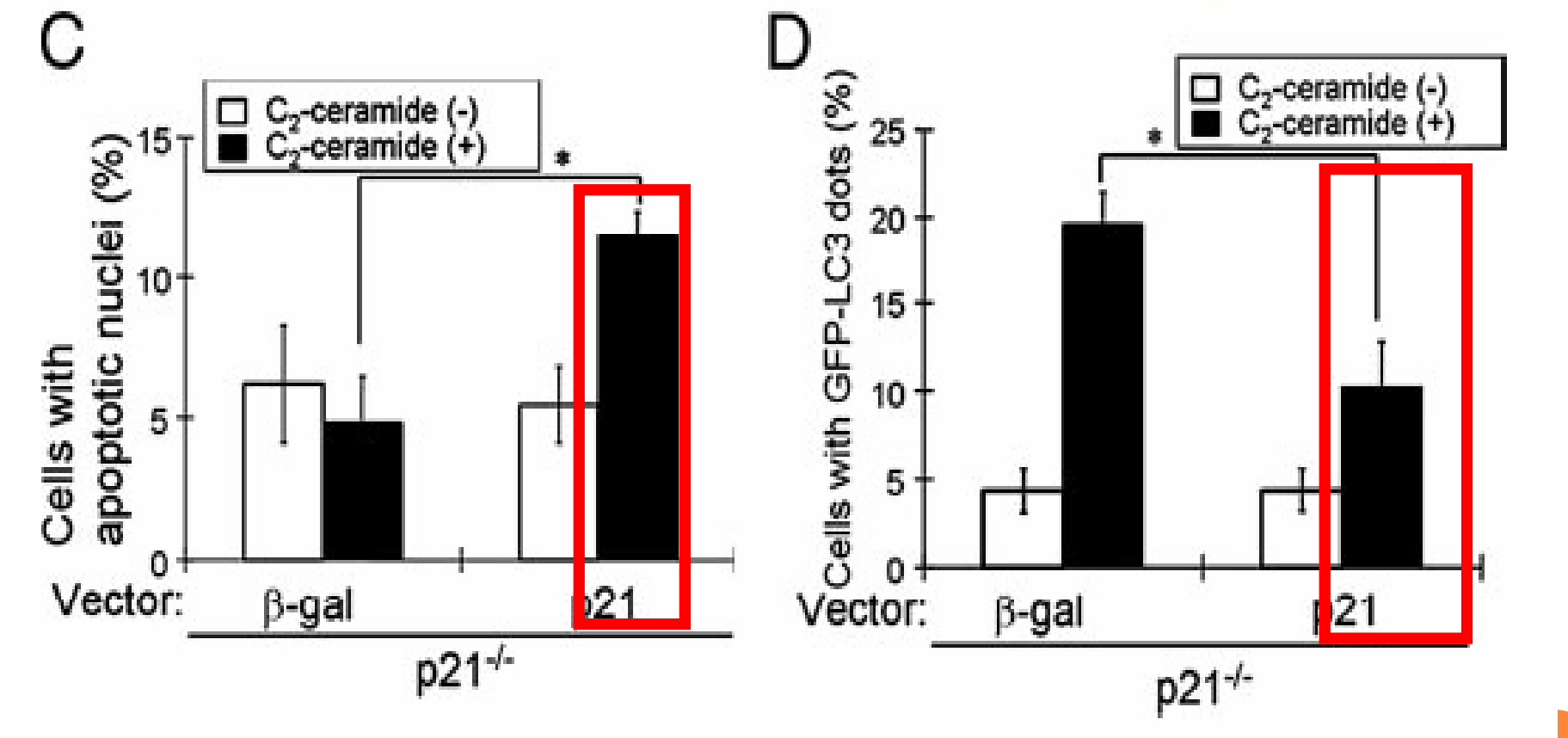
Silencing p21 suppresses the apoptosis pathway and turns to autophagy for p21+/+ MEFs.

How about p21 overexpression in p21-/- MEFs ?

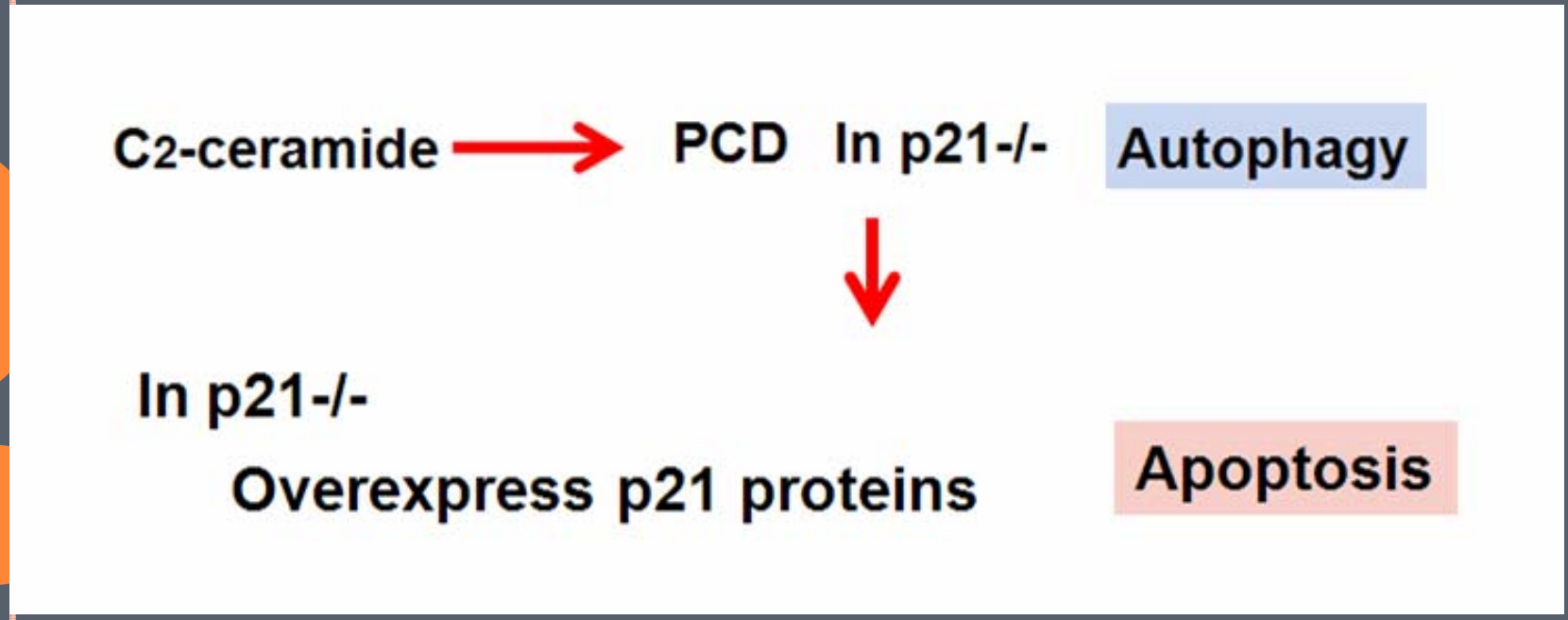


p21 overexpression reduce autophagy in p21-/-

Hoechst staining: to see apoptotic nuclear

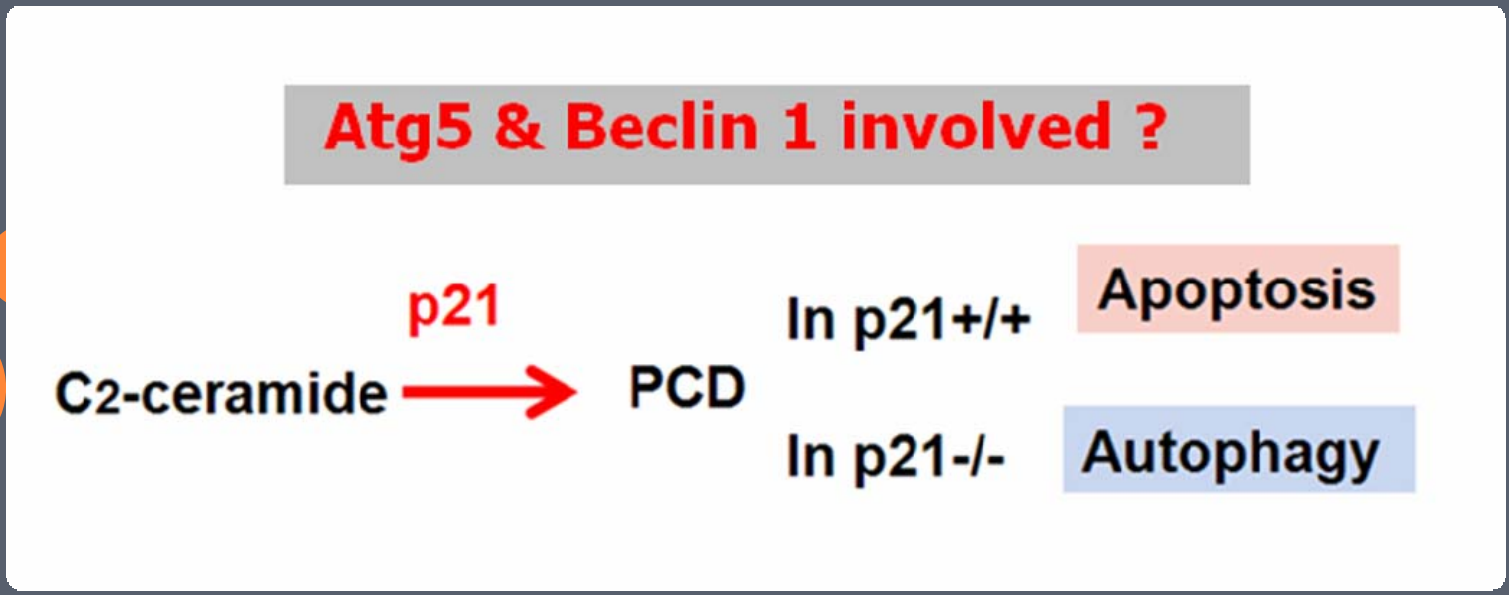


p21 overexpression suppresses the autophagy pathway and turns to apoptosis for p21-/- MEFs.



Autophagy-related (Atg) *genes* is important in the autophagy pathway.

How about their role in the **C2-ceramide induced PCD ?**



AUTOPHAGY-RELATED GENE (Atg gene)

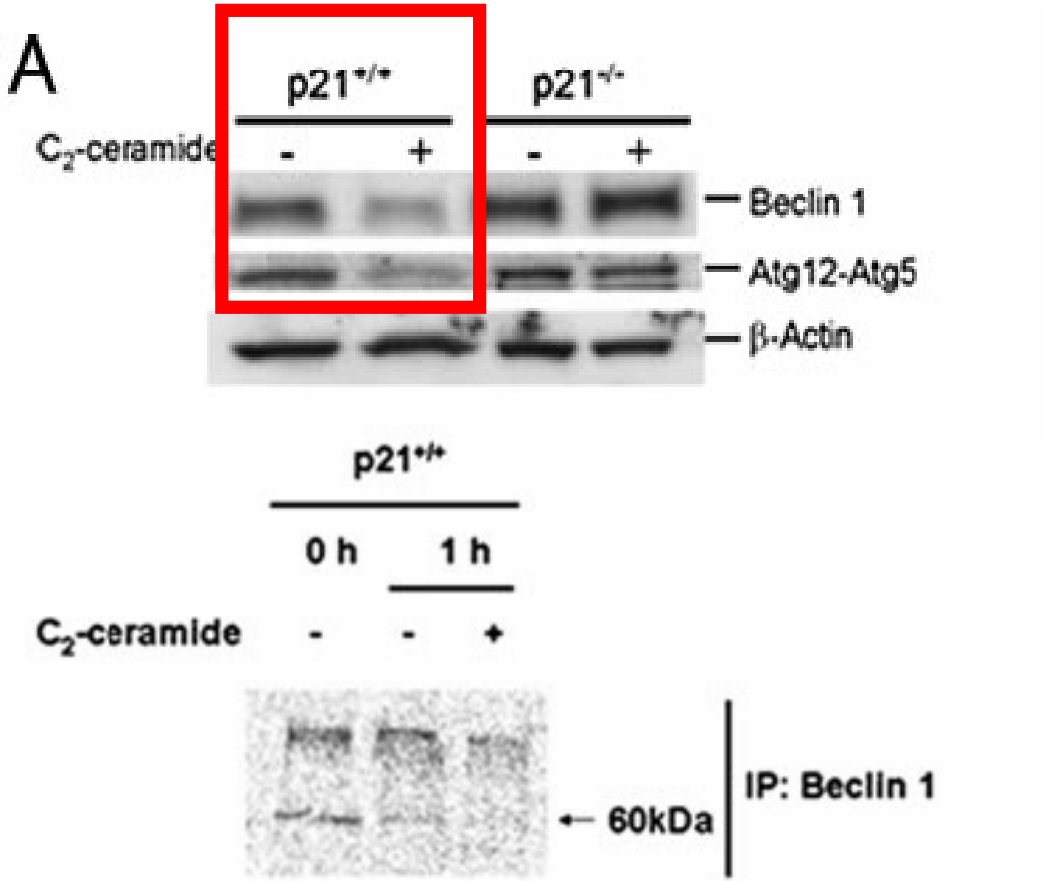
Atg5 : Atg5-Atg12 complex

Biogenesis of autophagic vesicles (autophagosome)

Beclin 1 (Atg6)：a Bcl-2 interacting protein

Form complex with PI3K to regulate autophagy.

Beclin 1 & Atg5 involved in C2-ceramide induced PCD

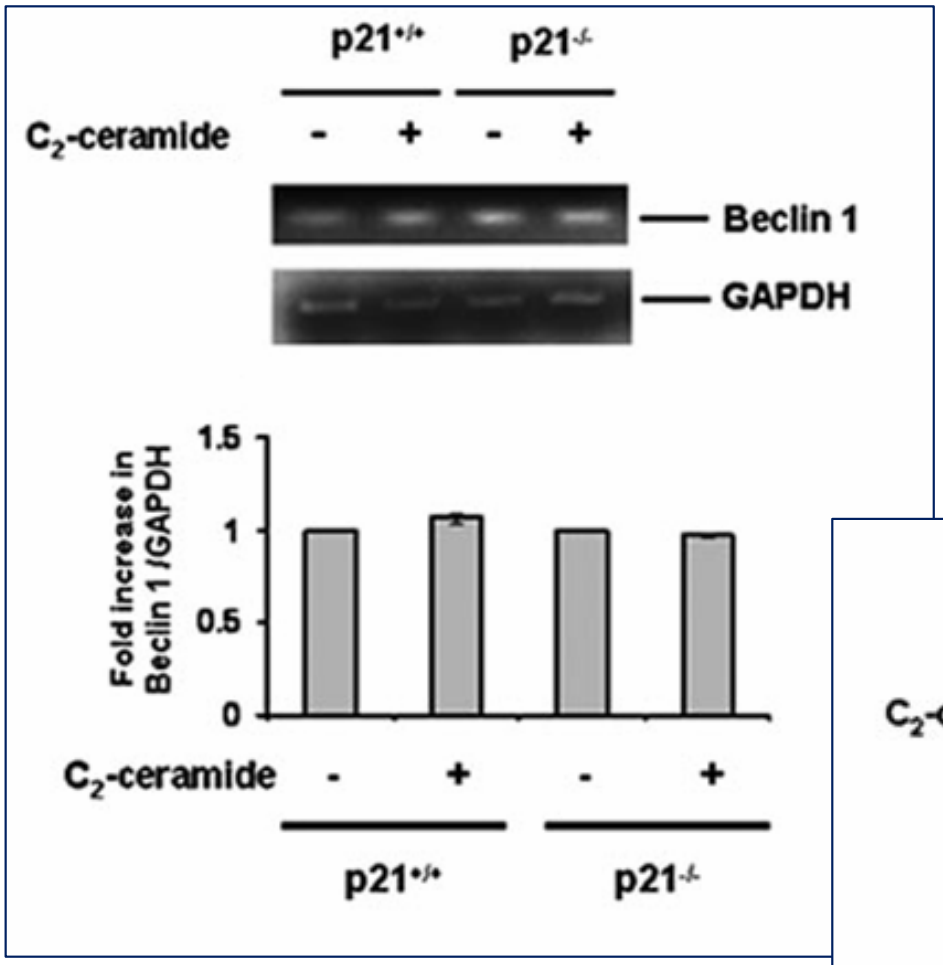


C2–ceramide affects Beclin 1 stability.

C2–ceramide affect Beclin 1 stability

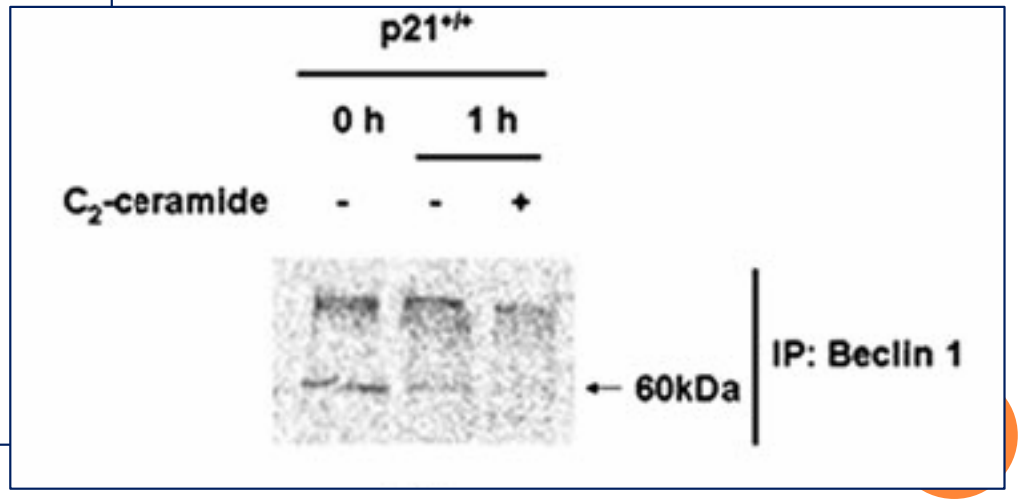
(transcription level ?)

↓



→ **NOT by transcription level**

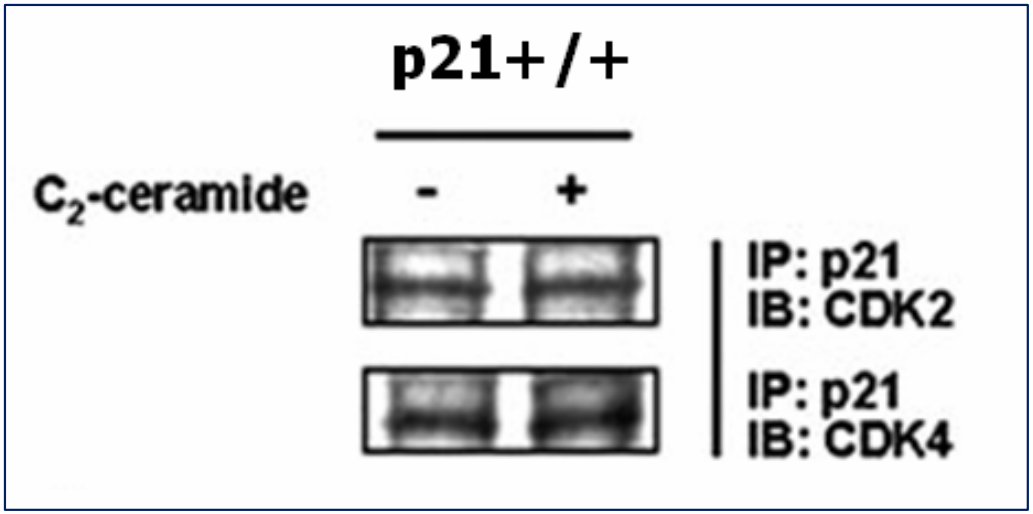
↓



C2–ceramide induces apoptosis in p21+/+

(By CDK activity ?)

↓

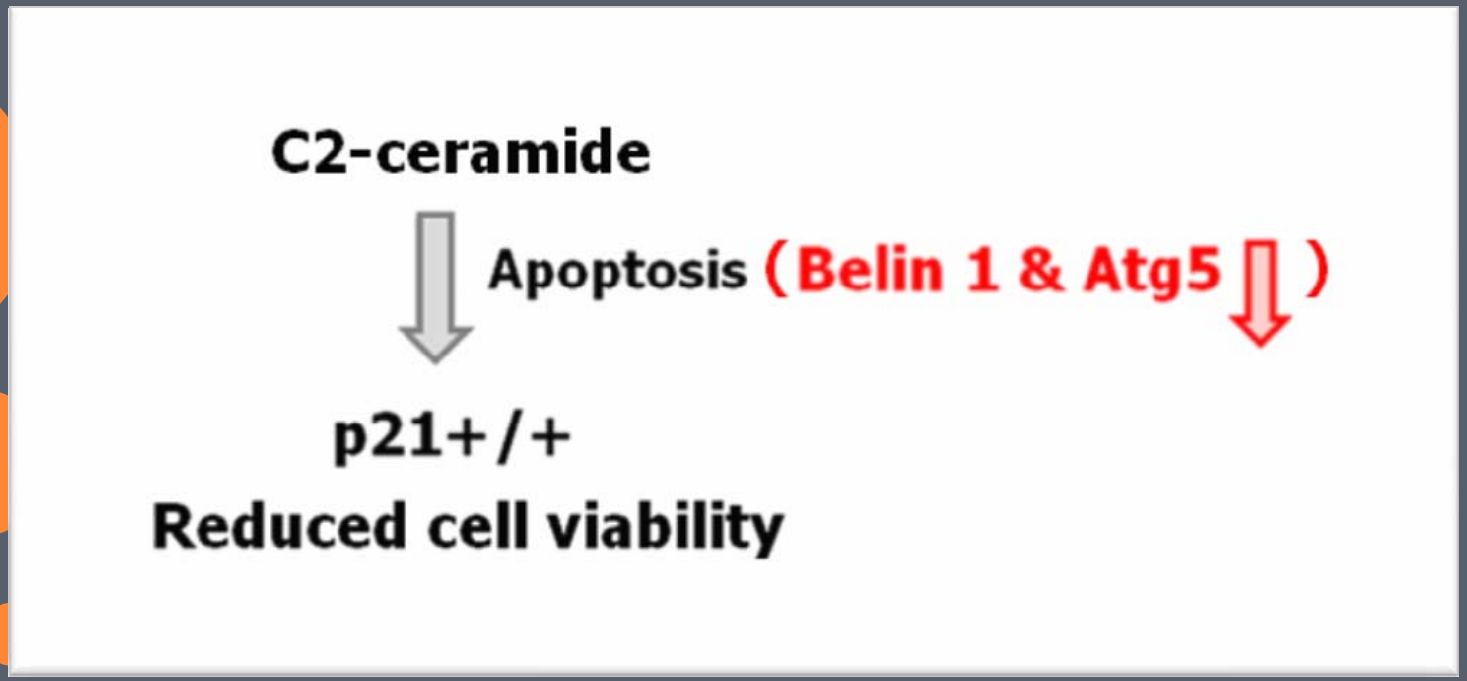


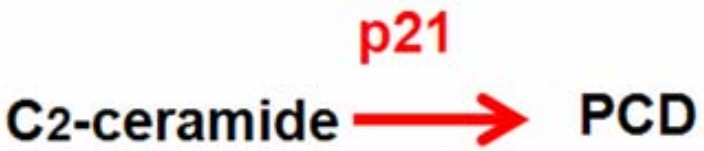
↓

C2–ceramide induces apoptosis in p21+/+

NOT by CDK activity

C2–ceramide induces apoptosis in p21+/+ not by CDK activity , but by destabilizing Atg proteins.



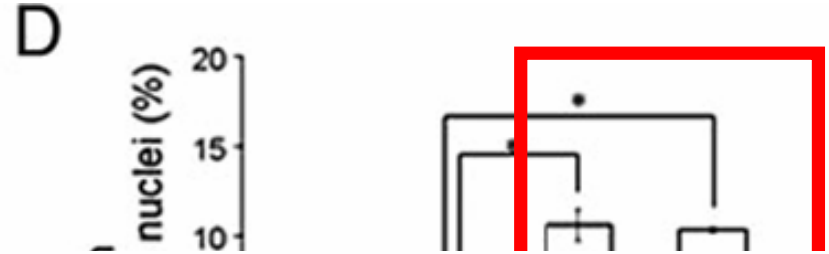


In p21 -/-

Inhibit Atg5 or Beclin 1 ...?

Beclin 1 & Atg5 involved in C2-ceramide induced PCD

p21-/- cells



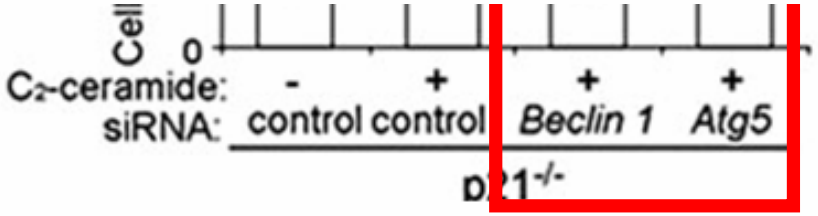
p21

C2-ceramide → PCD In p21-/- **Autophagy**

Atg5 & Beclin 1 **high**

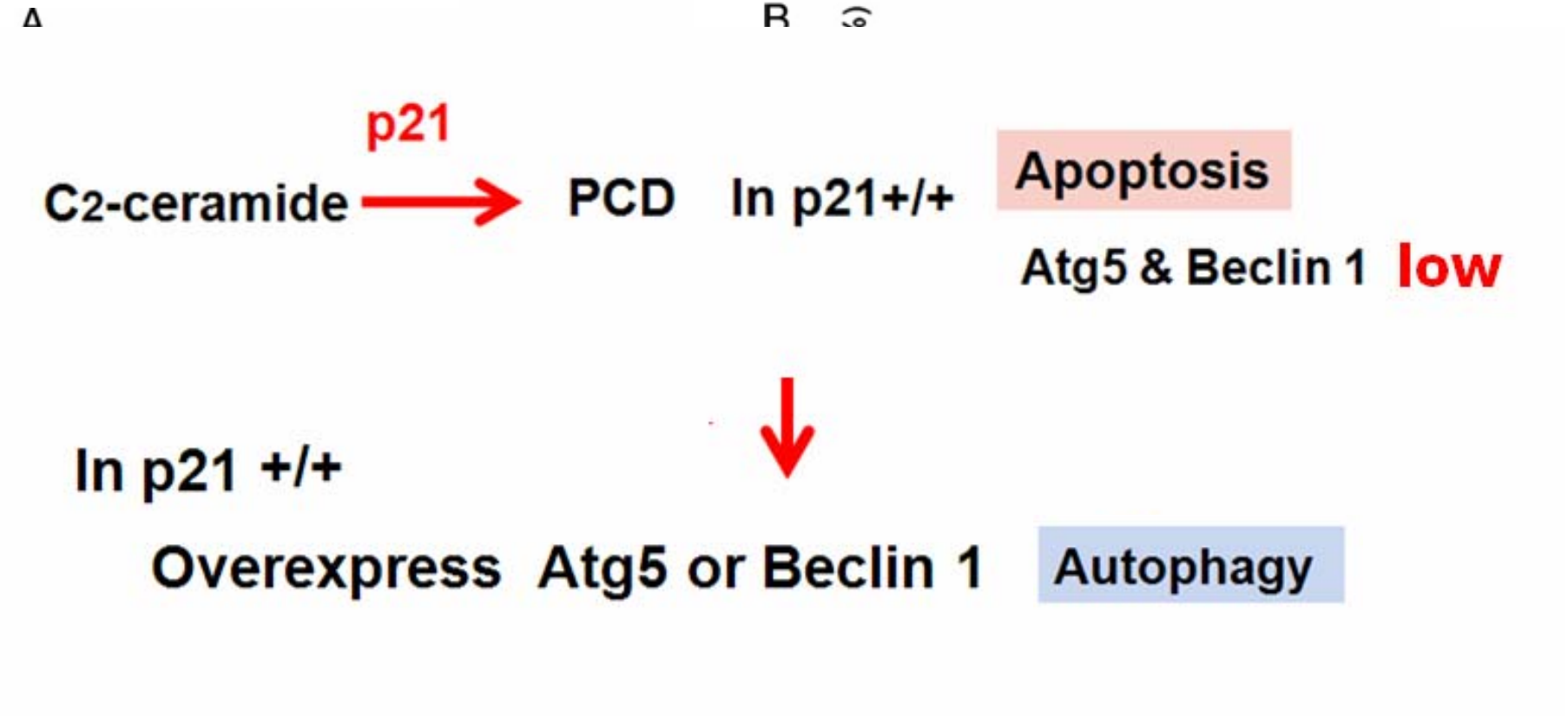
In p21 -/-

Inhibit Atg5 or Beclin 1 **Apoptosis**



Beclin 1 & Atg5 involved in C_2-ceramide induced PDC

P21+/+ cells

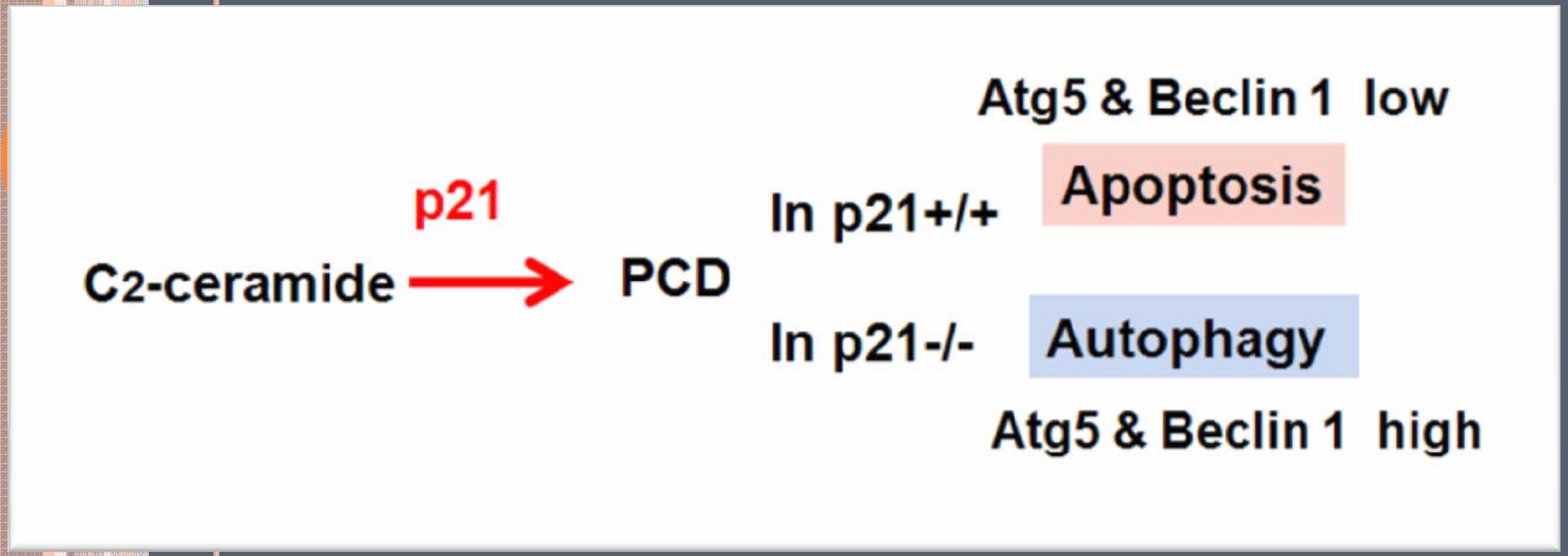


↓ apoptosis, ↑ autophagy

C2-ceramide induced apoptosis needs

↑ Beclin 1 and Atg5 ,

while autophagy is the opposite.



Conclusion

Atg5 & Beclin 1 low

p21

C_2-ceramide → PCD

In p21+/+ **Apoptosis**

In p21-/- **Autophagy**

Atg5 & Beclin 1 high

In p21 +/+

Inhibit p21 expression **Autophagy**

Overexpress Atg5 or Beclin 1 **Autophagy**

In p21-/-

Overexpress p21 proteins **Apoptosis**

Inhibit Atg5 or Beclin 1 **Apoptosis**

Importance of this research

Uncontrolled PCD would result in diseases

↓

Knowing more about the **regulation mechanism of PCD**

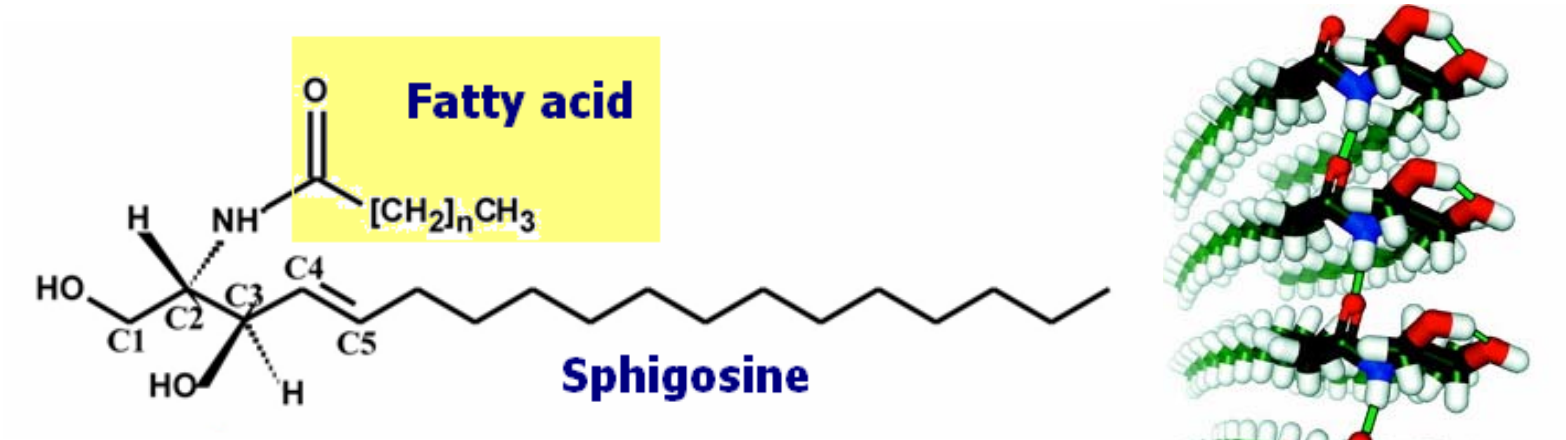
↓

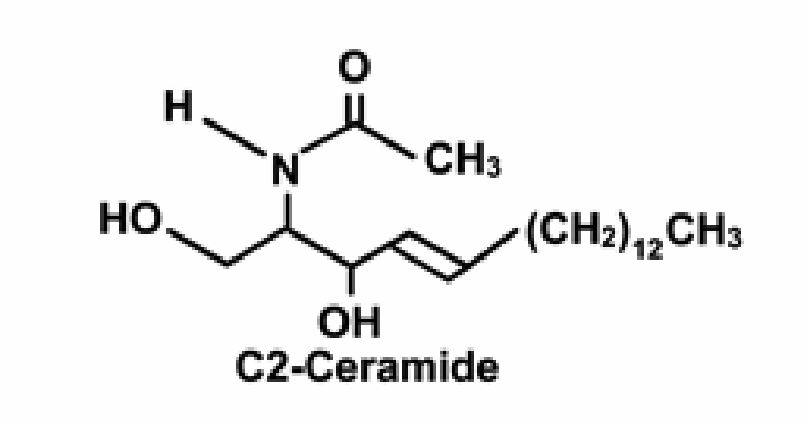
Preventing diseases occurrence

Thanks for your attention !!

Ceramide

The fourth large class of membrane lipids (polar)



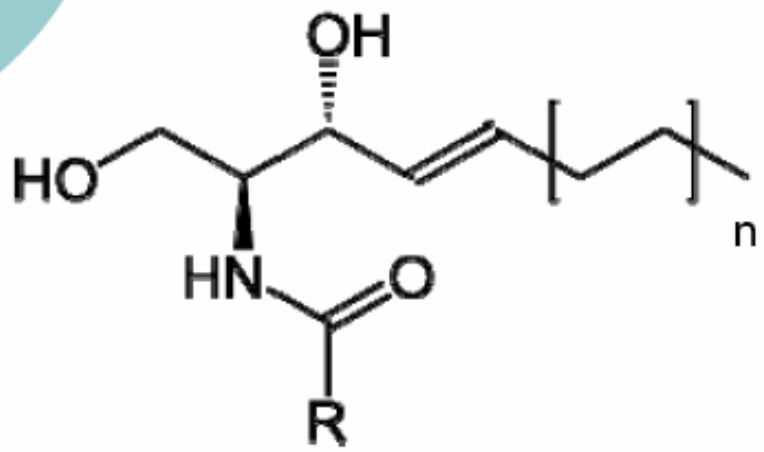


Ceramide in apoptosis

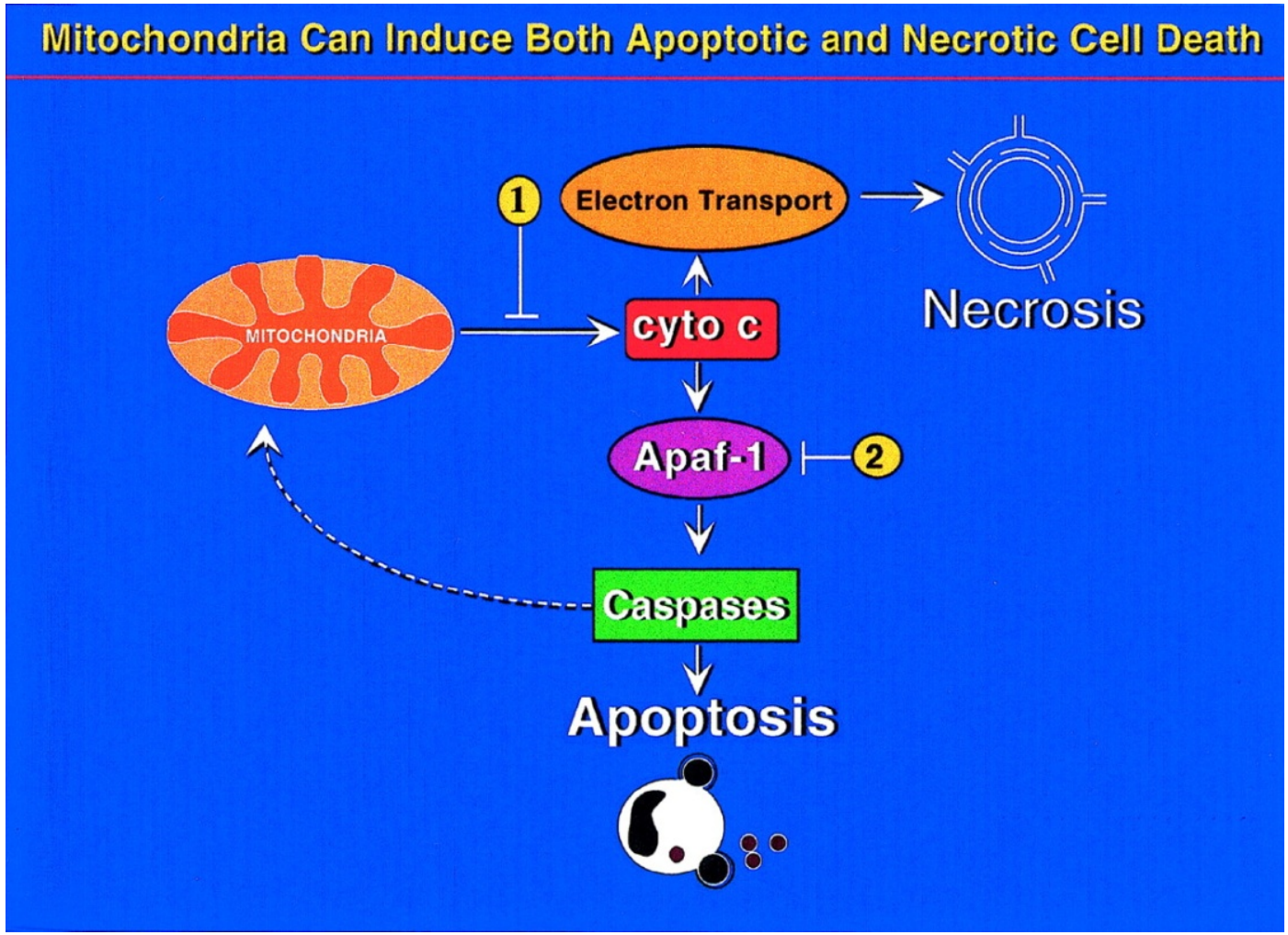
Ceramide induces **mitochondrial cytochrome C release** before transmembrane depolarization and caspase-3 activation [20,21]. Recent evidence shows that ceramide specifically **forms channels in mitochondrial outer membranes, facilitating mitochondrial protein release** [22]. Ceramide induces caspase-dependent [21,23] as well as caspase-independent apoptosis [24,25]. In addition, ceramide treatment leads to activation of the stress-activated protein kinase (SAPK/JNK) [26,27]. Prosurvival pathways are also affected by elevated cellular concentration of ceramide. Ceramide suppresses Ras/Raf1/MEK1 activation [28]. Studies show that ceramide is involved in dephosphorylation and inactivation of PI3 kinase/Akt [23]. It is suggested that **ceramide-mediated activation of phosphatases (ceramide-activated protein phosphatase) such as PP1 and PP2 [29-31] is involved in PI3 kinase/Akt inactivation** [32].

Molecular Vision 2007; **13**:1618-1626

Ceramides



- Sphingolipid
- Implicated in
  - Differentiation
  - Cell cycle arrest
  - Apoptosis
  - Senescence
- 2nd-messenger-functions
- Produced by several stress stimuli, including chemotherapy and γ-irradiation


Mitochondria Can Induce Both Apoptotic and Necrotic Cell Death
1
Electron Transport
Necrosis
MITOCHONDRIA
cyto c
Apaf-1
2
Caspases
Apoptosis

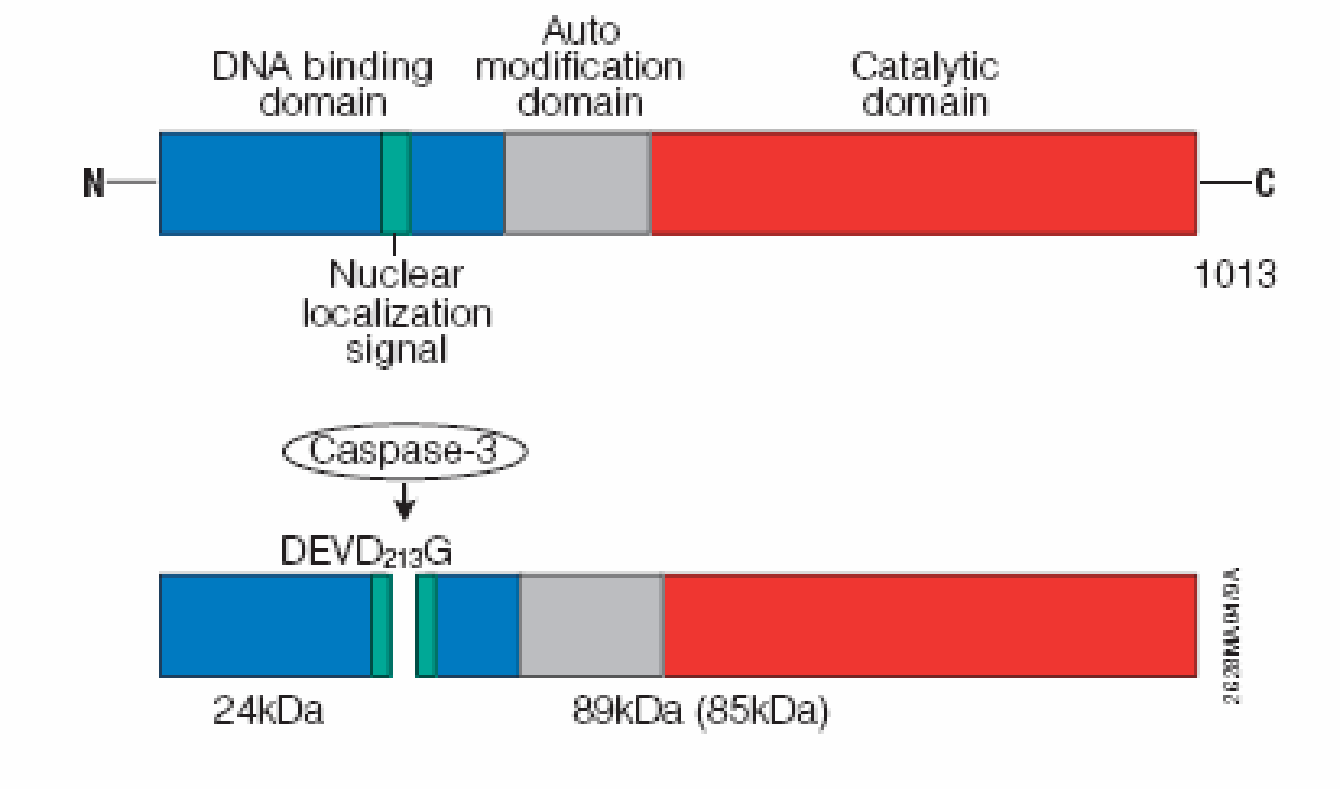
PARP

PARP，poly(ADP-ribosyl) transferase ，located in nuclear and in charged of DNA repairment, preventing DNA form lysis. **It's very important for the genomic stability and cell viability.**

It's the main target of caspase-3 *in vivo*. After cleaved by caspase, PARP lost it's activity .
And the cleaved PARP is a significant indicator for apoptosis, and it's considered to be a marker of caspase-3 activation.

Genes and Development Vol. 11, No. 18, pp. 2347-2358

PARP



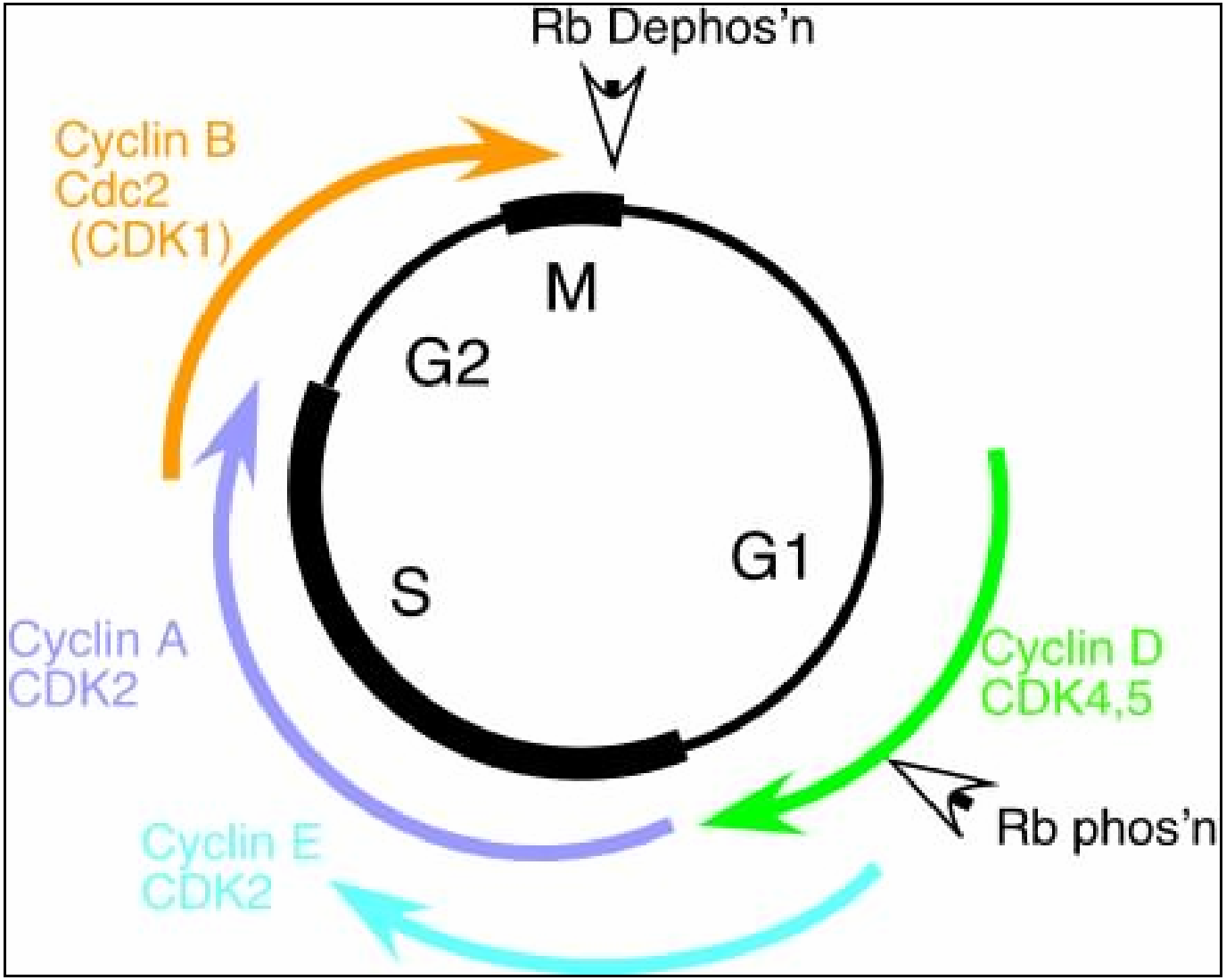
After cleaved by caspase **in to p24 and p89 inactive fragment**, to prevent excessive NAD consumption and ATP loss.

TUNEL

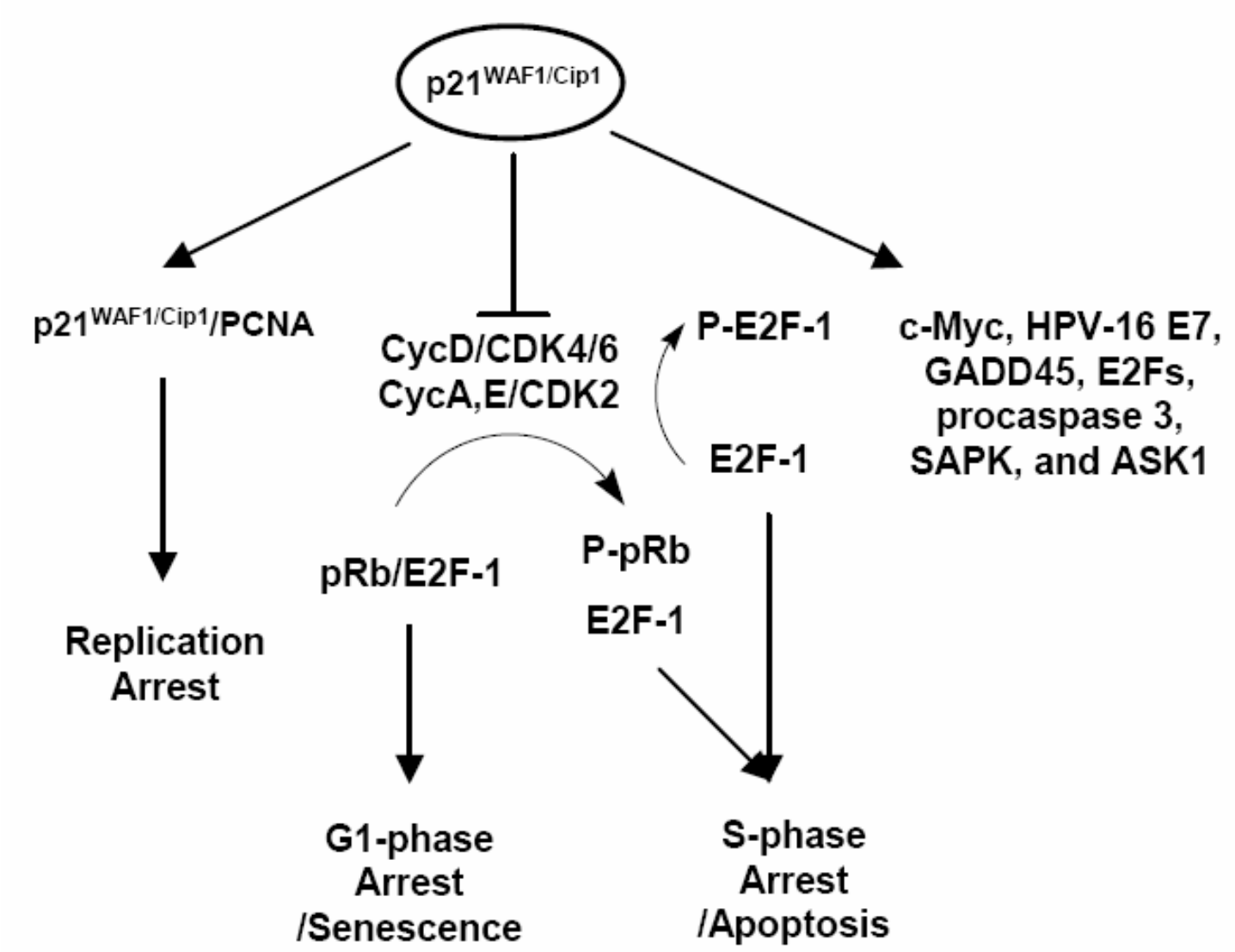
One reason for the inconsistent
results using the TUNEL assay, is that **small non-nuclear calcium-containing vesicles present in certain lesions can bind nucleotides** .

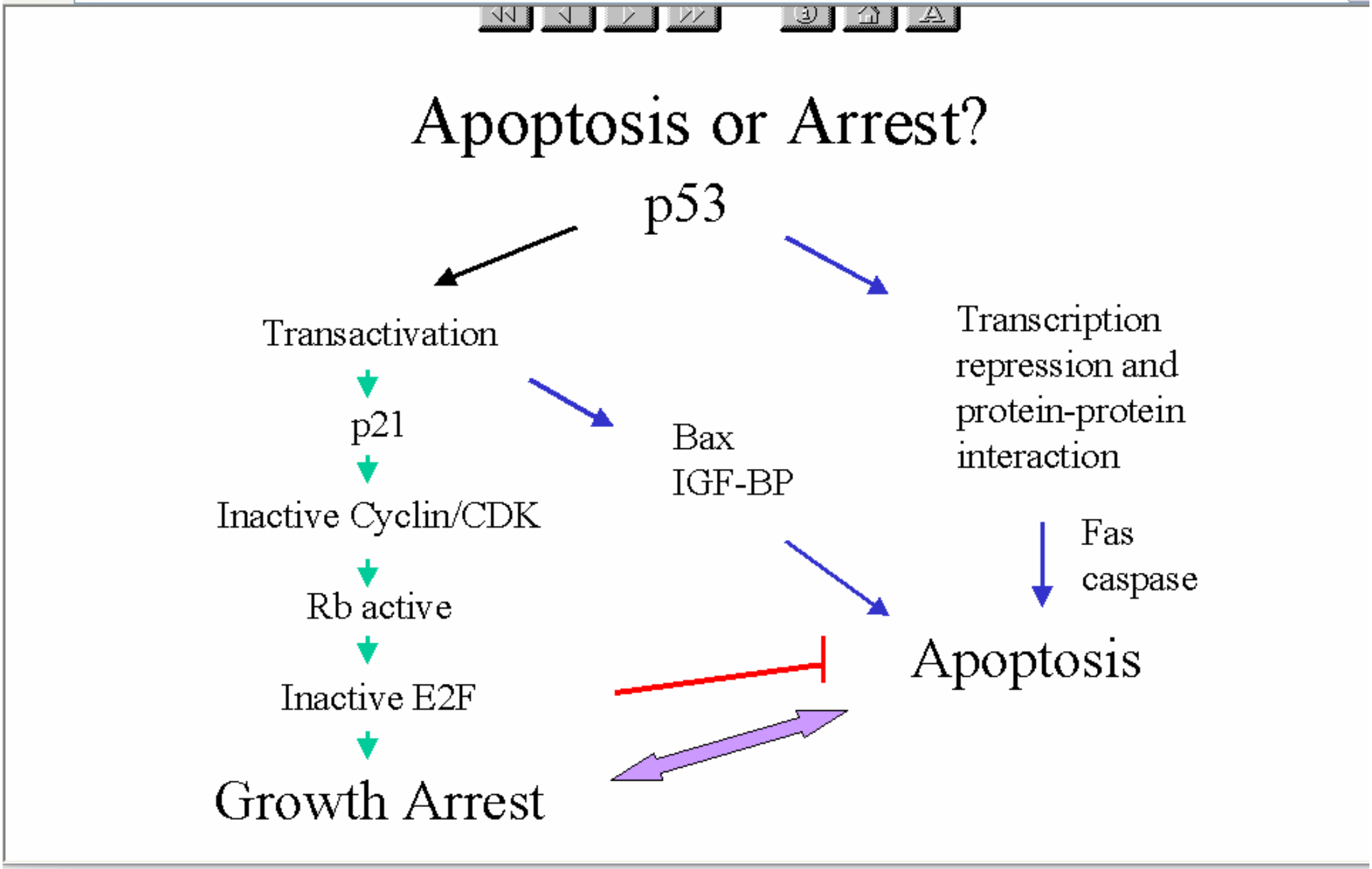
In addition, TUNEL staining is not specific for apoptotic cells; it was recently demonstrated in human atherosclerotic **plaques that nonapoptotic nuclei showing high levels of RNA synthesis/splicing can belabeled by the TUNEL technique .**

CDK function and regulation

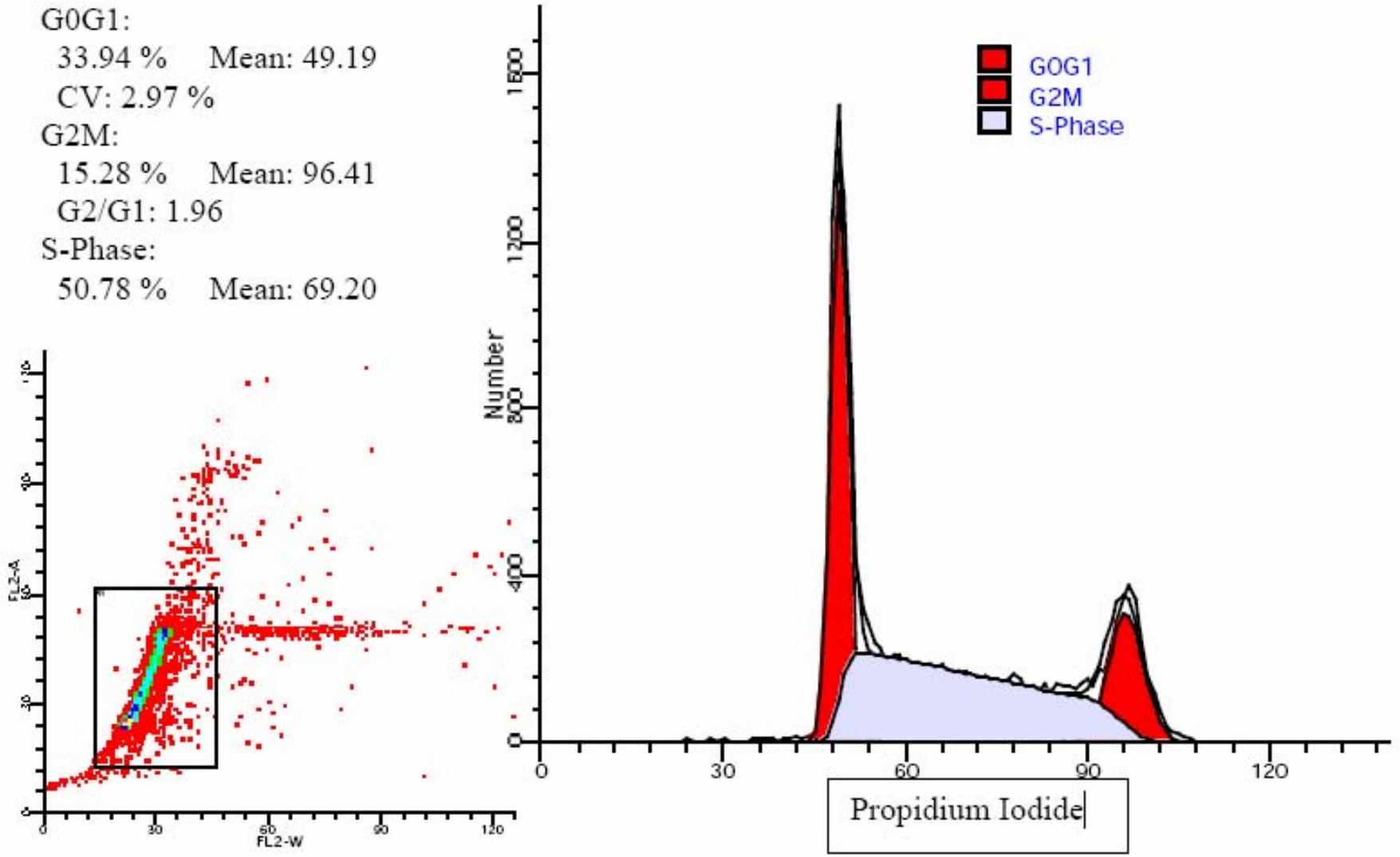


CIP/KIP family

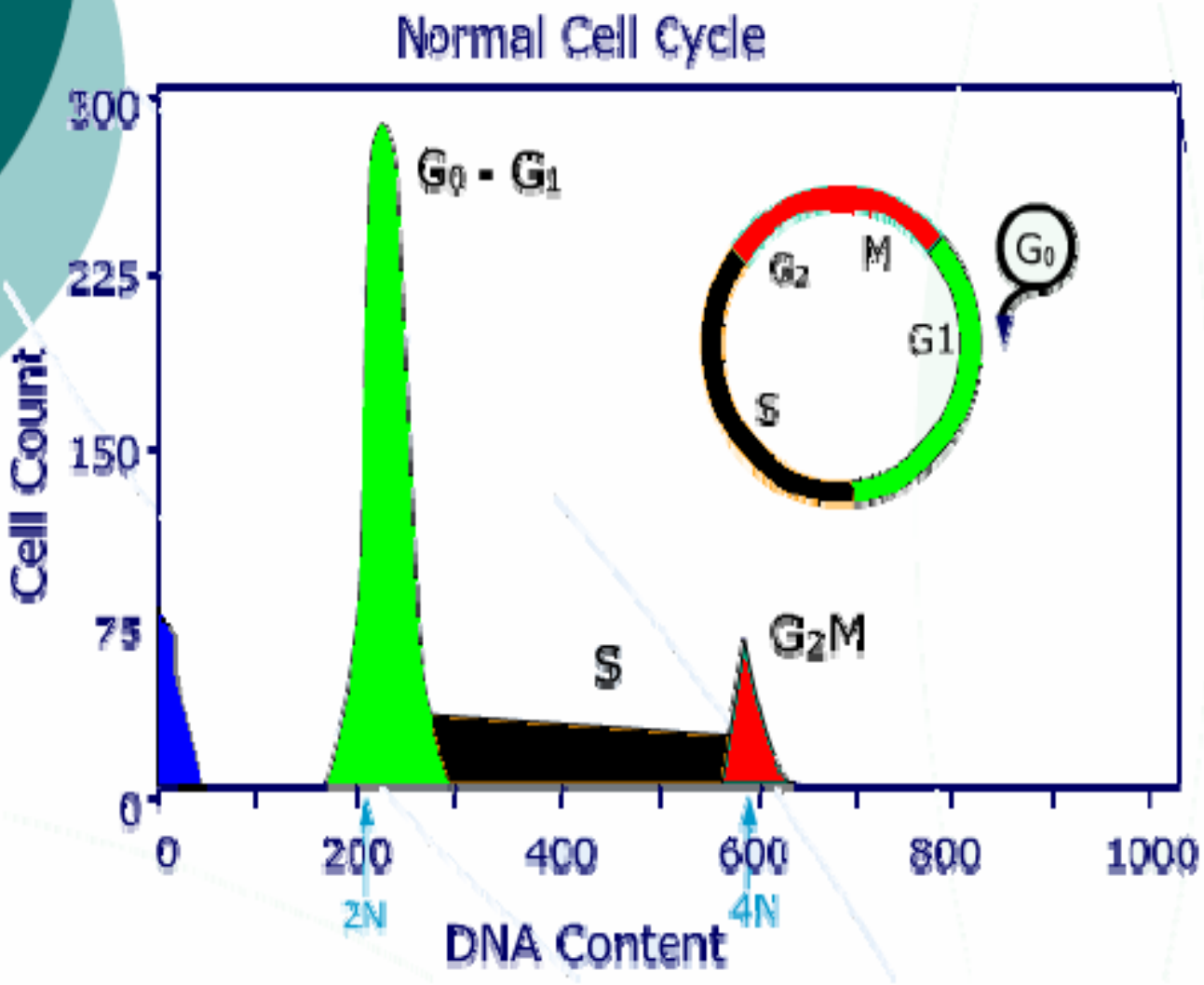




Sub-G1 phase


G0G1:
33.94 % Mean: 49.19
CV: 2.97 %
G2M:
15.28 % Mean: 96.41
G2/G1: 1.96
S-Phase:
50.78 % Mean: 69.20
FL2-A
FL2-W
Number
GOG1
G2M
S-Phase
Propidium Iodide

FACScan analysis of DNA content



- G0/G1 phase: diploid/DNA content of 2n
- G2/M phase: DNA content of 4n
- S-phase cells: DNA content >2n and < 4n
- Apoptotic cells: subdiploid (<2n) can also be quantified using this method

http://www.cyto.purdue.edu/class/e-lectures/jpr2/jpr2.html

G0

G_0

Many times a cell will leave the cell cycle, temporarily or permanently. It exits the cycle at G_1 and enters a stage designated G_0 (G zero). A G_0 cell is often called "quiescent**"**, but that is probably more a reflection of the interests of the scientists studying the cell cycle than the cell itself. **Many G_0 cells are anything but quiescent. They are busy carrying out their functions in the organism. e.g., secretion, attacking pathogens.**

Often G_0 cells are **terminally differentiated**: they will never reenter the cell cycle but instead will carry out their function in the organism until they die.

For other cells, G_0 can be followed by reentry into the cell cycle**. Most of the lymphocytes in human blood are in G_0**. However, with proper stimulation, such as encountering the appropriate antigen, they can be stimulated to reenter the cell cycle (at G_1) and proceed on to new rounds of alternating **S phases** and **mitosis**.

G_0 represents **not simply the absence of signals for mitosis but an active repression of the genes needed for mitosis**. **Cancer cells cannot enter G_0 and are destined to repeat the cell cycle indefinitely.**

GAPDH

GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE

HOECHST 33258

- **bisbenzimide**

Hoechst 33258 染色是染富含AT的區域。

AUTOPHAGY

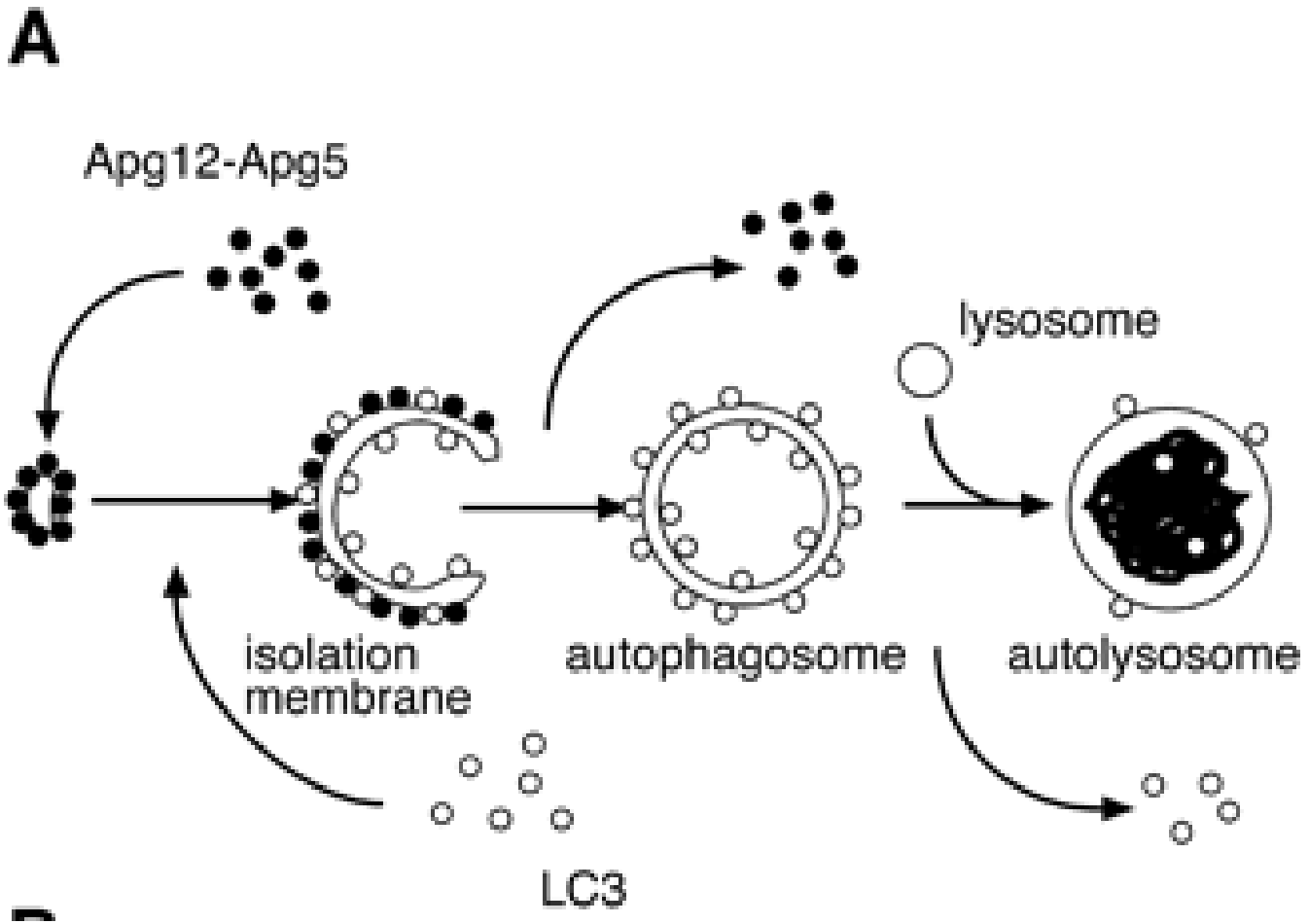
Microautophagy

The formation of autophagosomes is initiated by class III phosphoinositide 3-kinase and autophagy-related gene (Atg) 6 (also known as Beclin-1). In addition, two further systems are involved, composed of the ubiquitin-like protein Atg8 (also known as light chain (LC)3) and the Atg4 protease on the one hand and the Atg12-Atg5-Atg16 complex on the othe

Macroautophagy

Chaperone- mediated autophagy

Light chain 3-LC3 (Atg8)



J Cell Biol. 2001 Feb 19;152(4):657-68.

AVOS

AVO is a defense
mechanism that incr

vacuolar p

. In particular, the form
(AVOs) may be prever
proton ATPase ("V-H+
are based on the obse
accumulate AVOs and
surviving colonies of c
H+-ATPase decreases
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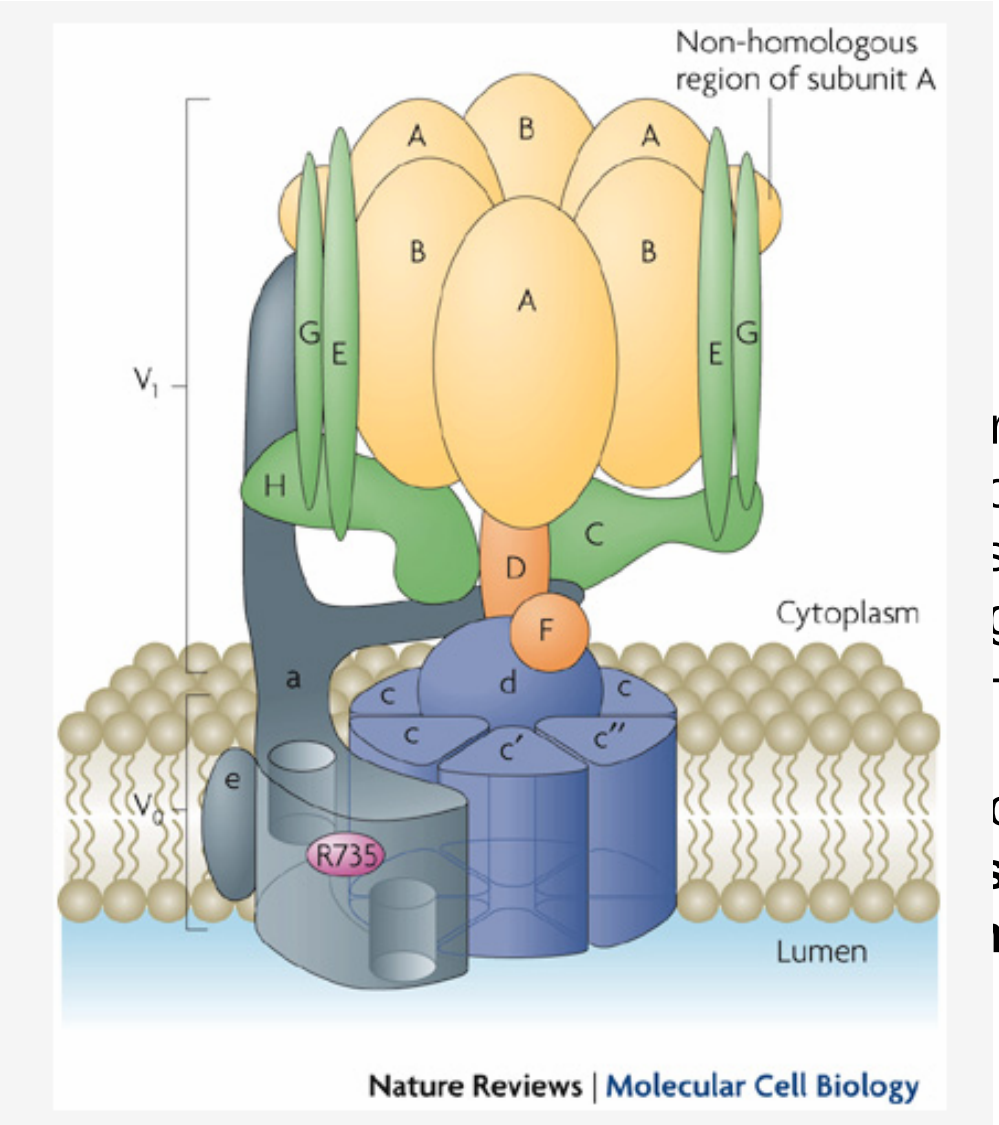

r organelles
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inhibition of V-
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