

## 2 蛋白質抽取 Protein extraction

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- 2.1 如何開始？ How to start?  
5W principles 基本原則
- 2.2 材料來源 Materials & sources  
材料取得與保存
- 2.3 均質及抽取 Homogenization & extraction  
確實做好第一步
- 2.4 鹽析及沉澱法 Salting-out & precipitation  
最經濟方便的方法

## ■ 如何開始？ How to start?

.....

先考慮以下諸點：

5W

- a. 要純化那一個蛋白質？ What ?
- b. 為何要純化此蛋白質？ Why ?
- c. 由何種材料純化？ Where, from ?
- d. 由那一個生長期？ When ?
- e. 如何純化此蛋白質？ How ?

# ■ 酵素純化過程的三個階段 Three stages

## (1) 粗蛋白 (crude protein) :

採樣 → 均質打破細胞 → 抽出全部蛋白，  
多用鹽析沉澱法。

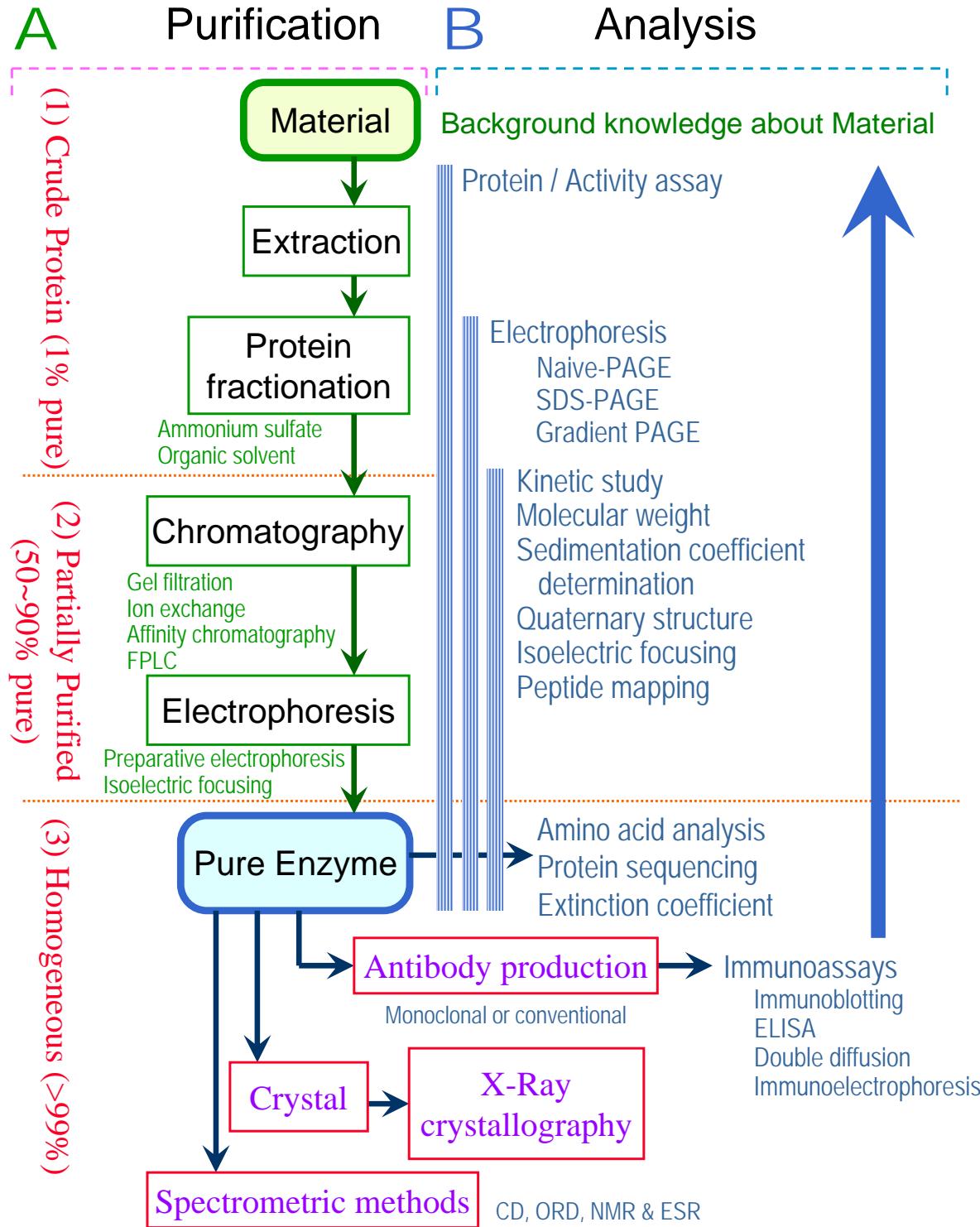
## (2) 部分純化 (partially purified) :

初步的純化，使用各種管柱層析法。

### (3) 均質酵素 (homogeneous) :

目標酵素的進一步精製純化，可用製備式電泳或 HPLC 等。

# 酵素純化階段及分析方法

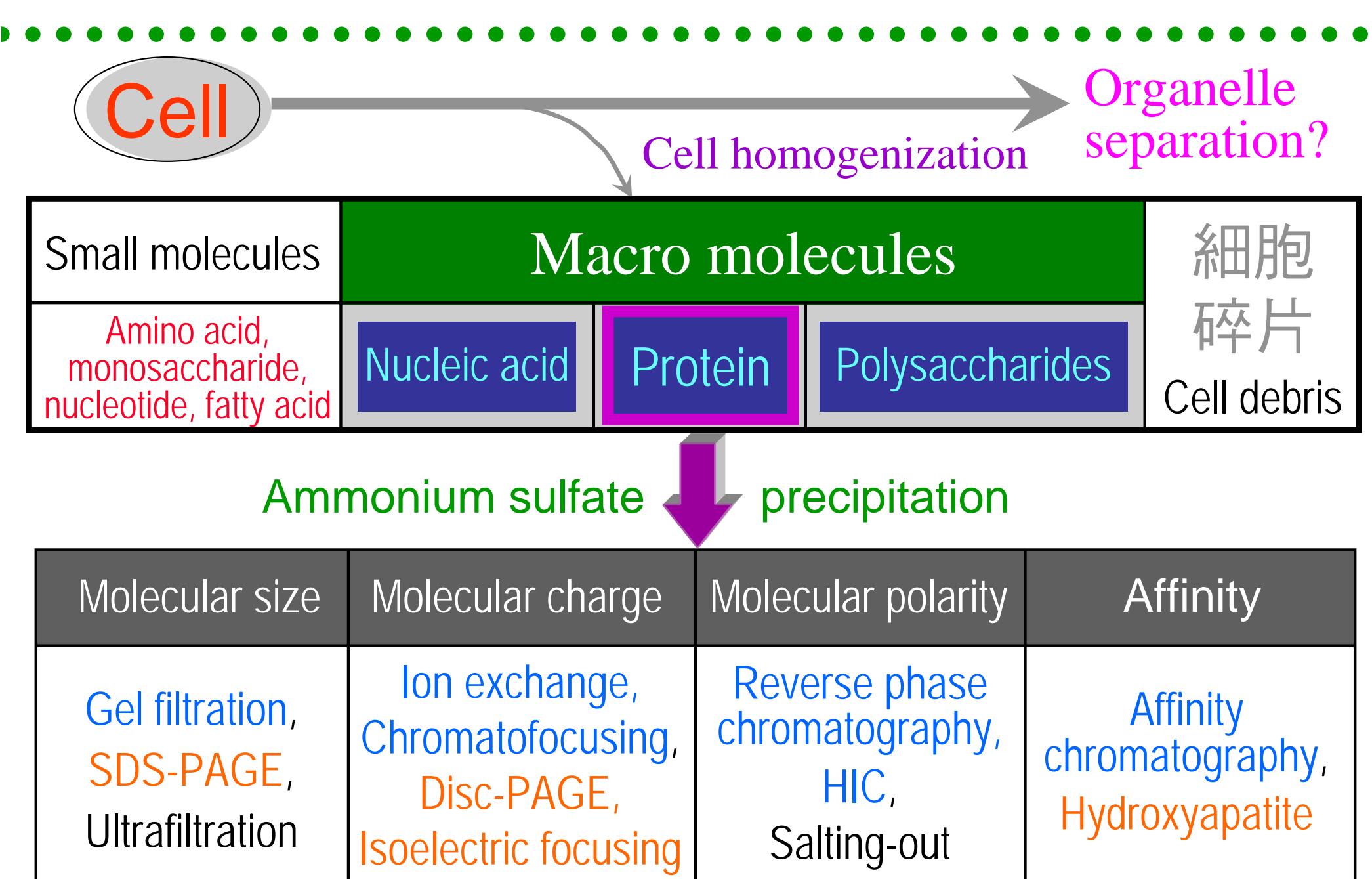


(1) 粗蛋白  
Crude protein

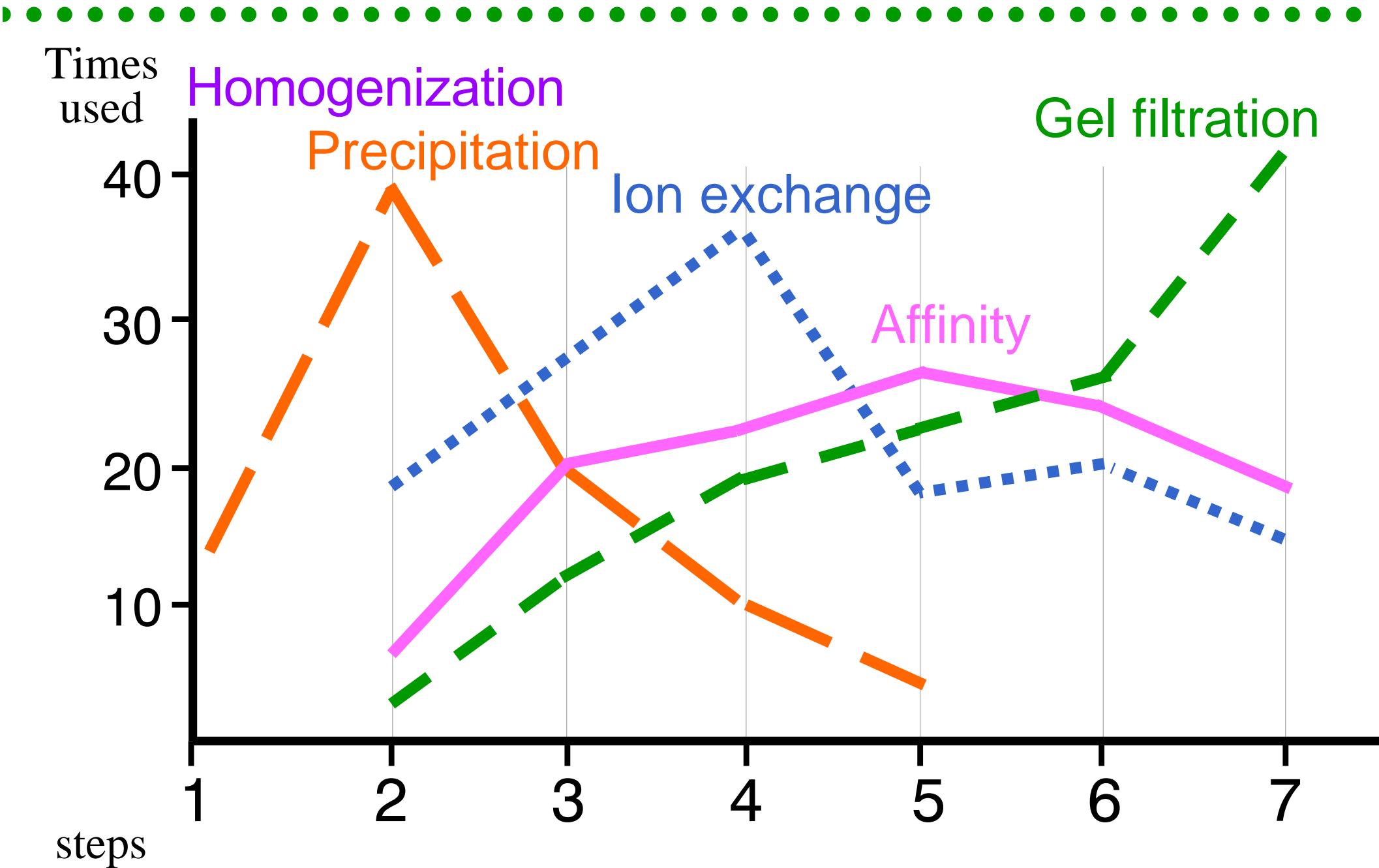
(2) 部分純化  
Partially purified

(3) 均質酵素  
Homogeneous

# ■ 各種純化或分析方法的原理



■ 各種純化方法的應用次序 Which step first?



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## ■ 目標材料之選擇 What's your starting material?

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● Which organism?

動物、植物、微生物

● Which tissue?

根莖葉花果或組織培養

● Which organelle?

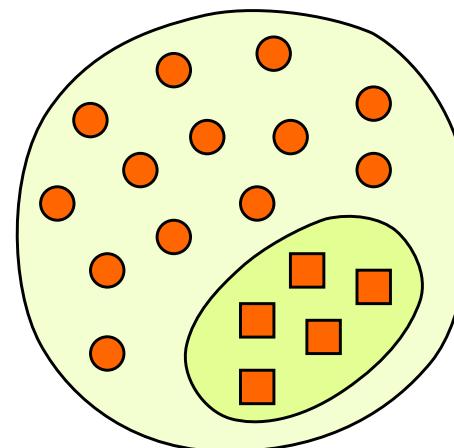
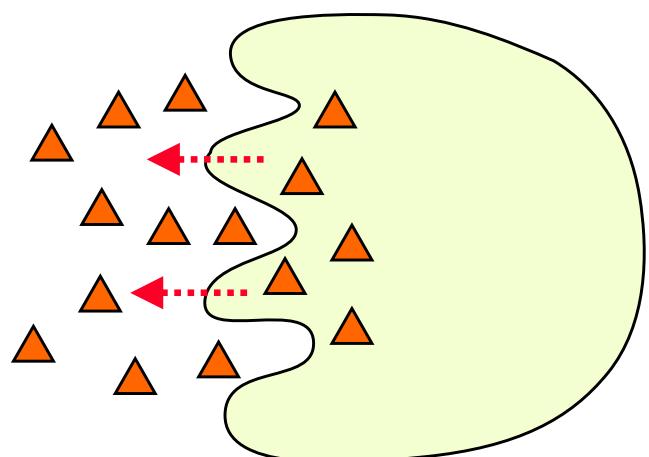
細胞核、液泡、葉綠體

● Secreted enzyme?

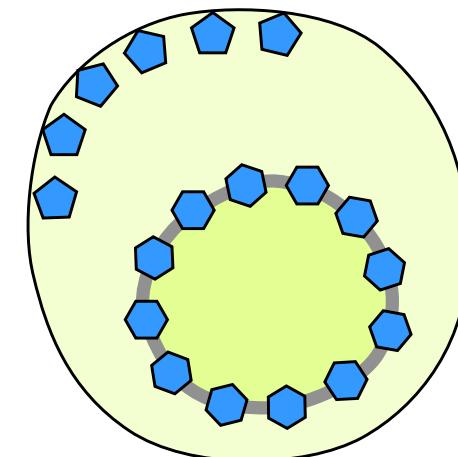
有關酵素的穩定性

● Membrane protein?

影響抽取策略的設計



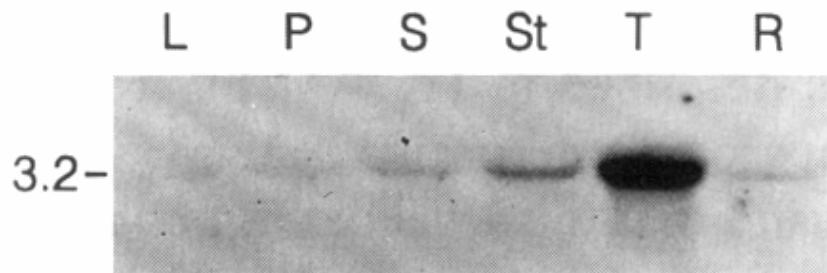
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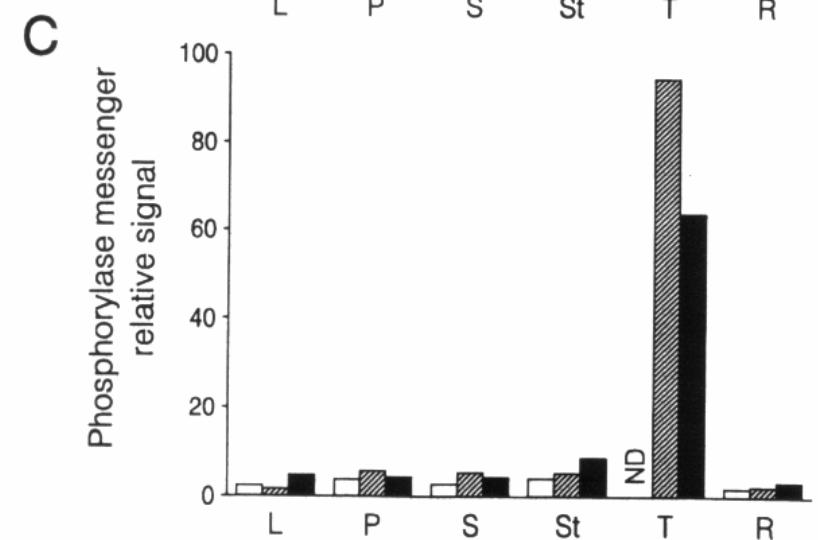
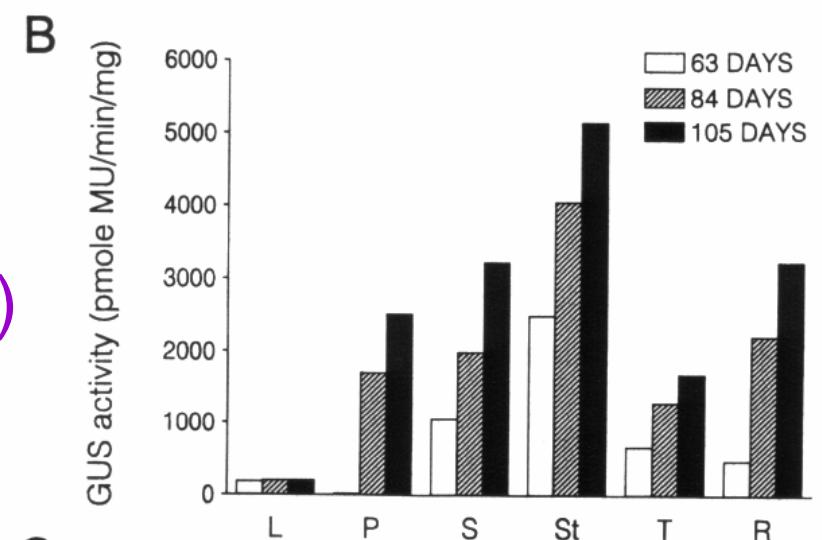
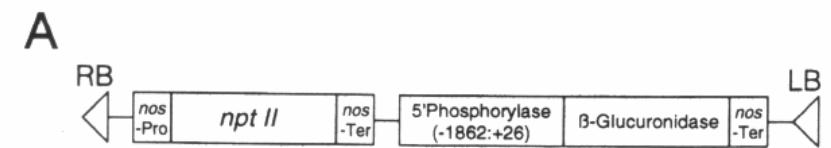
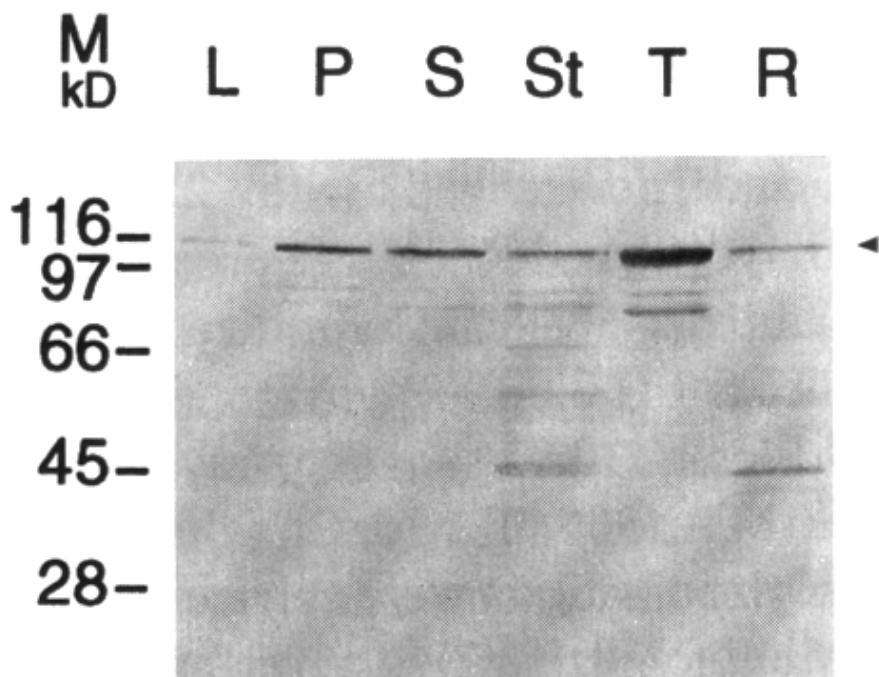
St Pierre,B.; Bertrand,C.; Camirand,A.; Cappadocia,M.; Brisson,N. (1996) Plant Molecular Biology 30: 1087-1098  
The starch phosphorylase gene is subjected to different modes of regulation in starch-containing tissues of potato



## SP RNA expression (potato tissues)

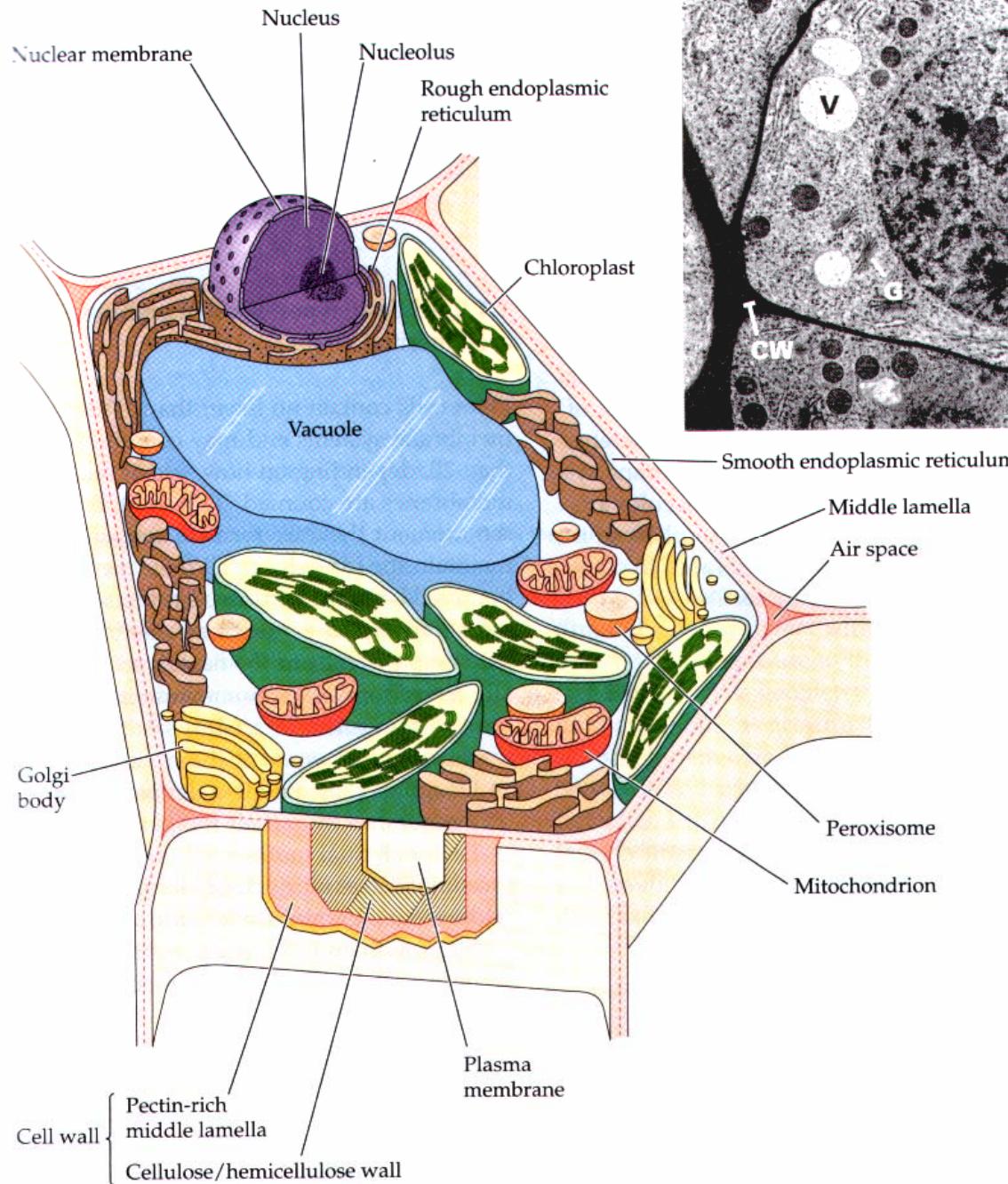


## SP protein expressed (potato tissues)



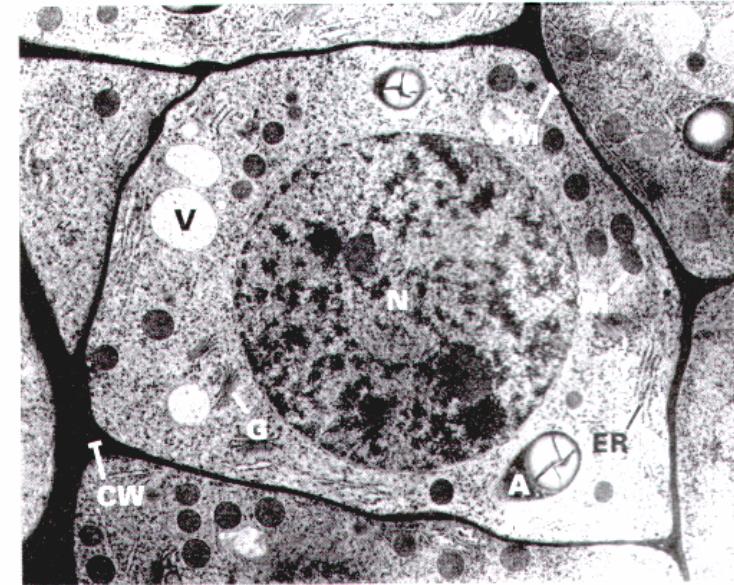
# 一個典型植物細胞

(A) Mesophyll



A typical plant cell

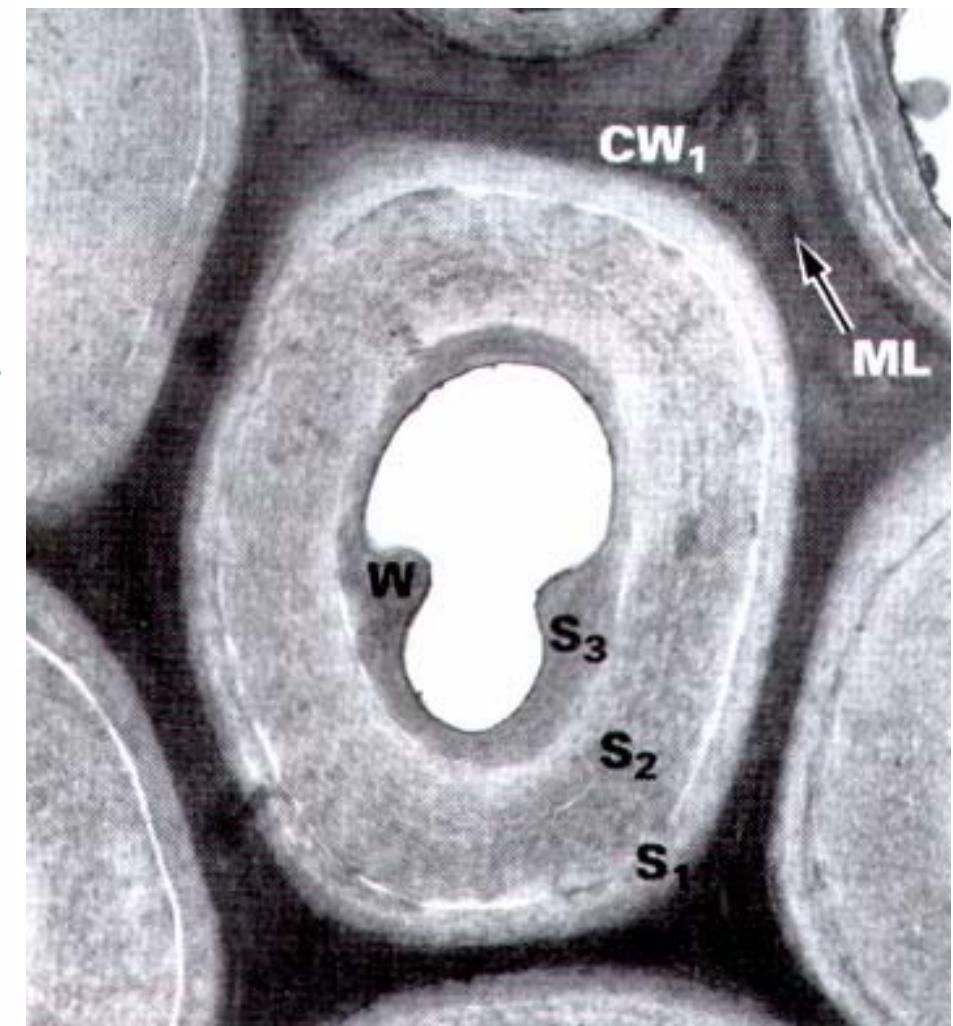
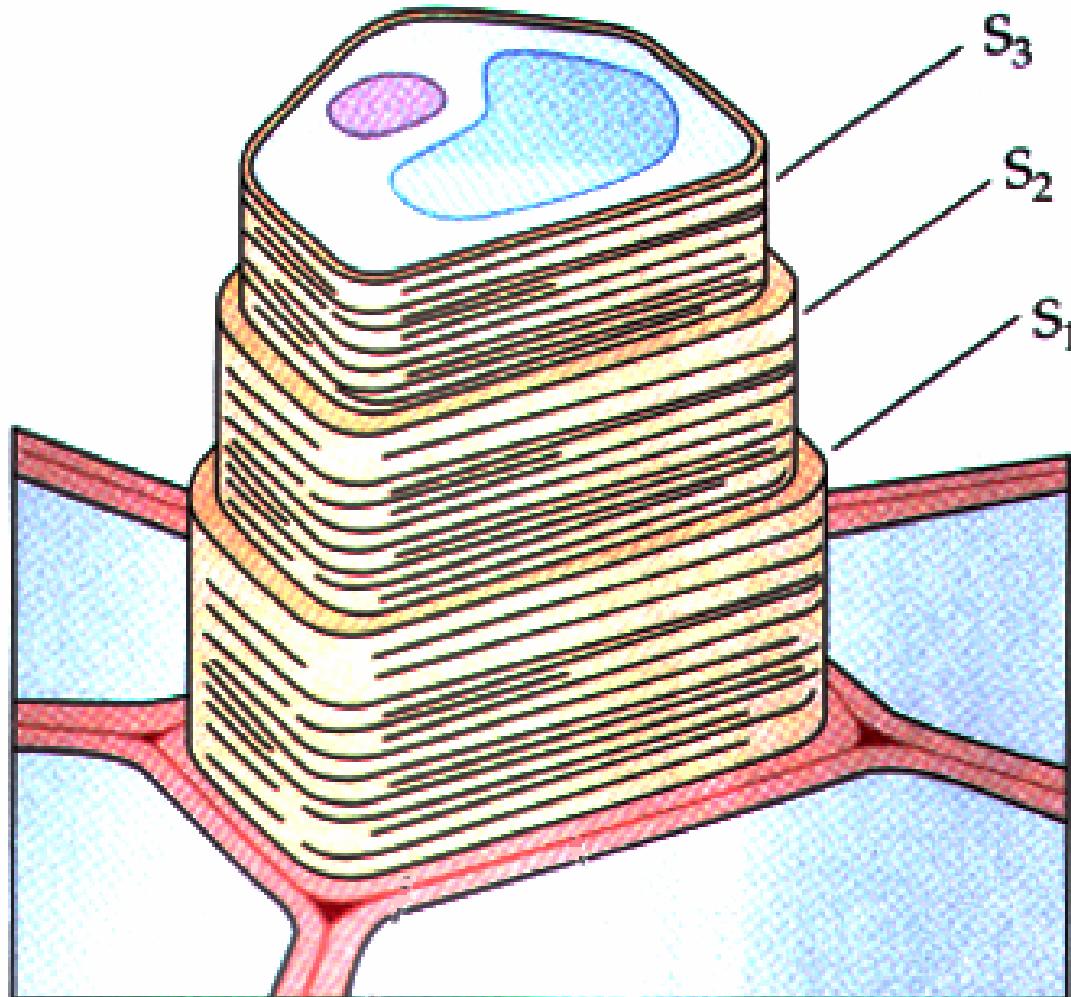
(B)



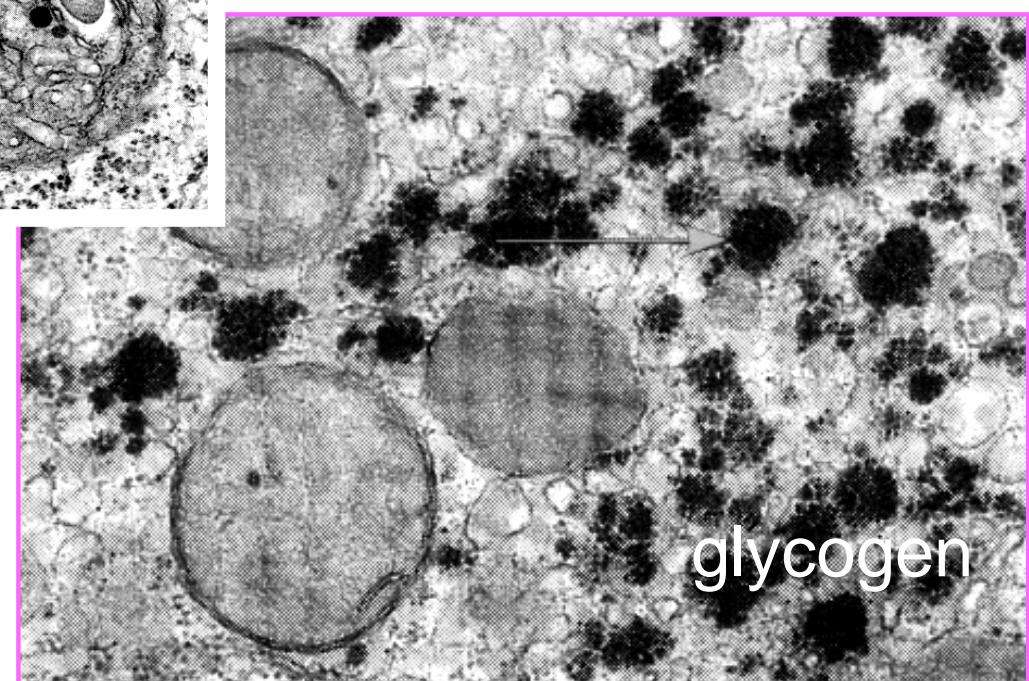
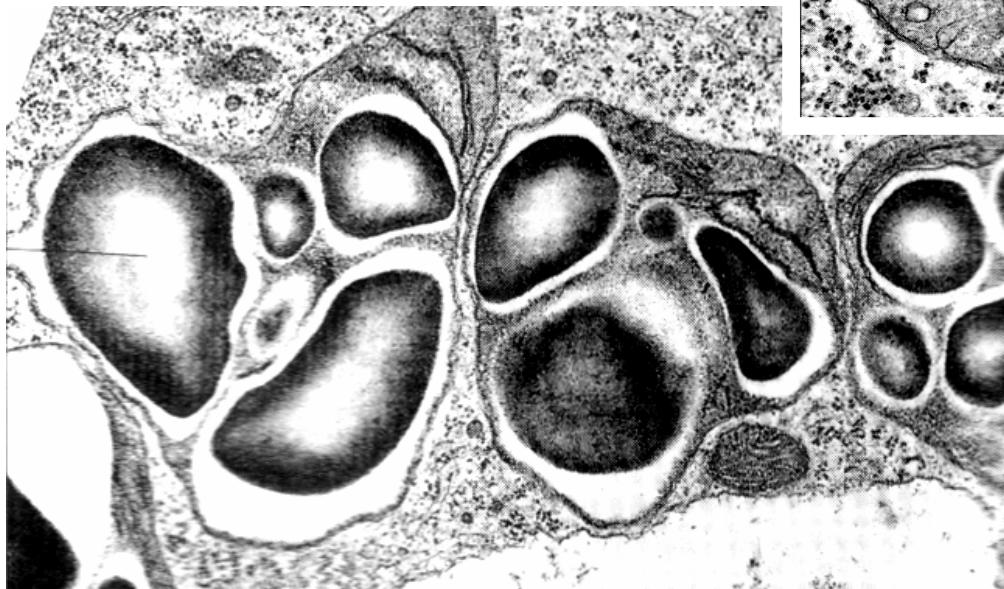
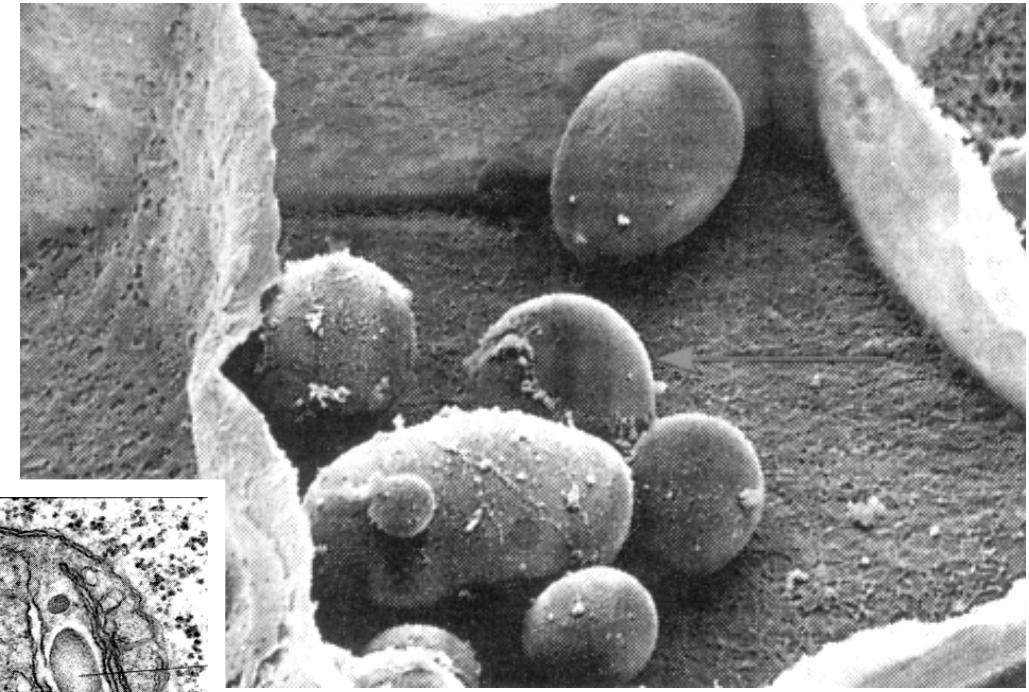
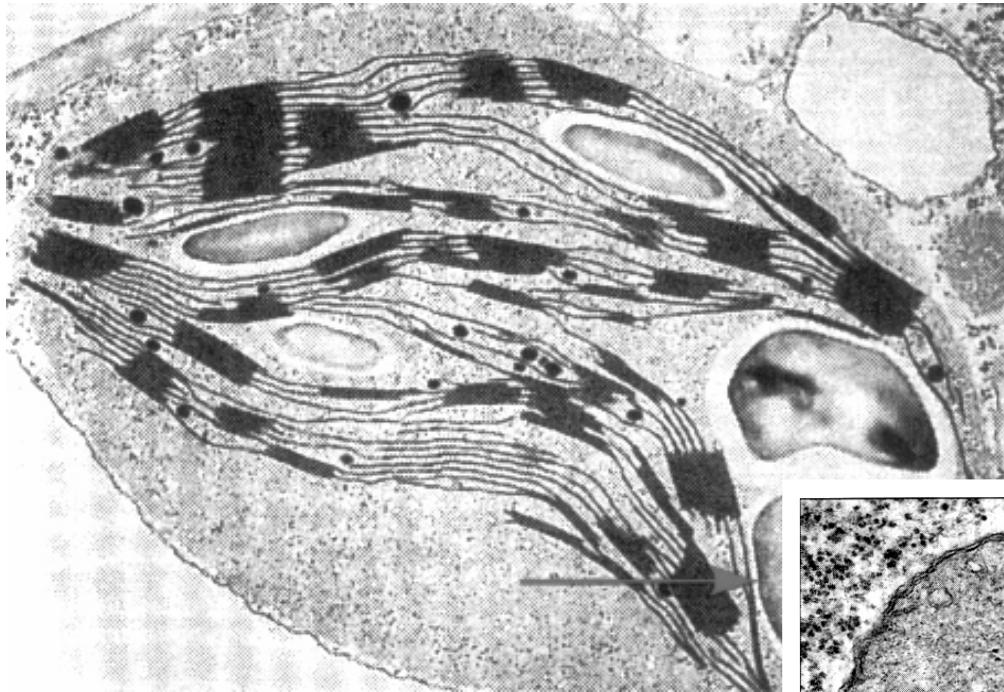
## ■ 植物的細胞壁 Plant cell wall is a barrier

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- Old plant cell wall is very tough to break



● Transformed from proplastid to amyloplast and chloroplast

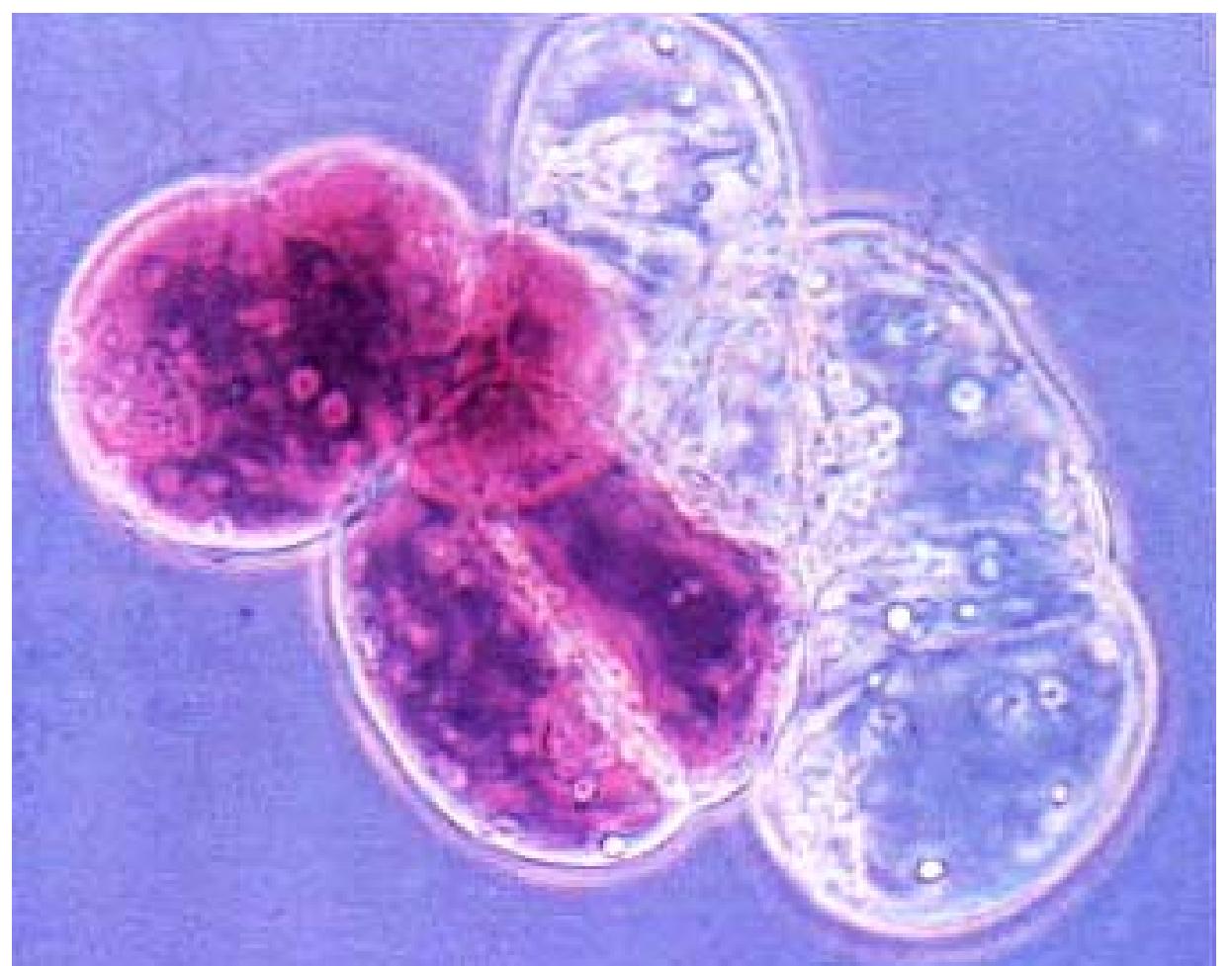


## ■ 植物色素在純化上的問題 Pigment is a problem

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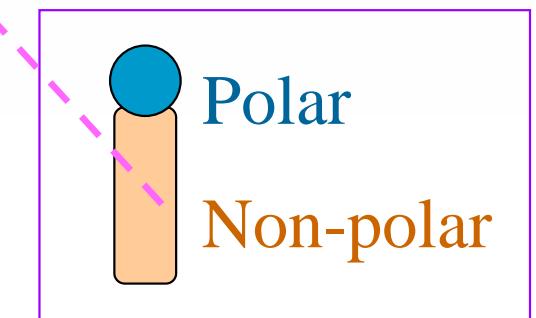
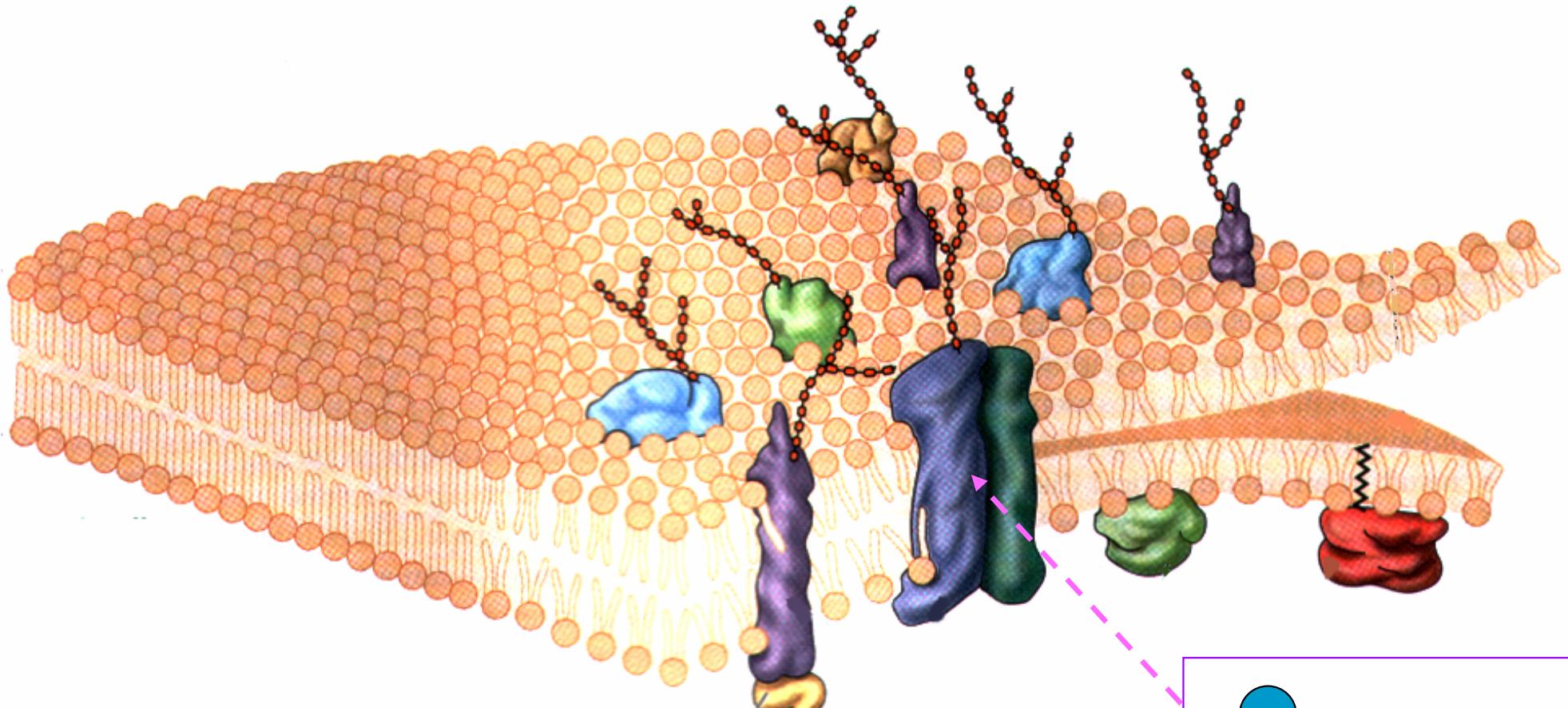
- Pigment stains the gel during purification



## ■ 細胞膜蛋白質抽取較困難 Membrane proteins

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- Detergent (Triton X) is used to solubilize membrane proteins



## ■ 植物材料問題 Special problems for plant materials

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- 細胞壁 Cell wall 較難打破
- 葉綠體 Chloroplast 特有的酵素
- 液泡 Vacuole 有許多干擾物質

蛋白酶 (proteases)

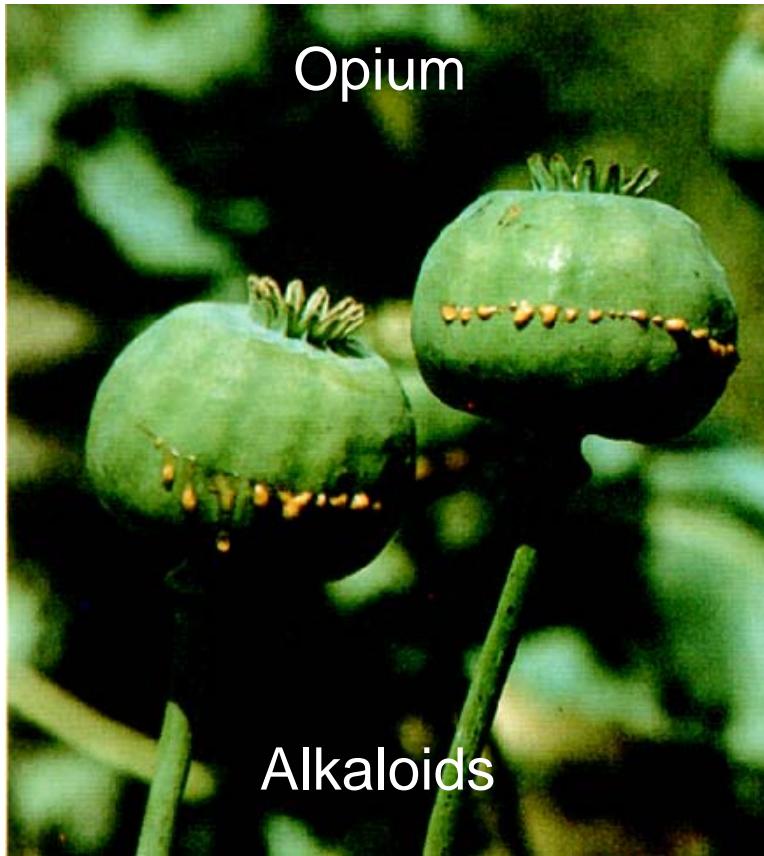
多酚化合物 (polyphenols):

Alkaloid 生物鹼

Flavonoid 類黃酮

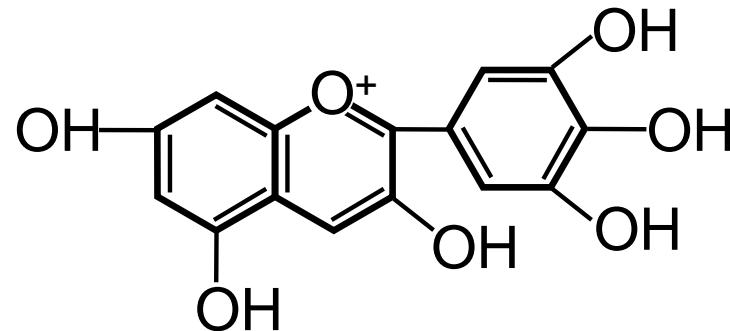
Tannin 單寧

# 植物一次代謝物有重要藥理作用



牛頓雜誌 (1991) 第 164 期, p.48-49

## Polyphenolic compounds



Plant secondary metabolites  
are active ingredients of some  
important medical herb



## ■ 植物色素之產生與去除 How to remove pigment

.....

**$\beta$ -mercaptoethanol**

inhibit

Phenol oxidase

Decrease enzyme catalysis

Low temperature

Oxidation

***Phenolic compound*** →→→ **Pigment**

adsorb

吸附

**Polyvinylpolypyrrolidone  
(PVPP)**

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# ■ 打破細胞的方法 101 ways to break the cell

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## ● Dry way:

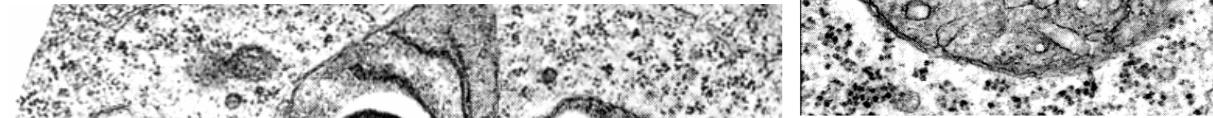
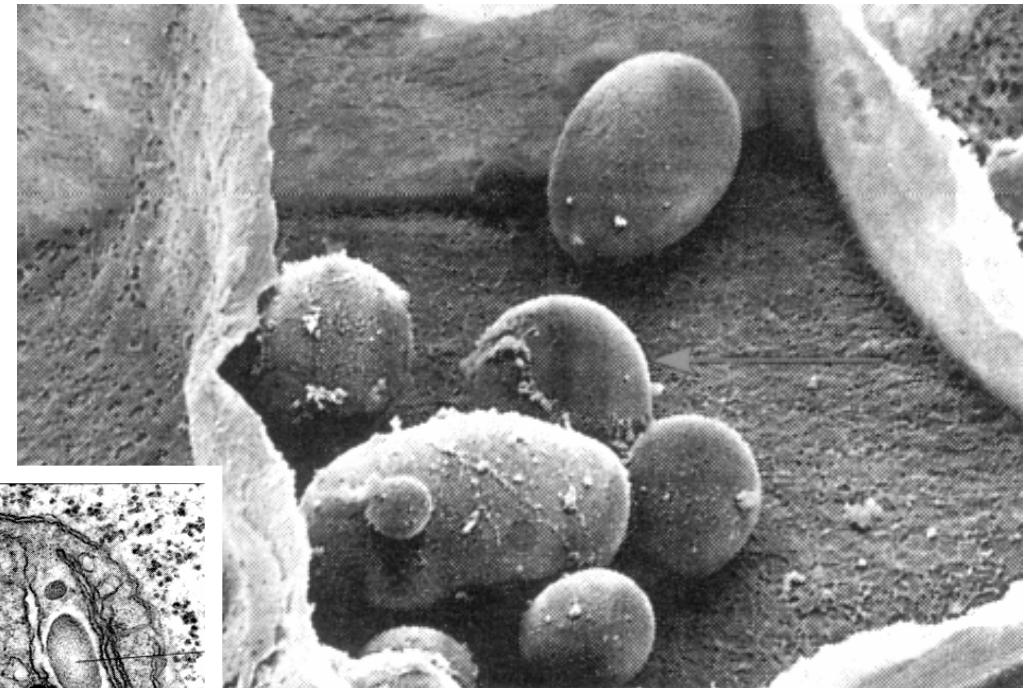
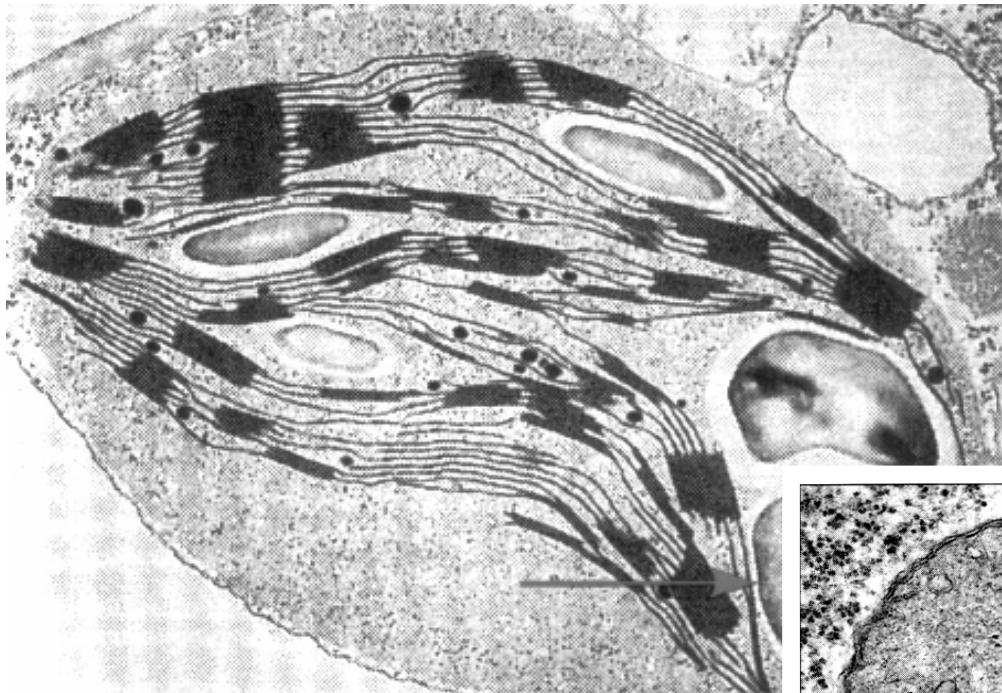
液態氮研磨 (grinding in liquid nitrogen), 磨粉機 (coffee grinder), 球磨機 (ball mill)

## ● Wet way:

均質器 (homogenizer), 果汁機 (Waring blender),  
Polytron, 研砵 (mortar), 玻璃球 (glass bead mill),  
超音波震盪 (ultrasonication), French press

Is your target protein released from the cell?

● 某些胞器的分離相當困難 Some organelles are difficult to isolate



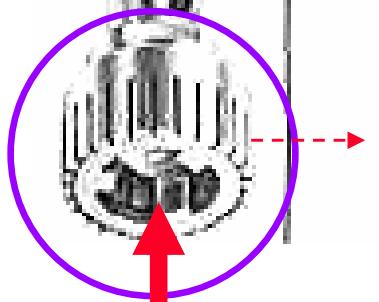
▼ Homogenizer for breaking the cell but leave organelles intact



## ■ 研磨樣本常用方法 Two popular methods

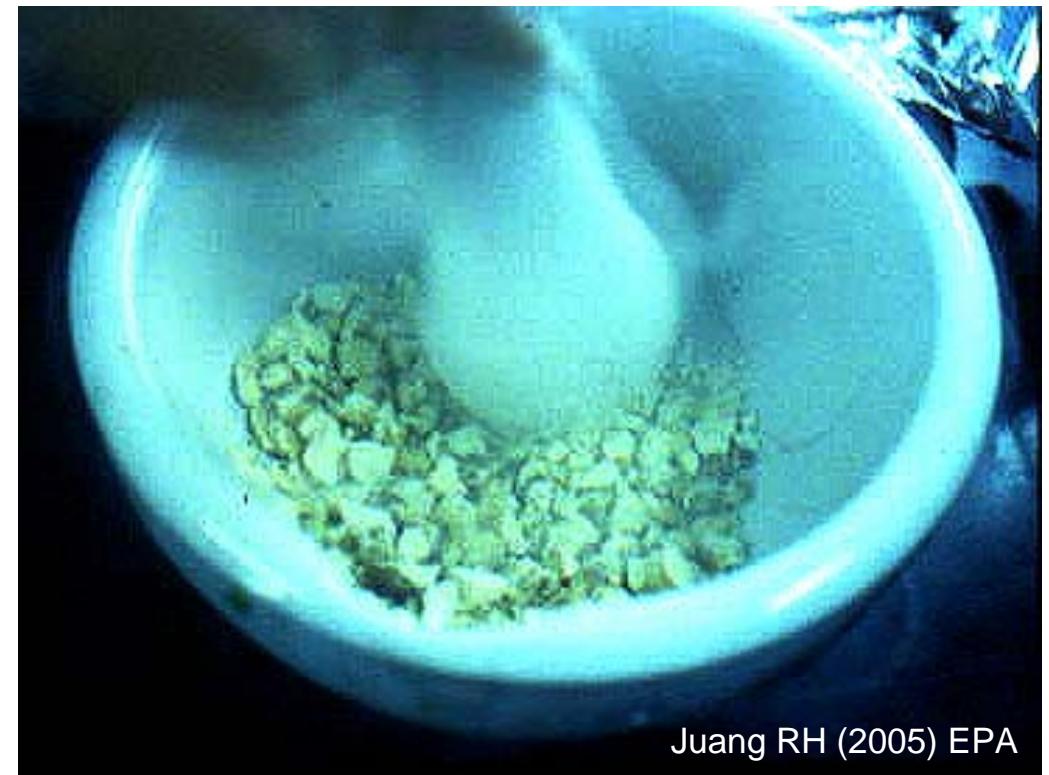


Various size of probe



● Use liquid nitrogen

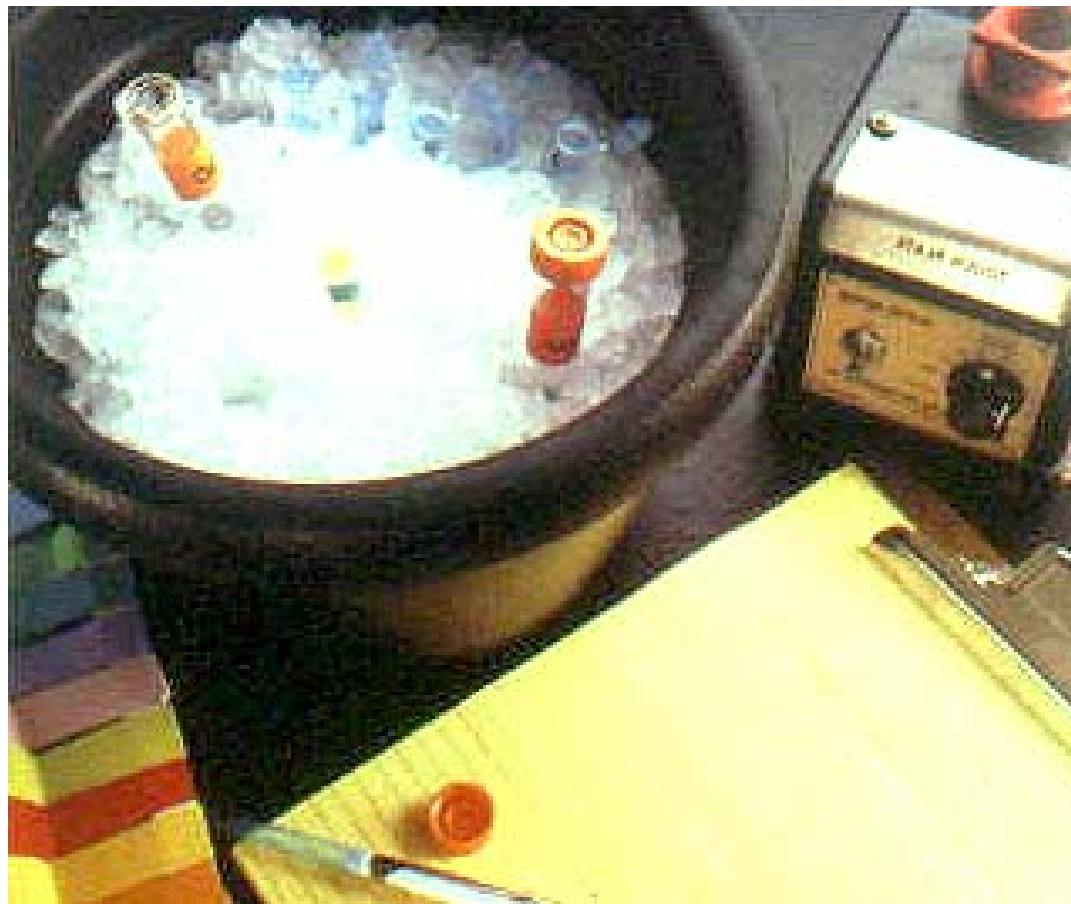
● Use Polytron



Juang RH (2005) EPA

## ■ 細胞打破之後 After breaking the cell.....

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(1) 降低溫度

Keep temperature low

(2) 儘速純化

Purify as soon as possible

(3) 避免氧化

Avoid oxidation

(4) 避免吸著

Avoid adsorption by flask

(5) 避免污染

Avoid contamination