植物病毒面面觀–是敵？是友？

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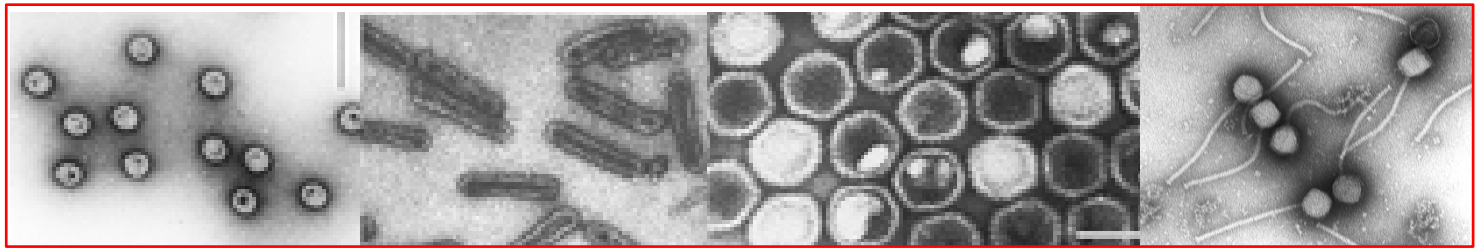
03.09.2024



病毒

10^3~10^5 bp

人

$3x10^9$ bp

動物

$>10^{10}$ bp

植物

$>10^9$ bp

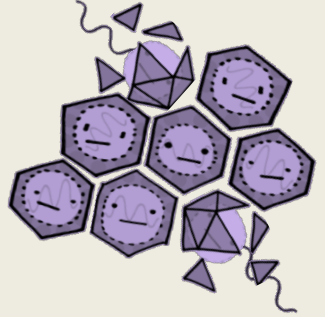
真菌

$3x10^7$ bp

細菌

$>10^7$ bp

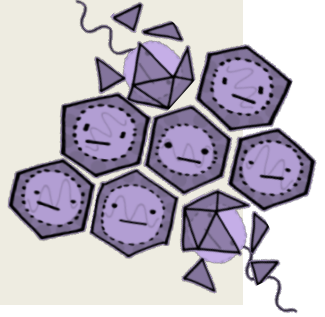
病毒（virus）



- 病毒是一種具有細胞感染性的超顯微粒子，只可在宿主的細胞系統進行自我複製。

- 病毒具有 DNA 或 RNA 基因體。

病毒基因體（viral genomes）

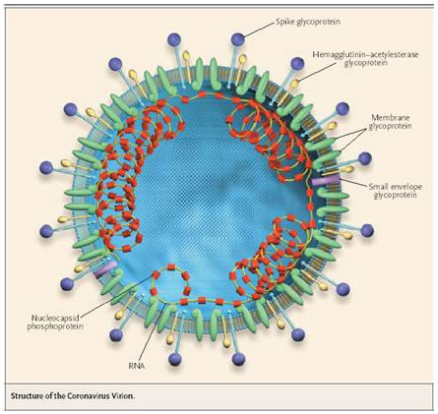


DNA： 雙股DNA
　　單股DNA

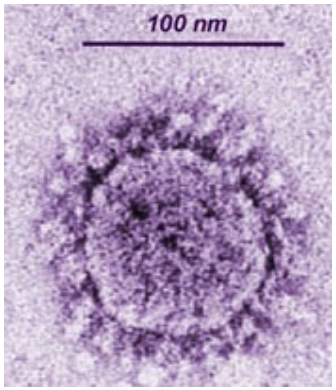
RNA： 雙股RNA
　　單股RNA

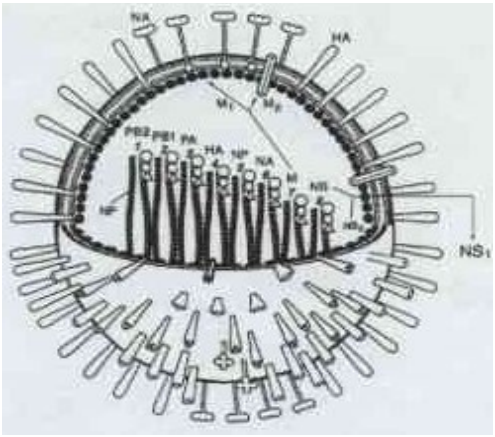
COVID-19, SARS

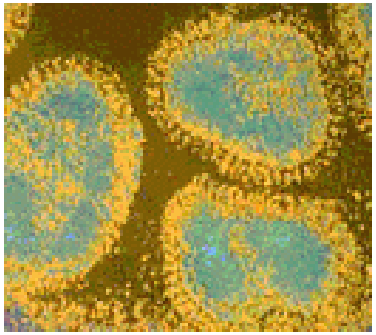
家禽流行性感冒病毒
Avian Flu

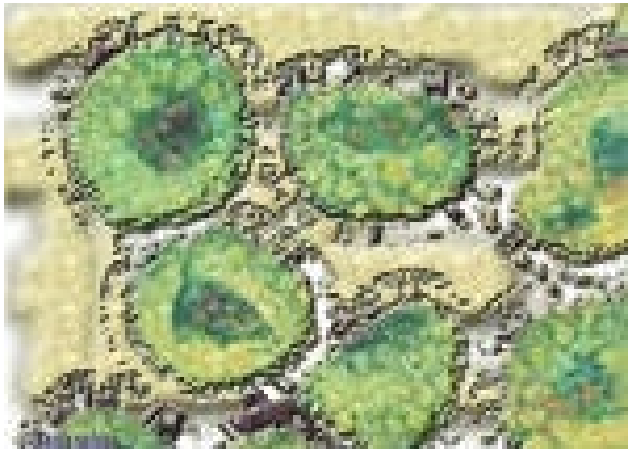


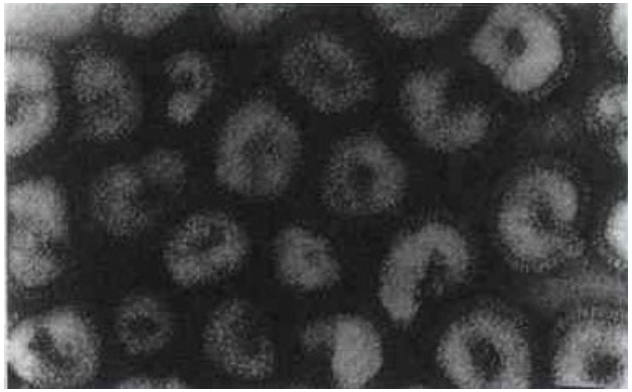
Structure of the Coronavirus Virion.











電子顯微鏡照片

"Rembrandt" tulips

「永遠的皇帝」

種植鬱金香狂(Tulipomania)

1636年，一棵價值三千荷蘭盾的鬱金香，可以交換:

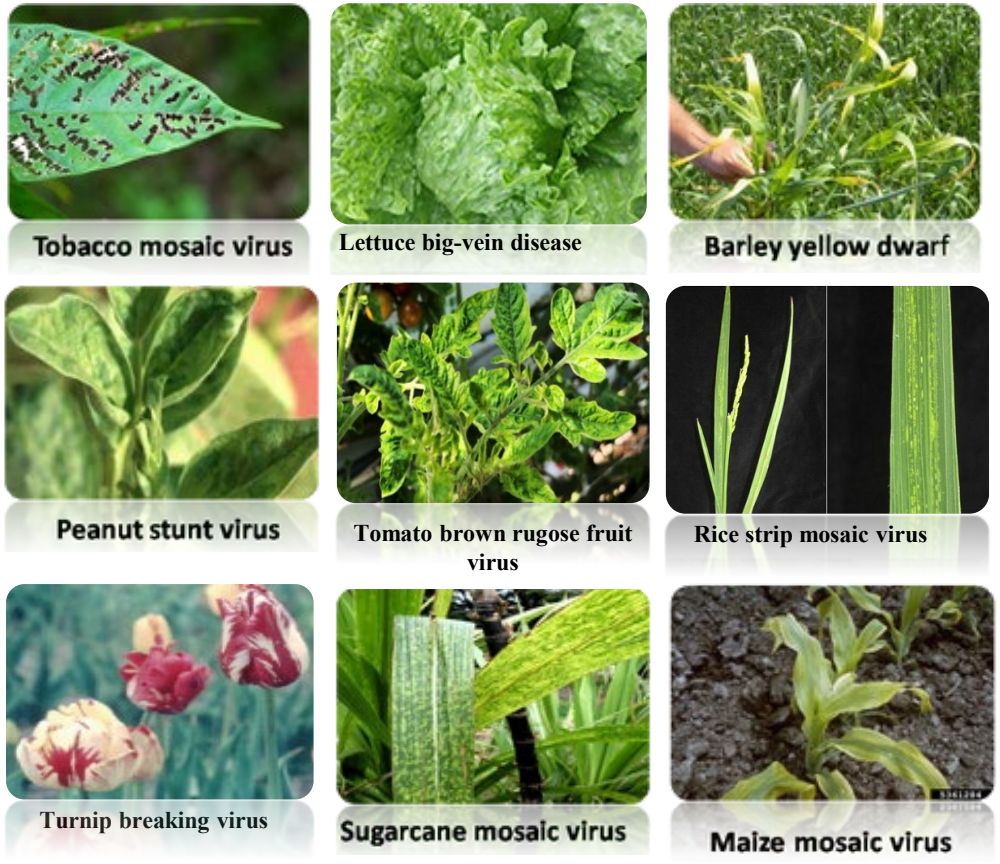
八隻肥豬
四隻肥公牛
十二隻肥羊
兩噸奶油
一千磅乳酪
一個銀製杯子
一套正式套裝
一張附有床墊的床外加一條船

A painting by Nicolas Robert (1614-1685) 目前收藏於劍橋大學的 Fitzwilliam Museum

植物病毒病害

- 每年全球經濟損失約300億美元
- 全世界約有 50% 植物病害皆由病毒引起

Agriculture & Food Security (2022) Vol 11,21



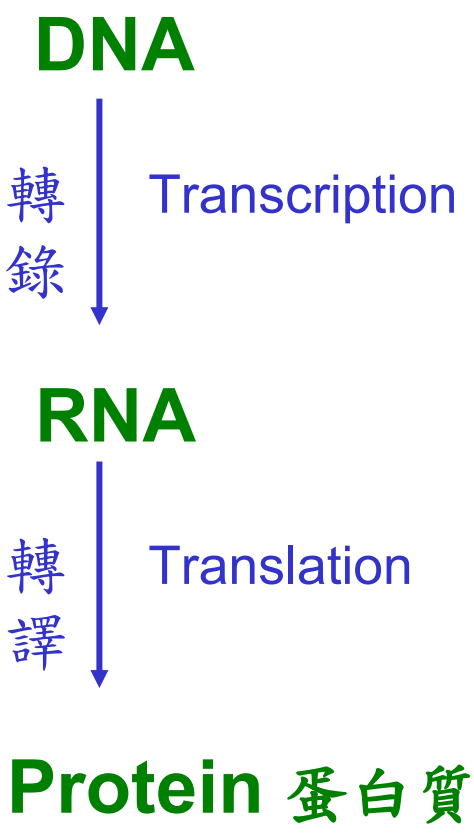
Modified from https://thebiologynotes.com/viral-diseases-of-leaf/

不同寄主的病毒基因體

基因體核酸	植 物		真 菌		動 物		細 菌	
	No.	%	No.	%	No.	%	No.	%
dsDNA	0	0	1	3.5	606	26.5	445	75.4
ssDNA	166	17.0	0	0	58	2.5	88	14.9
RT	31	3.2	0	0	112	4.9	0	0
dsRNA	45	4.6	27	93.0	383	16.7	1	0.2
(-)ssRNA	100	10.2	0	0	604	26.5	0	0
(+)ssRNA	635	65.0	1	3.5	525	22.9	57	9.6
Total	977		29		2288		591	

ds：雙股核酸
ss：單骨核酸
RT：反轉錄

生命中心定律(The central dogma)



cDNA<*$\overset{RT}{\text{----}}$RNA---→RNA→ protein

RNA---→RNA

* RT: reverse transcription
(反轉錄)

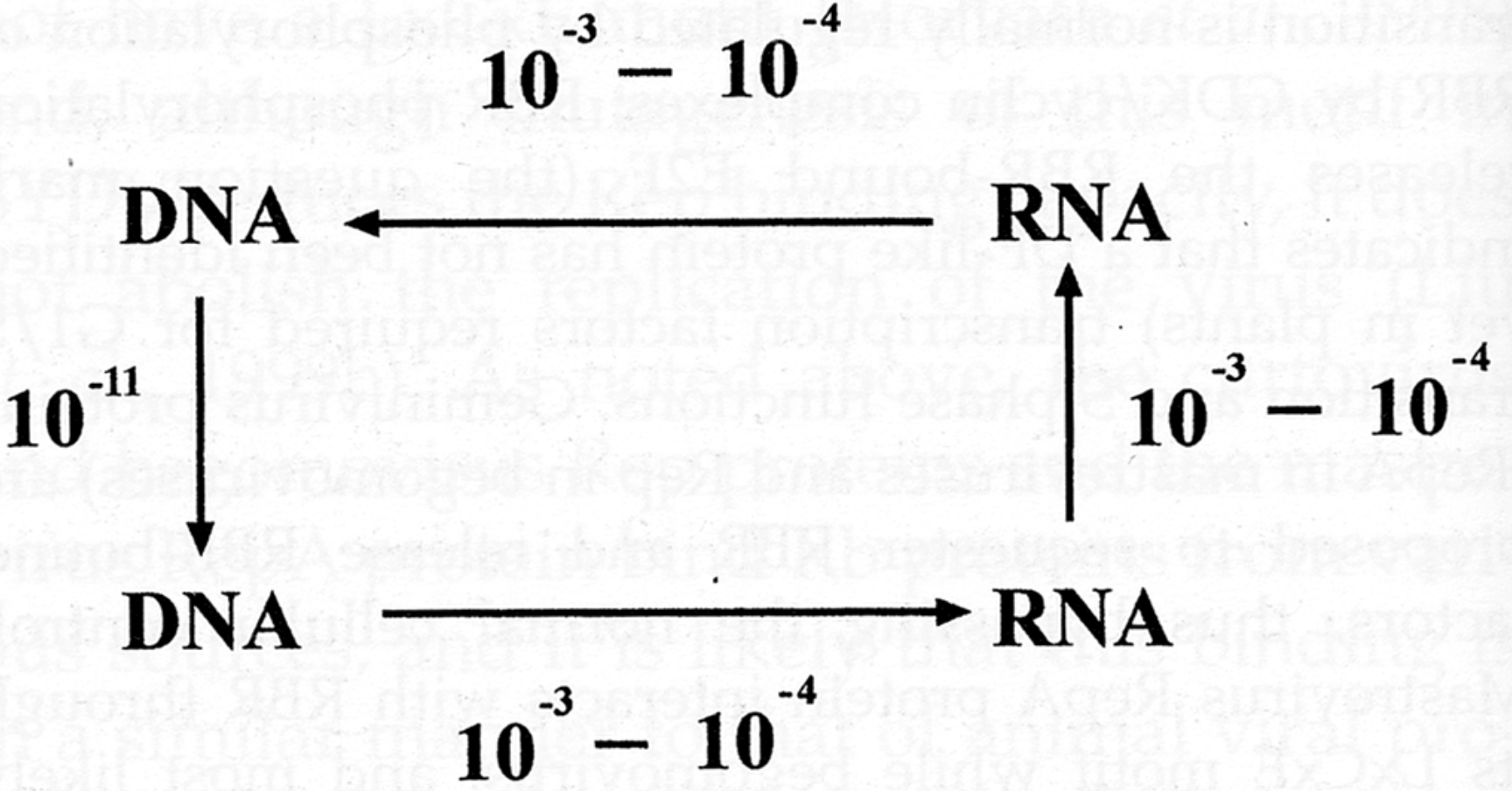
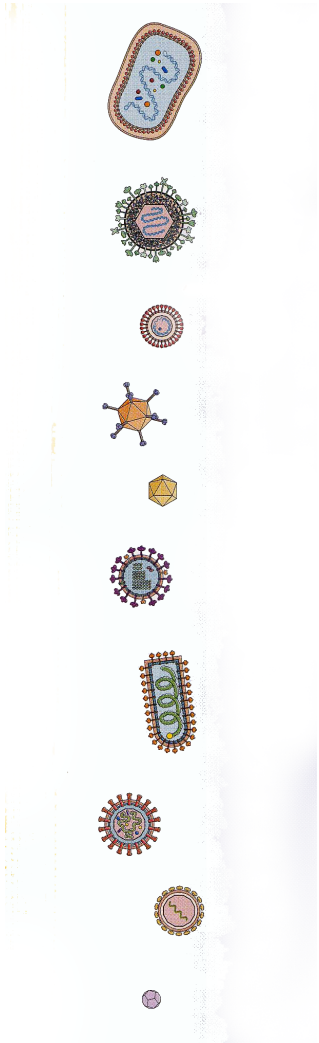


Fig. 8.29 Error rates of transcription within and between RNA and DNA.

植物病毒面面觀 ---是敵？是友？

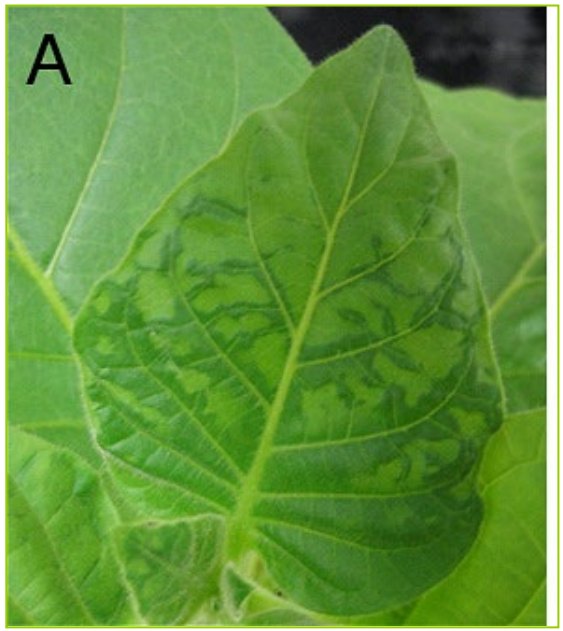


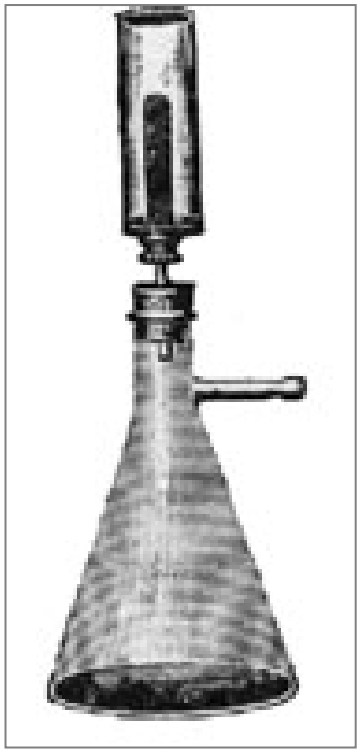
病毒是如何被發現的?

菸草嵌紋病毒

(Tobacco mosaic virus)

1886　利用感病菸草植物的汁液成功地感染健康的菸草(Mayer)
1892　發現病毒顆粒可以通透過 Chamberland filter (Ivanowski)
1898　證明 TMV 為病毒感染而非細菌感染 (Beijerinck)

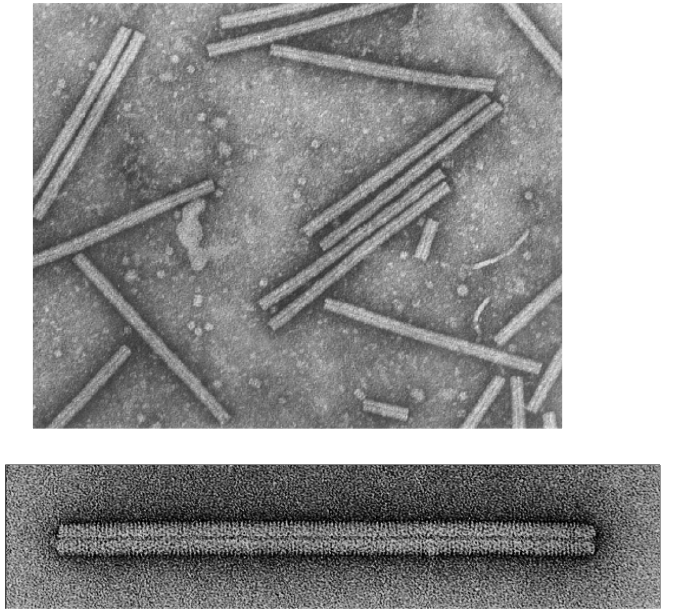




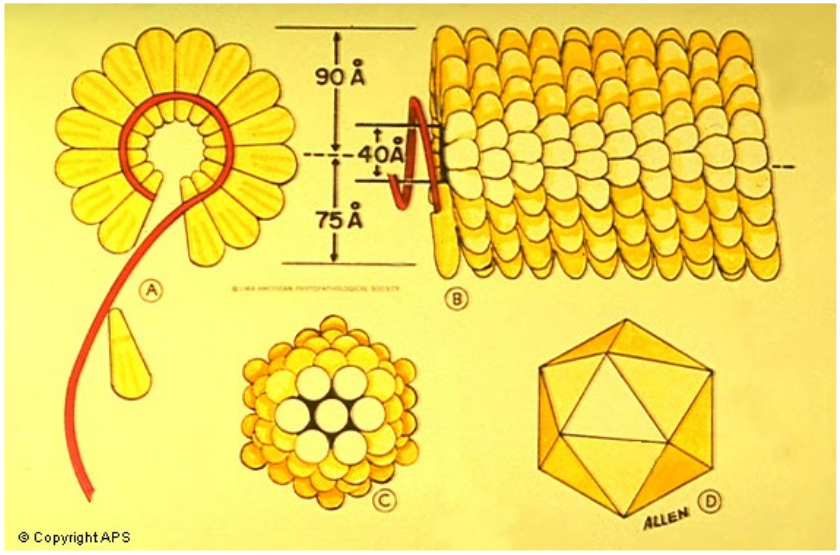
Chamberland filter

Tobacco mosaic virus
(300 nm x 17 nm)

1935　純化出的病毒顆粒具有蛋白質的特性 (Stanley*)
1936　發現病毒顆粒具有核酸-蛋白質成分 (Bawden & Pirie)
1956　純化出具有感染力的病毒RNA (Gierer* & Schramm*)



Electron micrograph



The structure of TMV

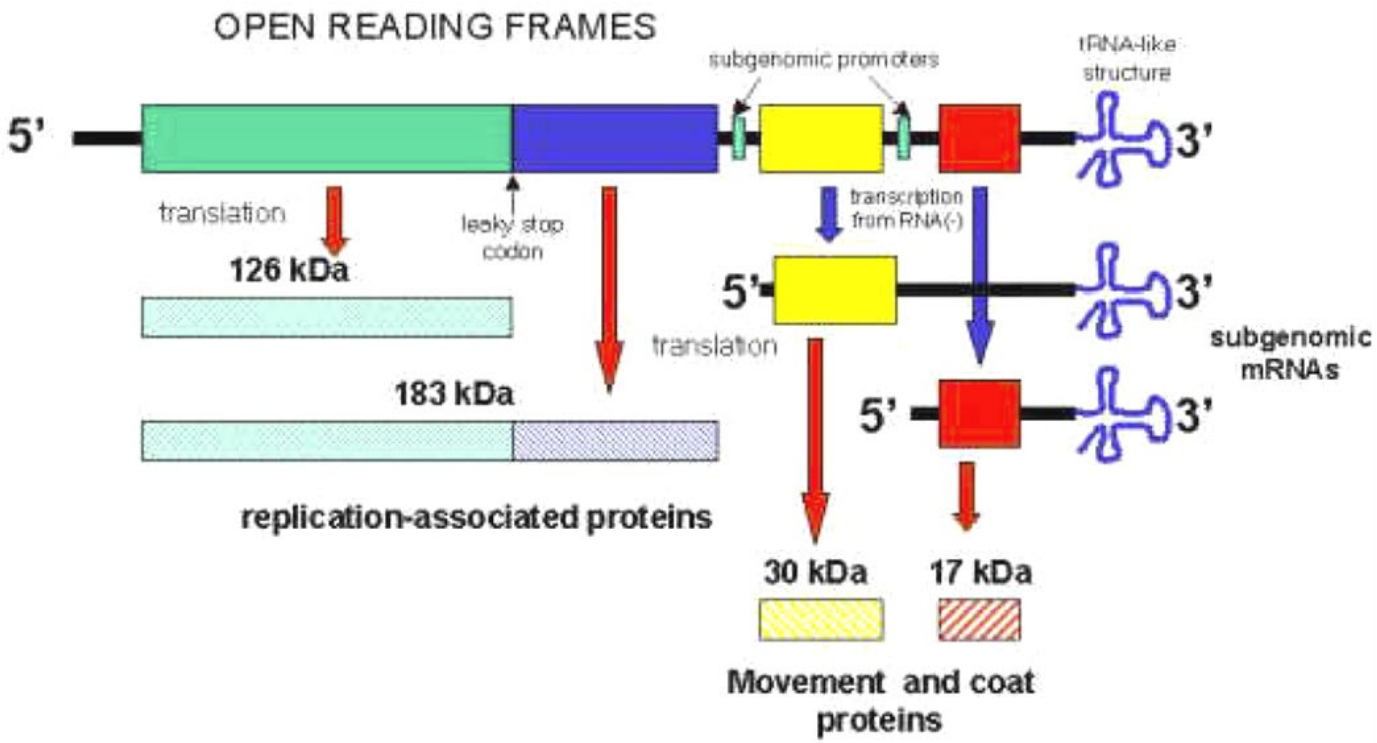
煙草嵌紋病毒（TMV）-已知最耐熱的植物病毒

- 煙葉汁液中，其致死溫度為93℃
- 乾葉中，加熱至120℃維持30分鐘，病毒仍有傳染力
- 煙草汁液中，TMV可存活4～5週
- 在無菌液中可存活5年以上
- 在乾的煙葉中其可保持傳染力50年以上
- TMV可在煙葉（包括田間作物和已加工成香煙和雪茄的產品）、煙枝及種皮上越冬。

1982定序出全長的TMV 基因組 RNA 序列 (Goelet et al.)

全長6400 核苷酸

1984 選殖 cDNA 及活體外合成全長具感染力的 BMV

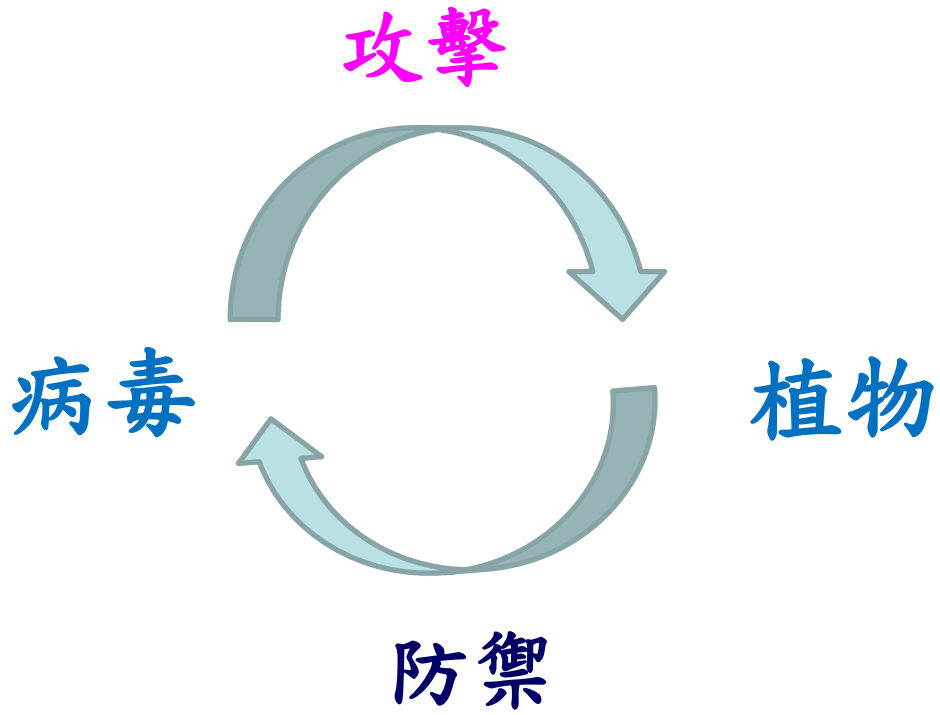


複製酶：183 &126 kDa

移動蛋白

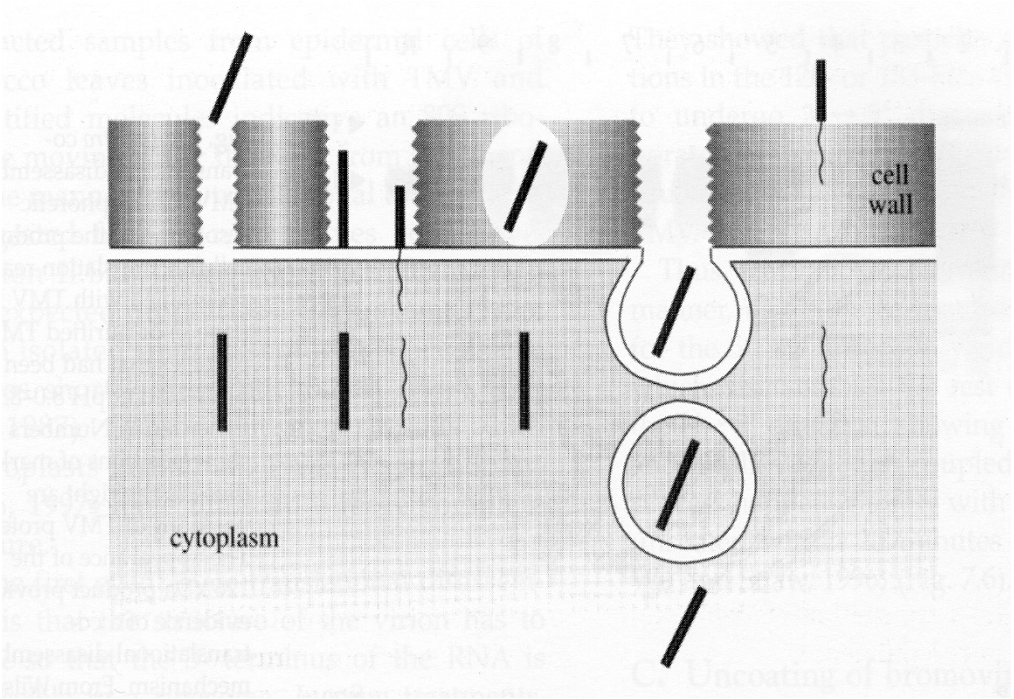
鞘蛋白

病毒與植物攻防戰

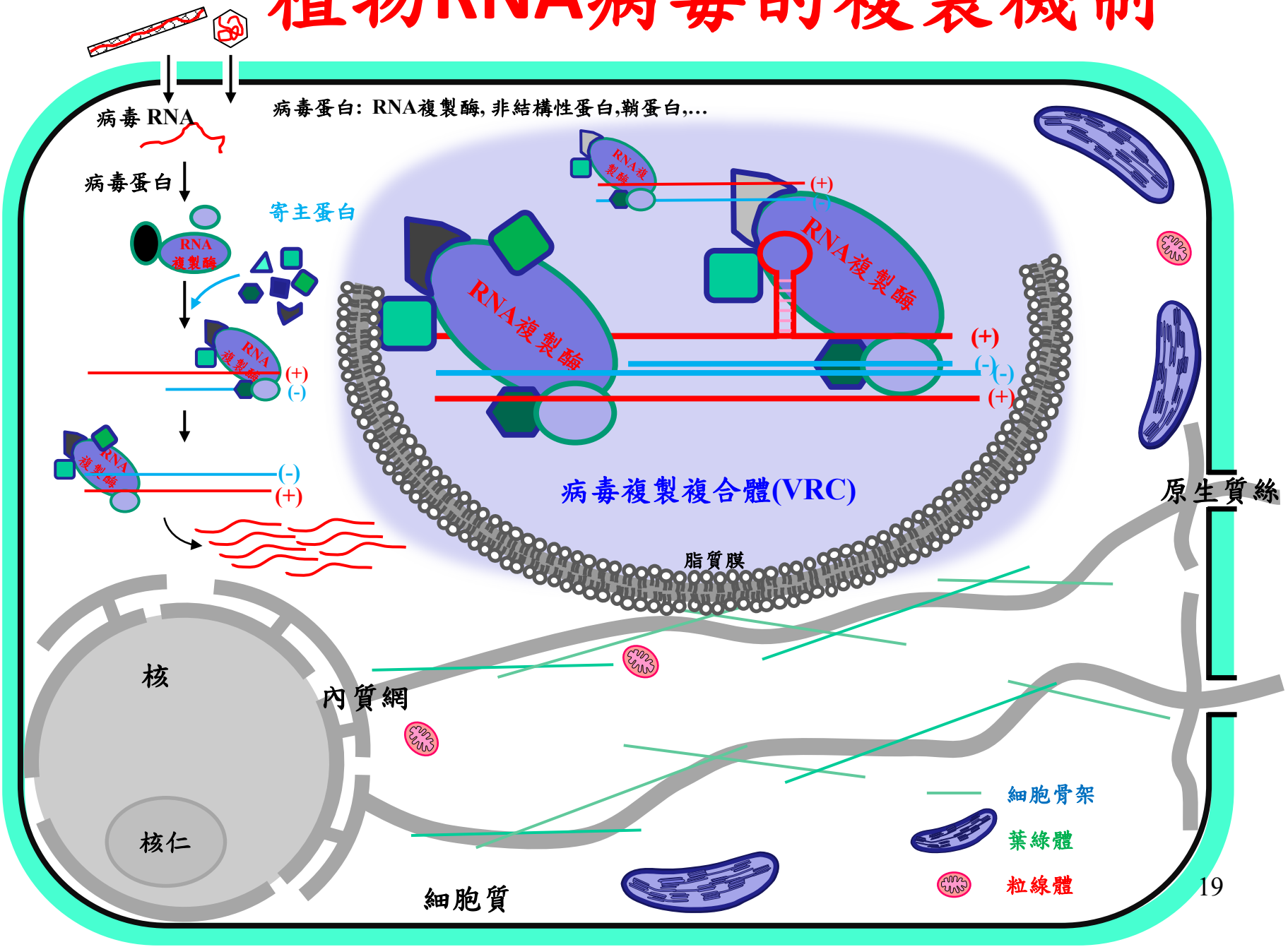


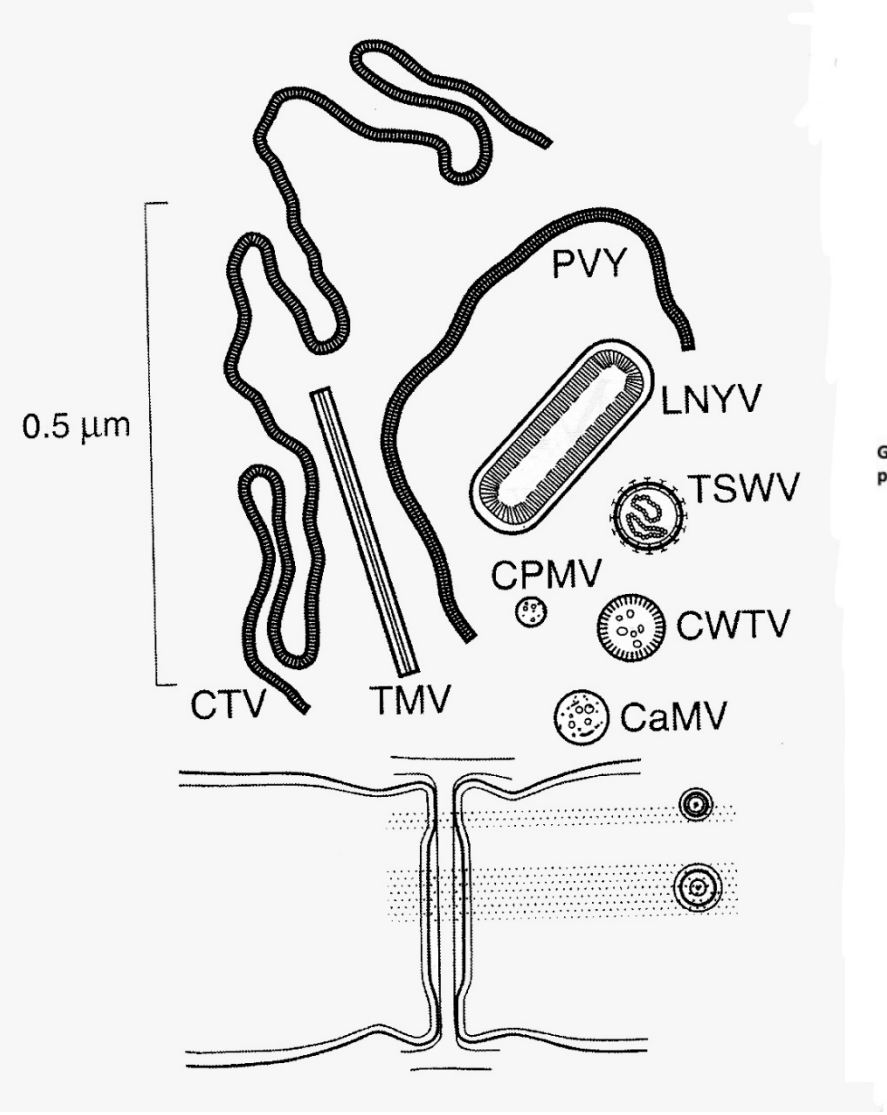
植物病毒之侵入

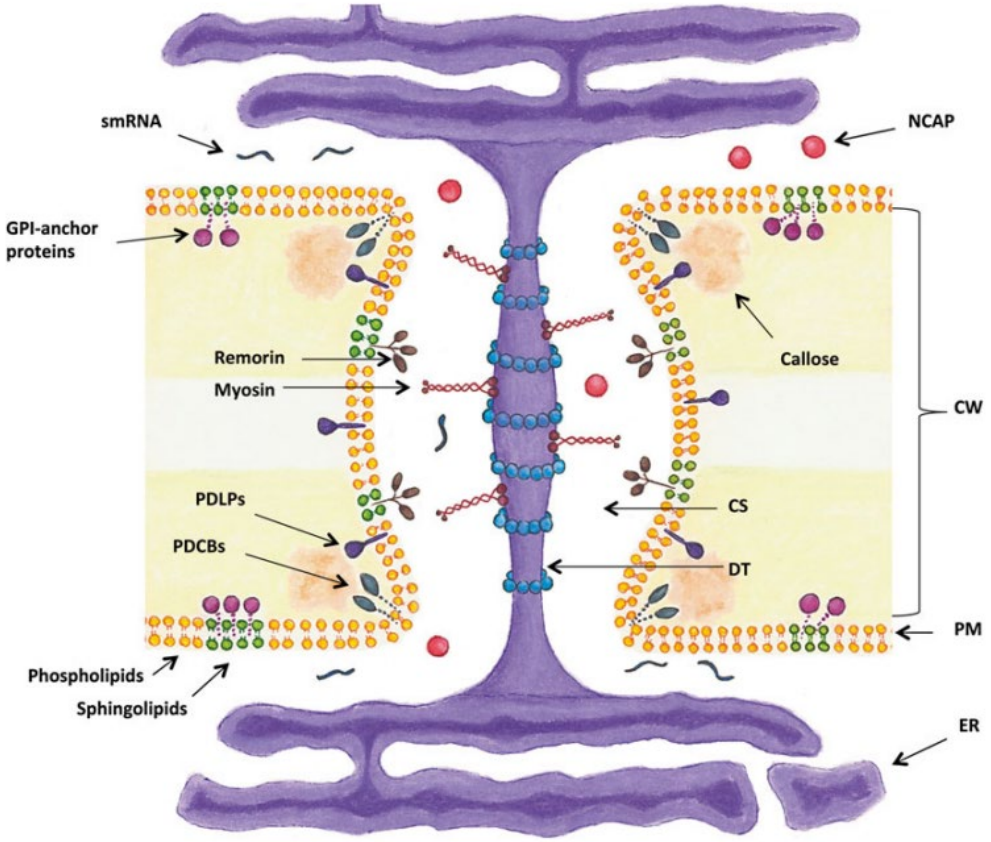
- 傷口侵入



植物RNA病毒的複製機制







TMV: 300 x 17 nm

原生質聯絡絲孔徑約1-5 nm

顯微注射螢光物質

(fluorescently labeled Lucifer yellow dextran)

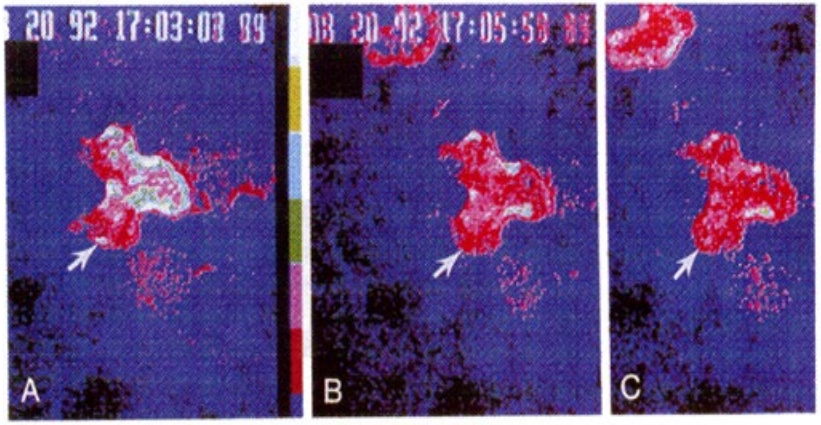
10 kDa

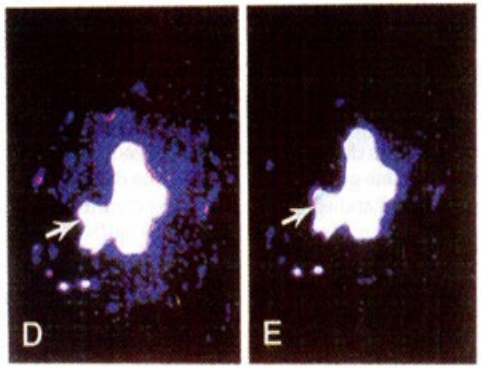
+ MP

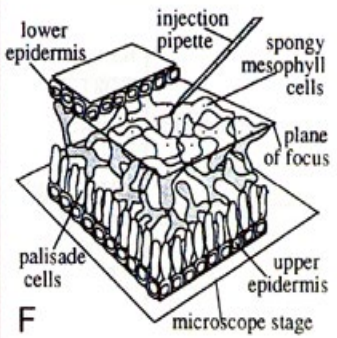
- MP

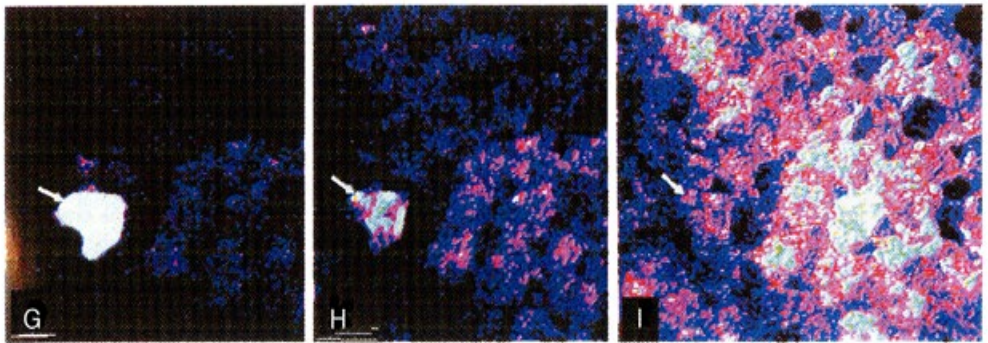
20 kDa

+ MP









TMV P30 移動蛋白是RNA結合蛋白

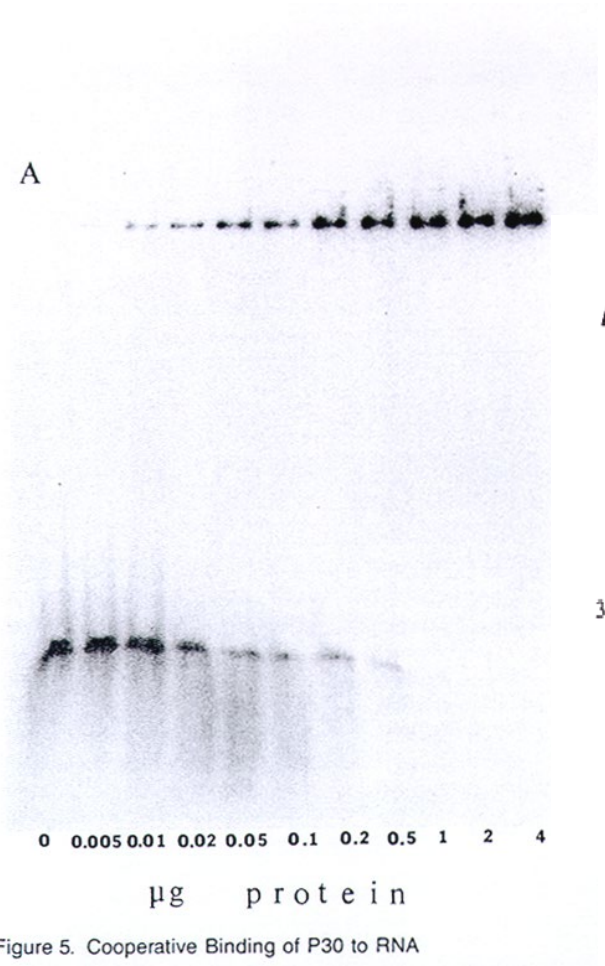
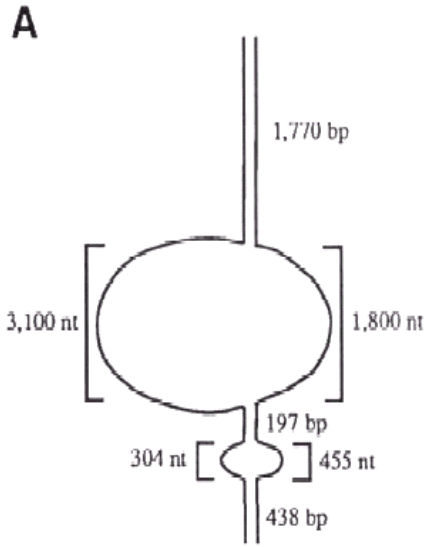
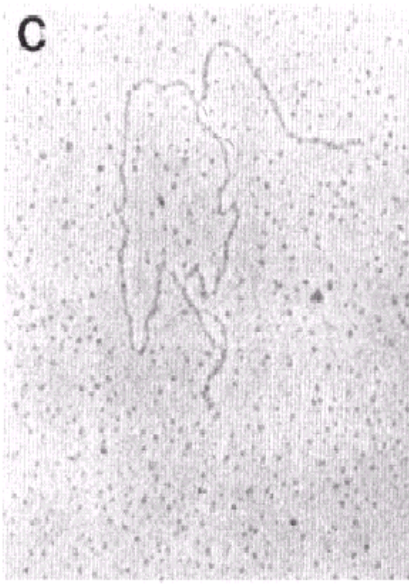
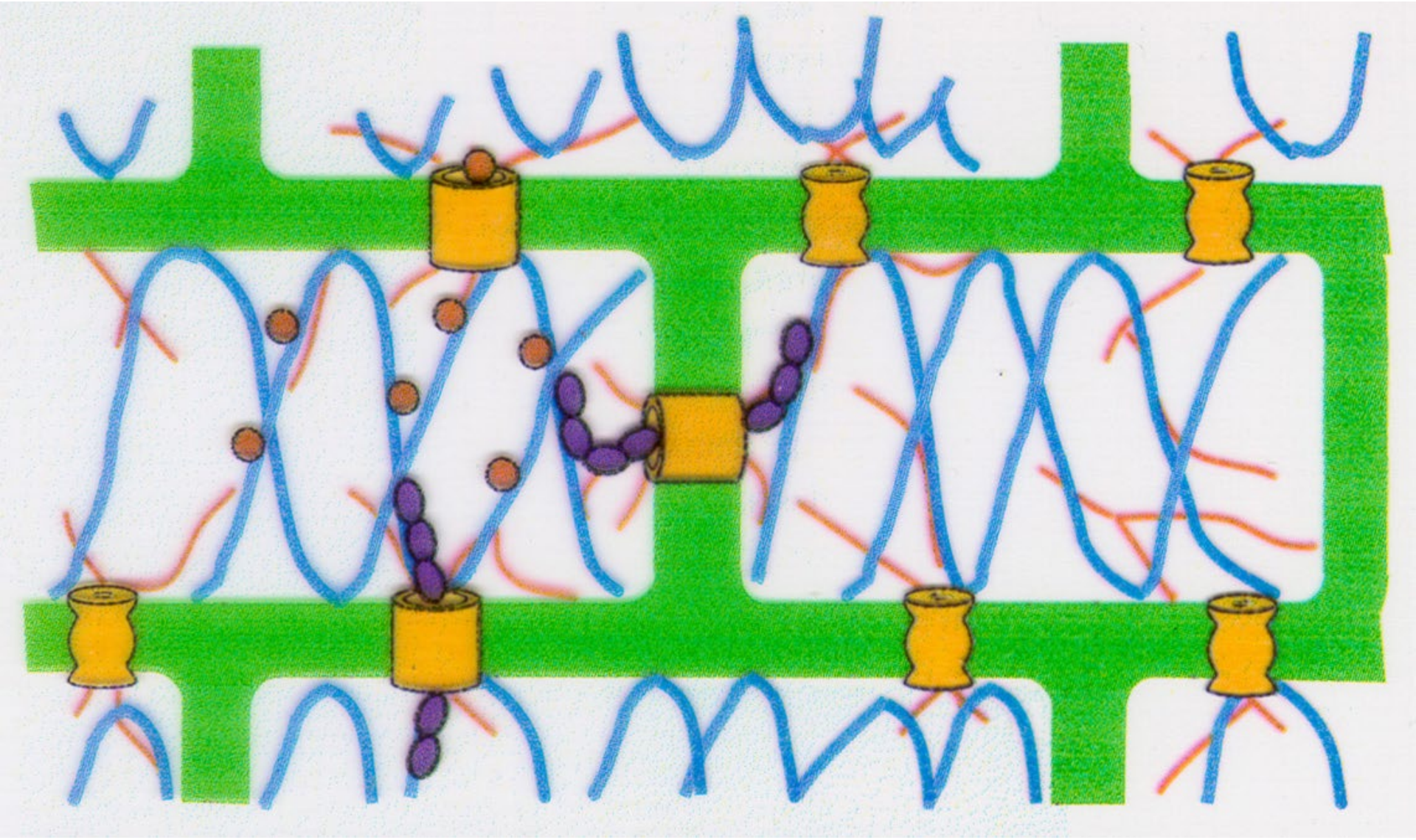


Figure 5. Cooperative Binding of P30 to RNA







TMV 在番茄幼苗的移動

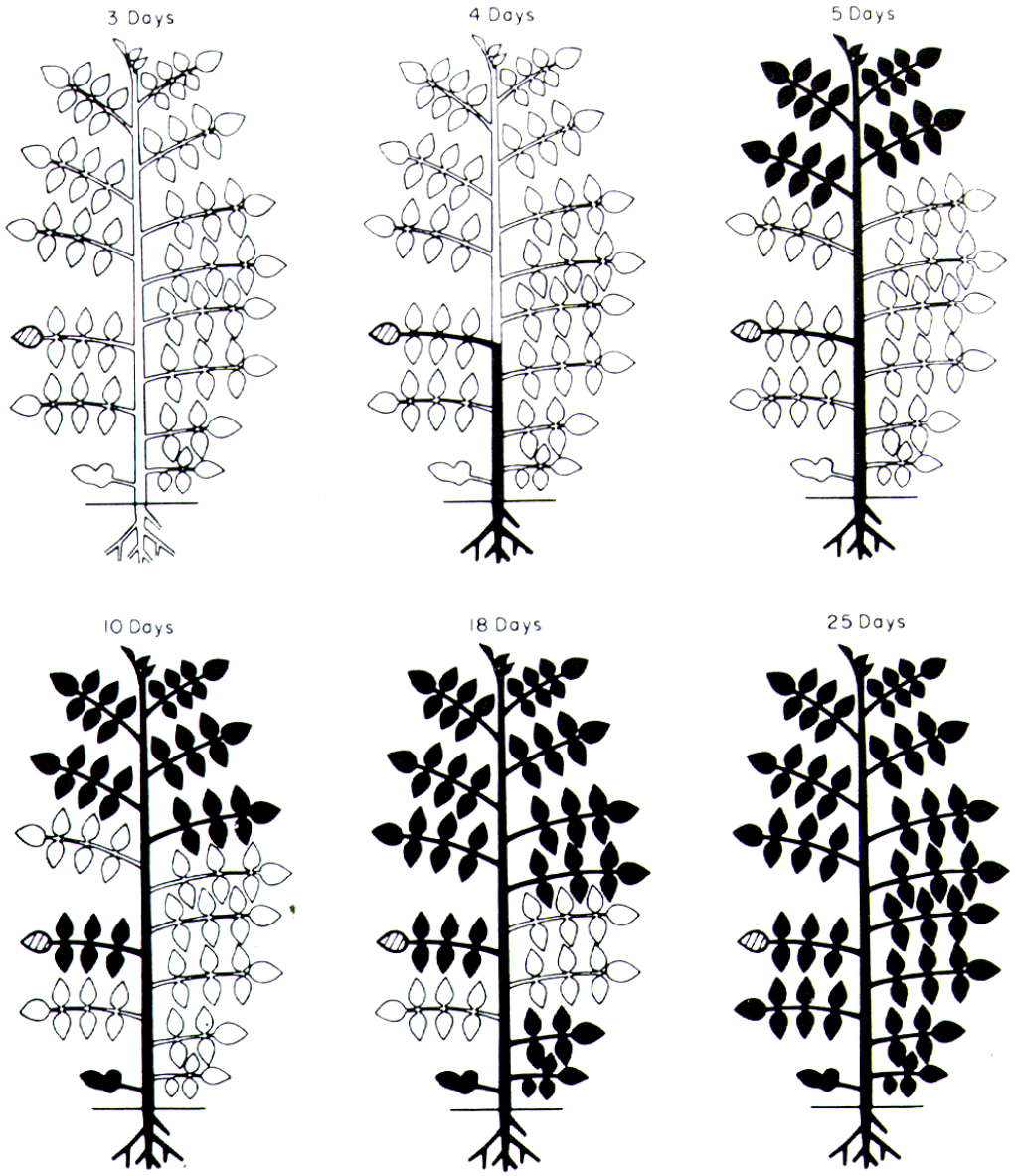
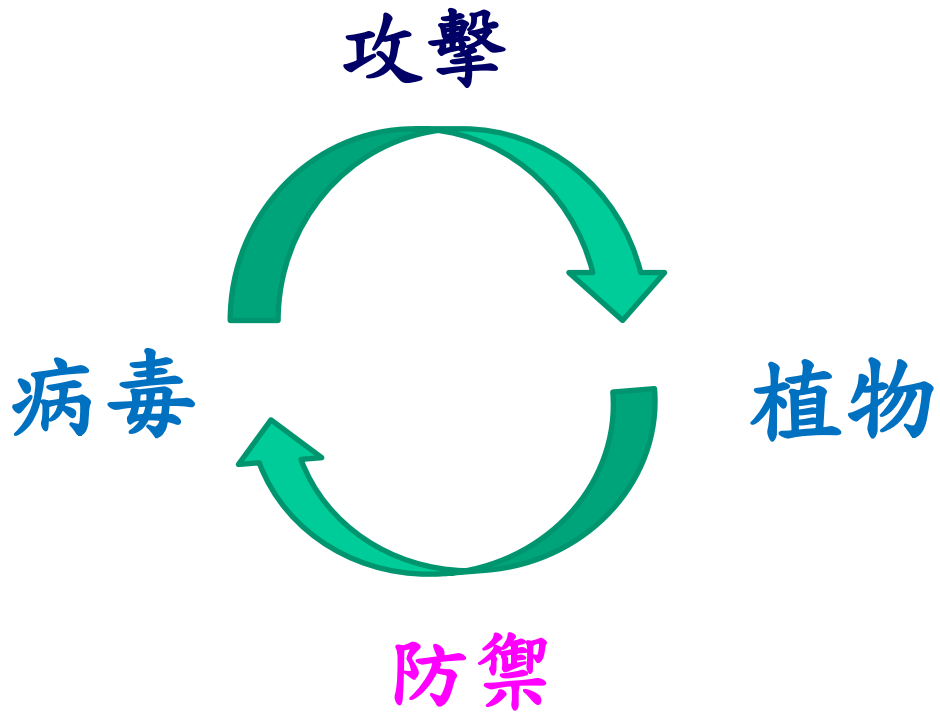


Figure 10.7 Diagram showing the spread of TMV through a medium young tomato plant. The inoculated leaf is shaded, and systematically infected tissues are shown in black. (From Samuel, 1934.)

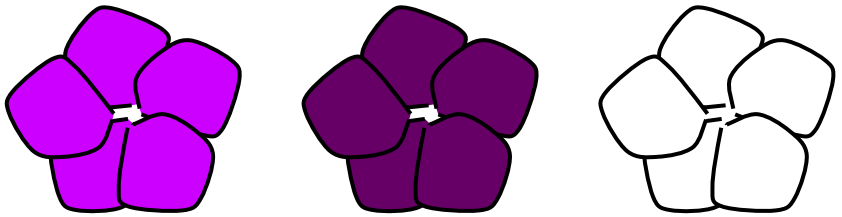
病毒與植物攻防戰



在植物發現小型干擾性核苷酸
(Small interfering RNAs, siRNAs)

研究轉殖基因靜默之機制

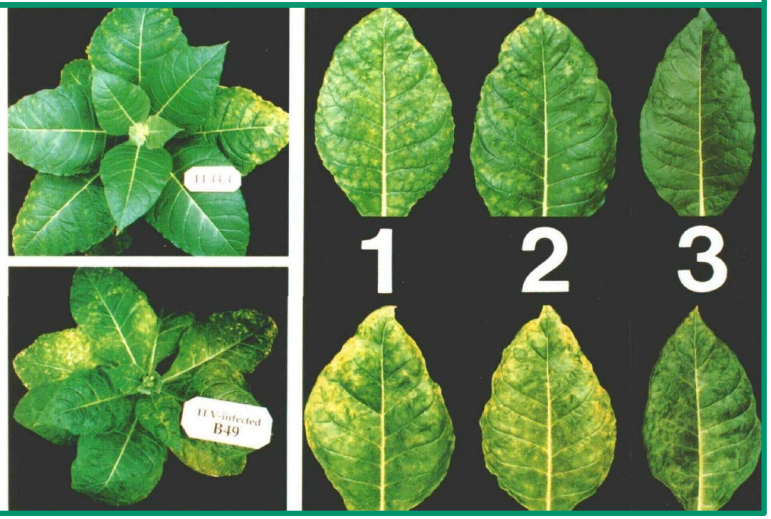
外加花色基因表現導致紫牽牛花花色改變



研究植物病毒抗性之機制

RNA 引發抗性

(RNA-mediated resistance)



Lindbo, J.A., Silva-Rosales, L., Proebsting, W.M., and Dougherty, W.G. (1993). Induction of a highly specific antiviral state in transgenic plants: Implications for regulation of gene expression and virus resistance. Plant Cell 5: 1749–1759.

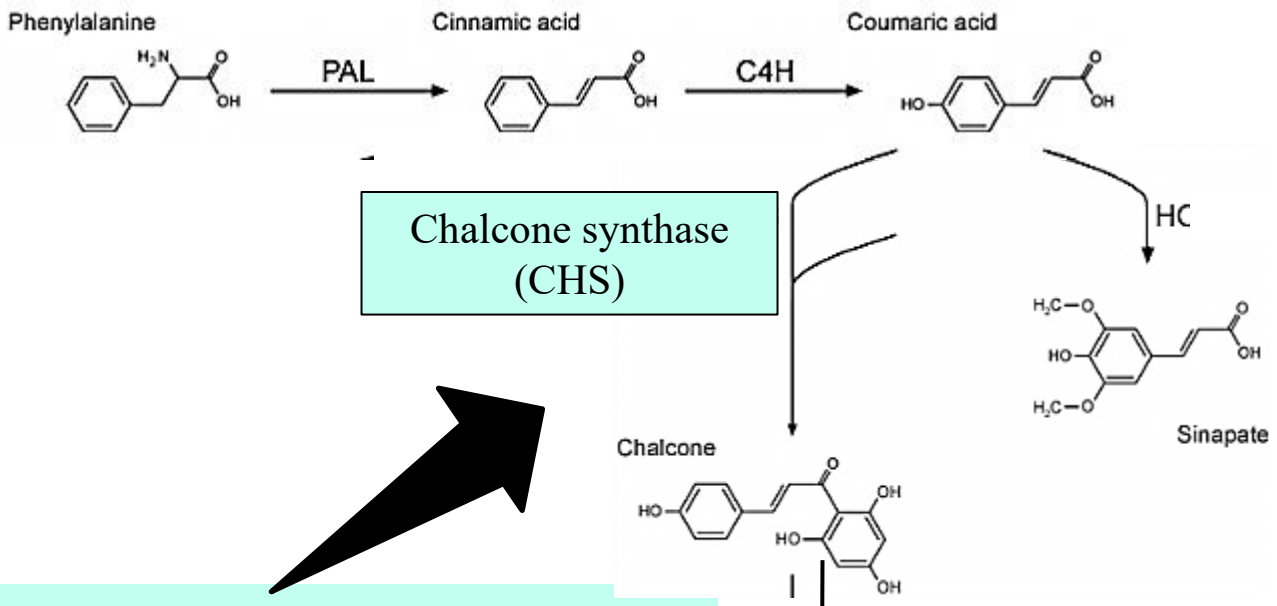
外加花色基因表現導致紫牽牛花花色改變提供我們什麼線索?

基因抑制 (Co-suppression)

Jorgensen and Mol

改變矮牽牛花花色

矮牽牛正常產生紫色花青色色素

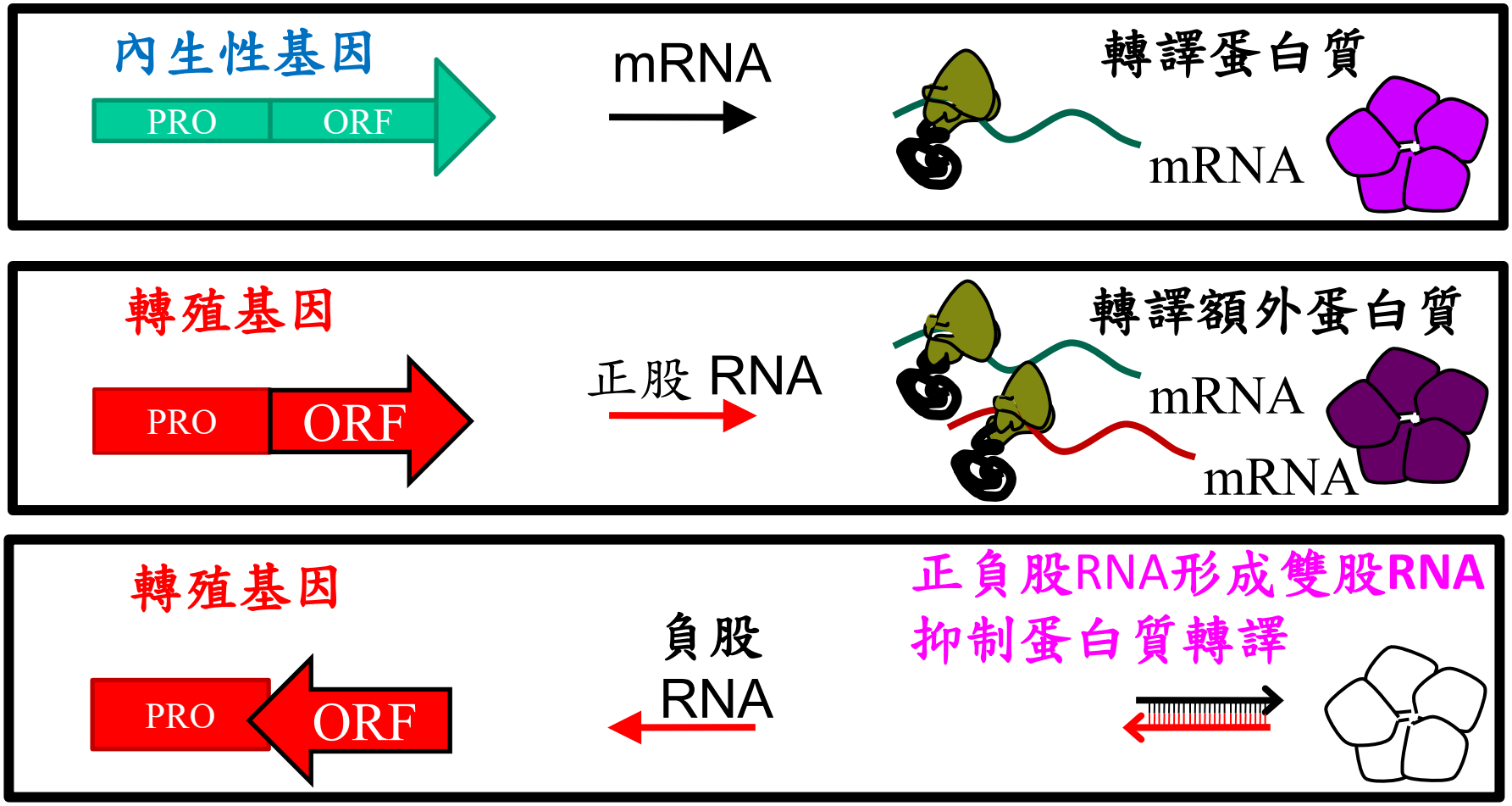


Chalcone synthase (CHS) is the enzyme at the start of the biosynthetic pathway for anthocyanins

Anthocyanins

Photo credit Richard Jorgensen; Aksamit-Stachurska *et al.* (2008) BMC Biotechnology 8: 25.

假說:外加正股RNA可以增加花色;負股RNA可以抑制花色素生成



意外地，無論正股或反股基因構築都抑制了花青素產生

Plants carrying CHS transgene

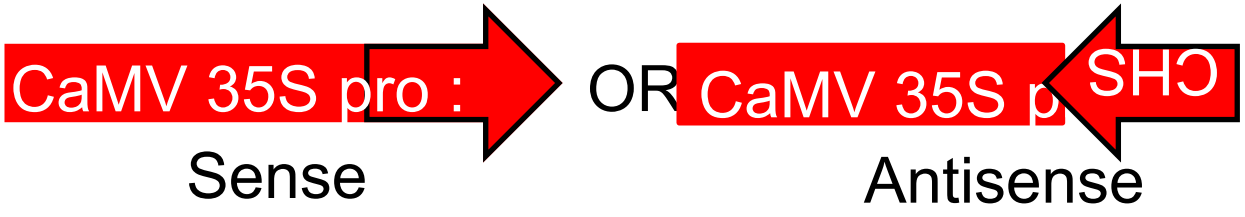


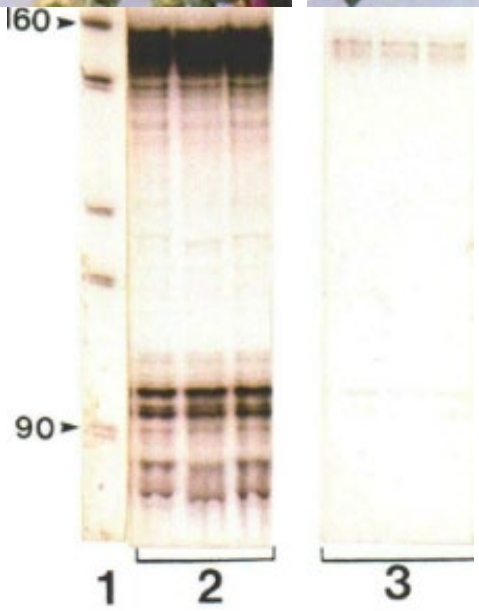
Photo credit Richard Jorgensen

靜默組織沒有內生性或外加轉殖RNA

紫花

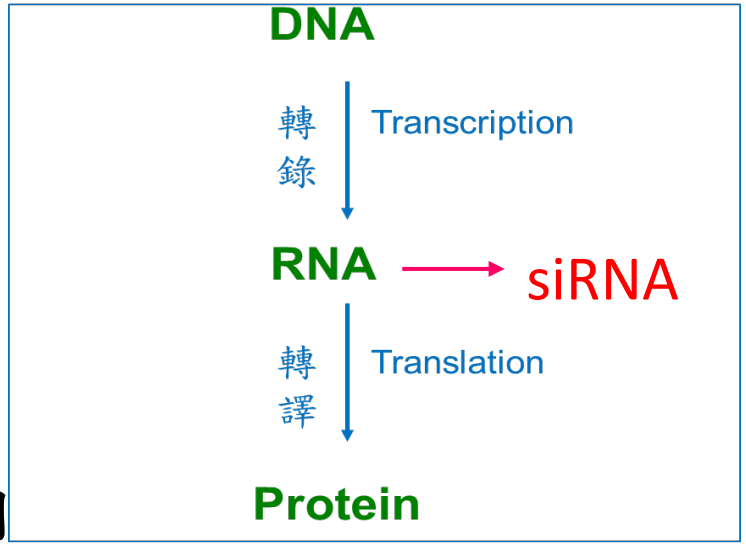
白花

這個現象，稱為共同抑制(**co-suppression**).

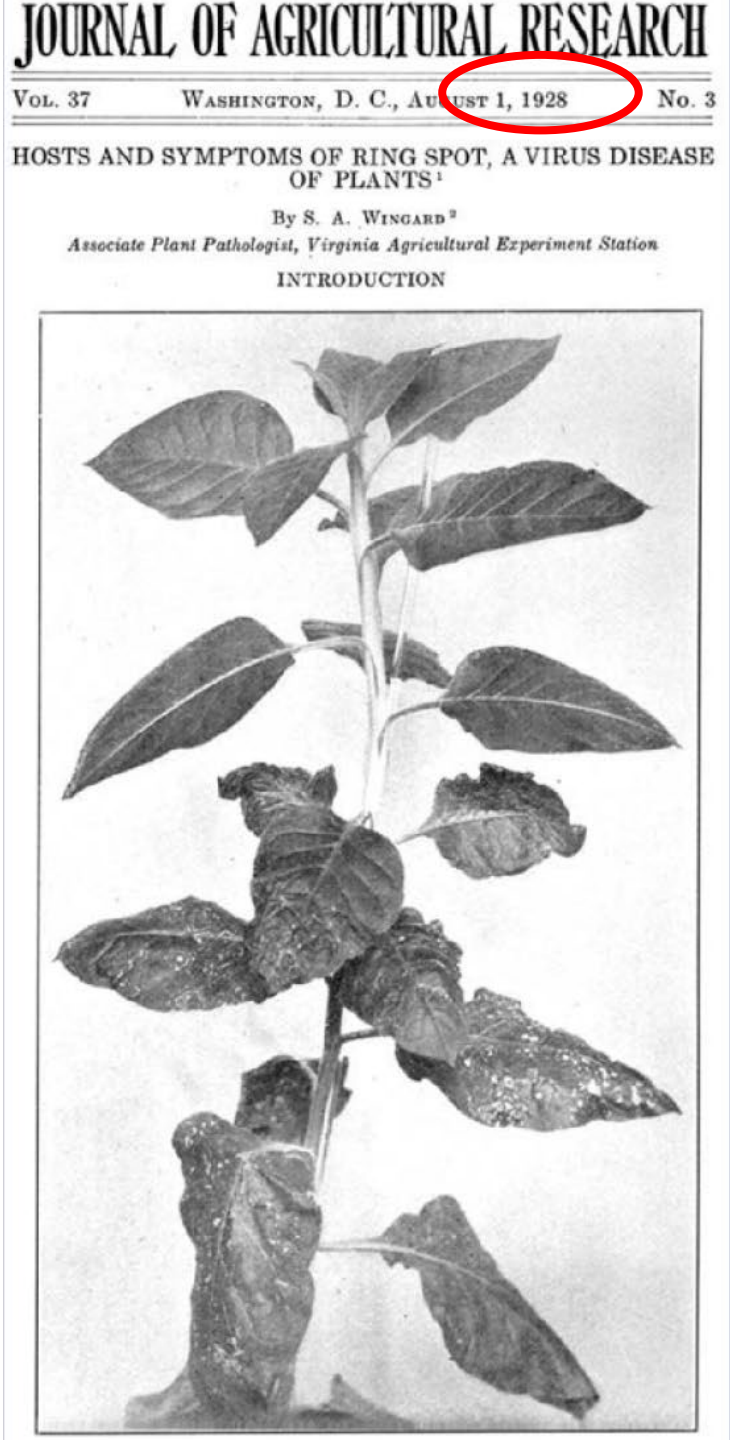


轉殖 RNA

內生性基因 RNA



Napoli, C., Lemieux, C., and Jorgensen, R. (1990) Introduction of a chimeric chalcone synthase gene into petunia results in reversible co-suppression of homologous genes *in trans*. Plant Cell **2**: 279–289.



JOURNAL OF AGRICULTURAL RESEARCH

VOL. 37 WASHINGTON, D. C., AUGUST 1, 1928 No. 3

HOSTS AND SYMPTOMS OF RING SPOT, A VIRUS DISEASE OF PLANTS[1]

By S. A. WINGARD[2]

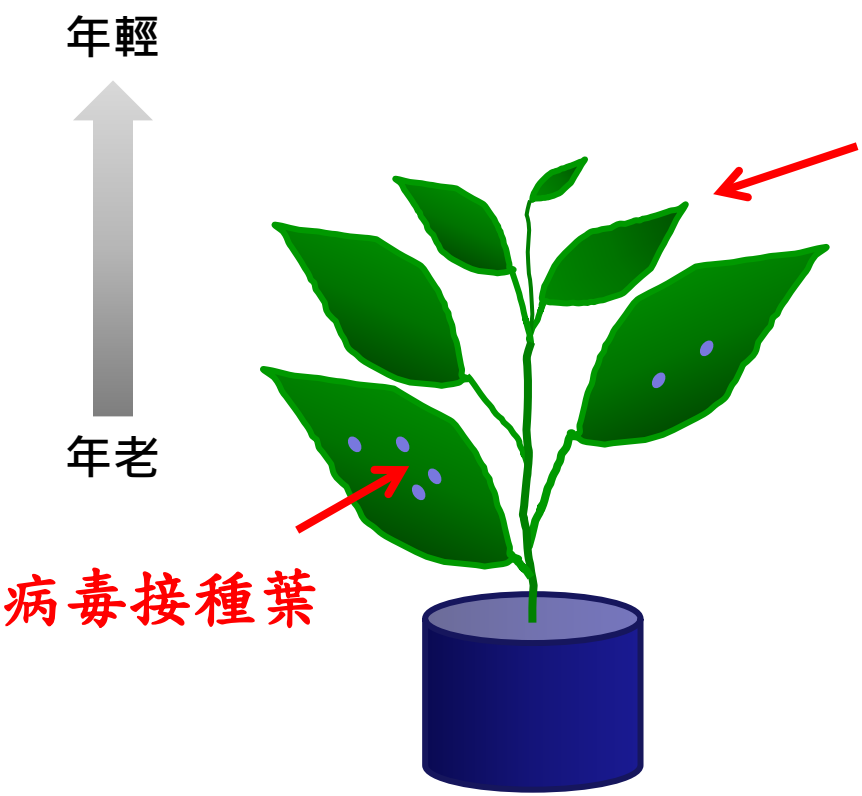
Associate Plant Pathologist, Virginia Agricultural Experiment Station

INTRODUCTION

Tobacco ringspot virus

Baulcombe, D. 2004
NATURE 431:356-363

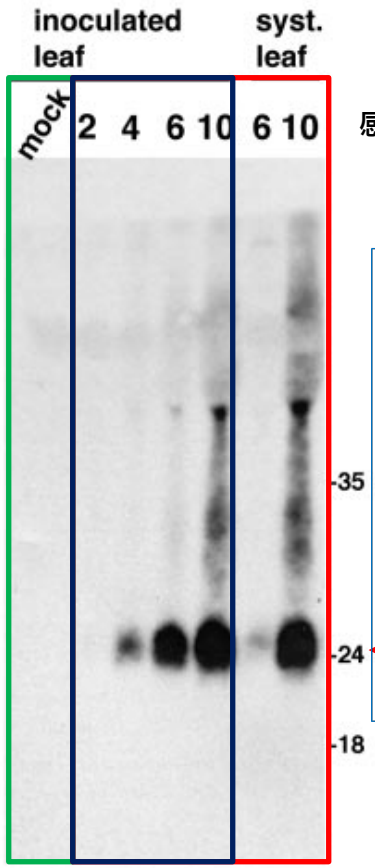
植物可以從病毒感染中恢復並變得具有抗病力

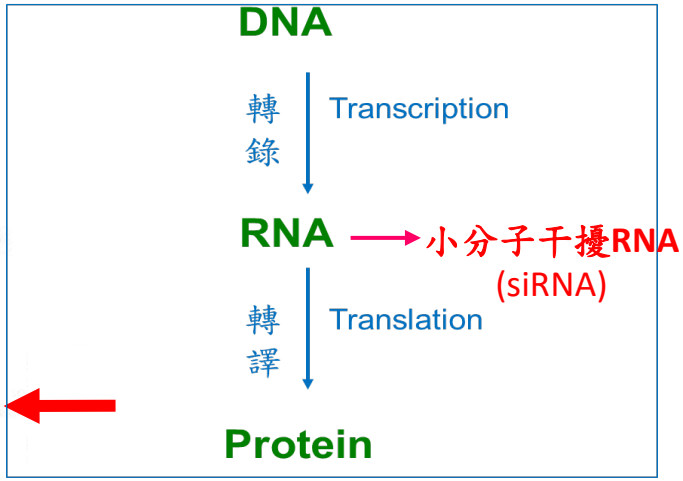


感病植株的新生葉不會有病徵，顯示植株已從感染中回復。

小片段RNA與病毒引起之基因靜默相關

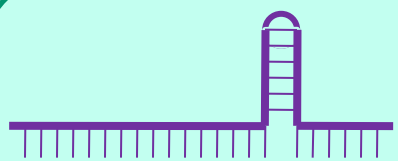
與病毒RNA同源的小片段RNA 存在於接種葉和遠端系統葉中，但不存在於對照組植株的葉中





From Ratcliff, F., Henderson, B.D., and Baulcombe, D.C. (1997) A similarity between viral defense and gene silencing in plants. Science 276: 1558–1560. Reprinted with permission from AAAS.

病毒引起之基因靜默

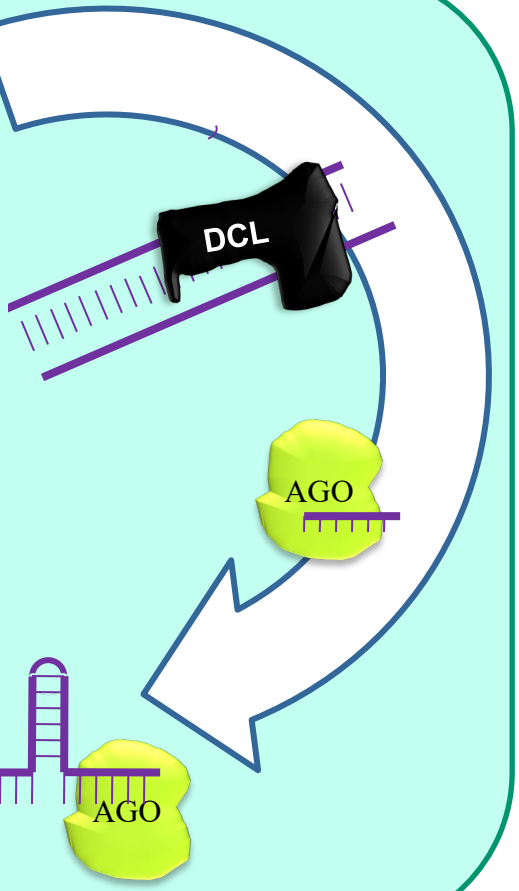


病毒單股RNA

病毒的RNA
複製酶
(RdRp)

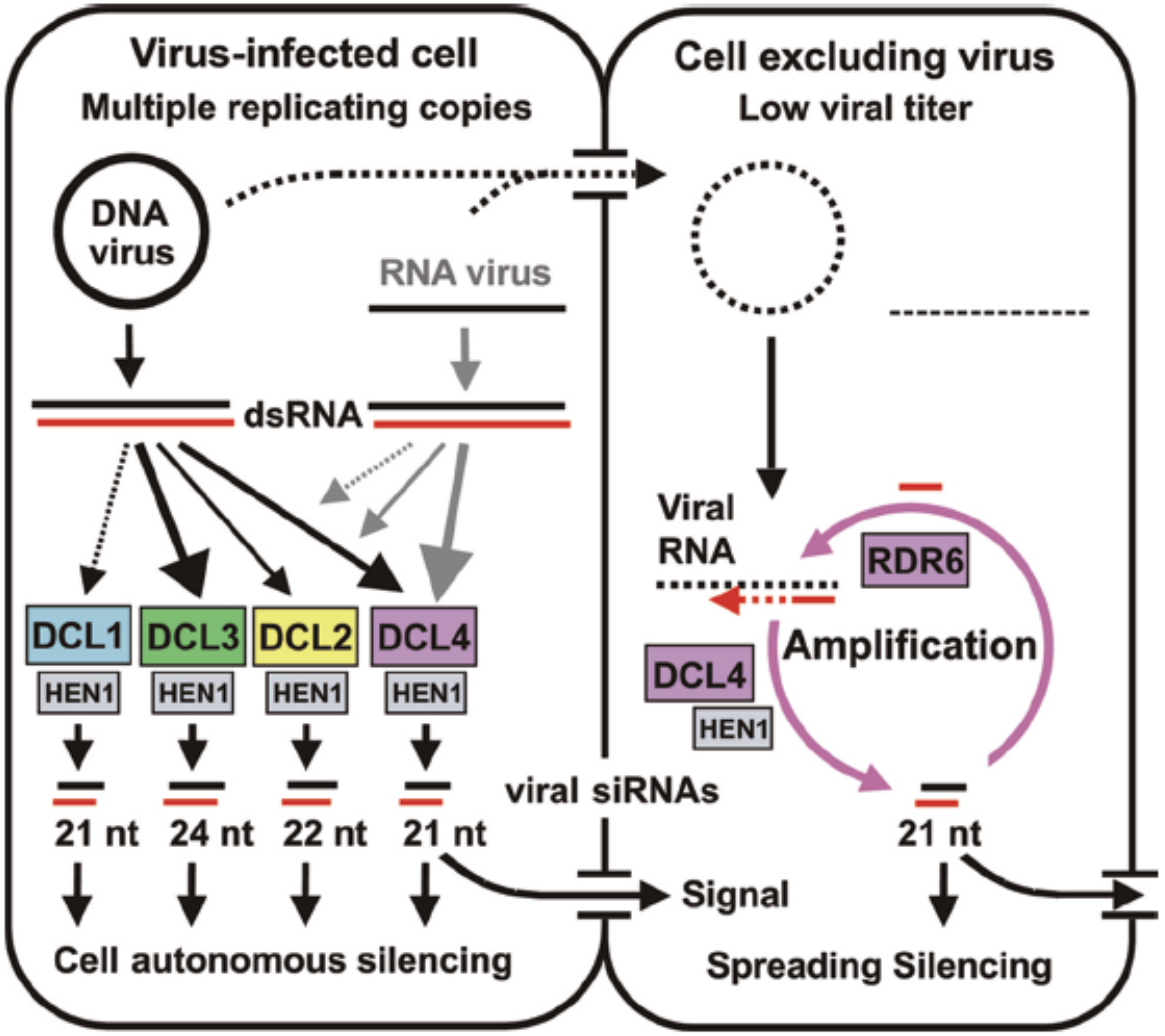
病毒雙股RNA

雙鏈 RNA 被 DCL蛋白質切割產生 小片段干擾RNA，其進一步與 AGO蛋白質結合以抑制病毒複製和累積



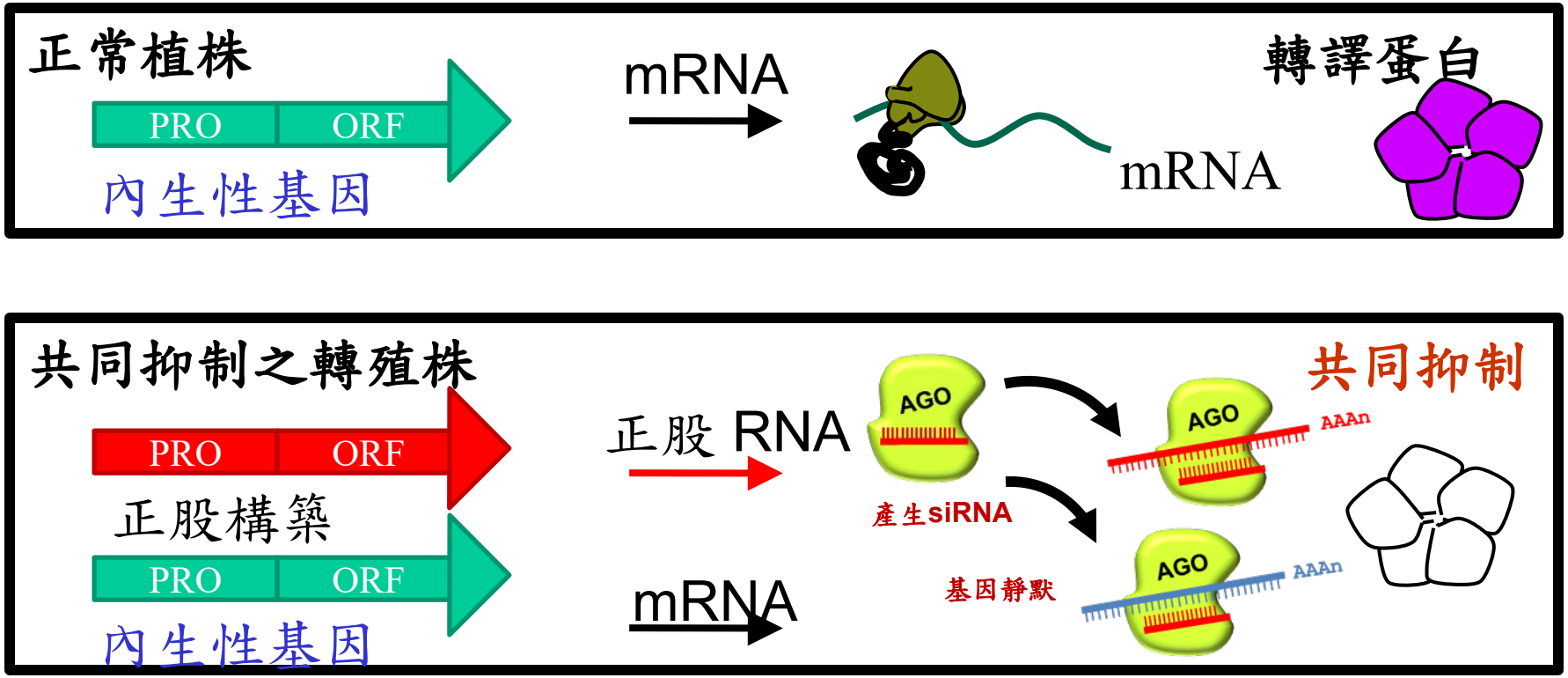
大部分植物病毒為RNA病毒，其複製過程中會產生雙股RNA中間產物

基因靜默(Gene silencing)是植物對抗病毒的防禦機制



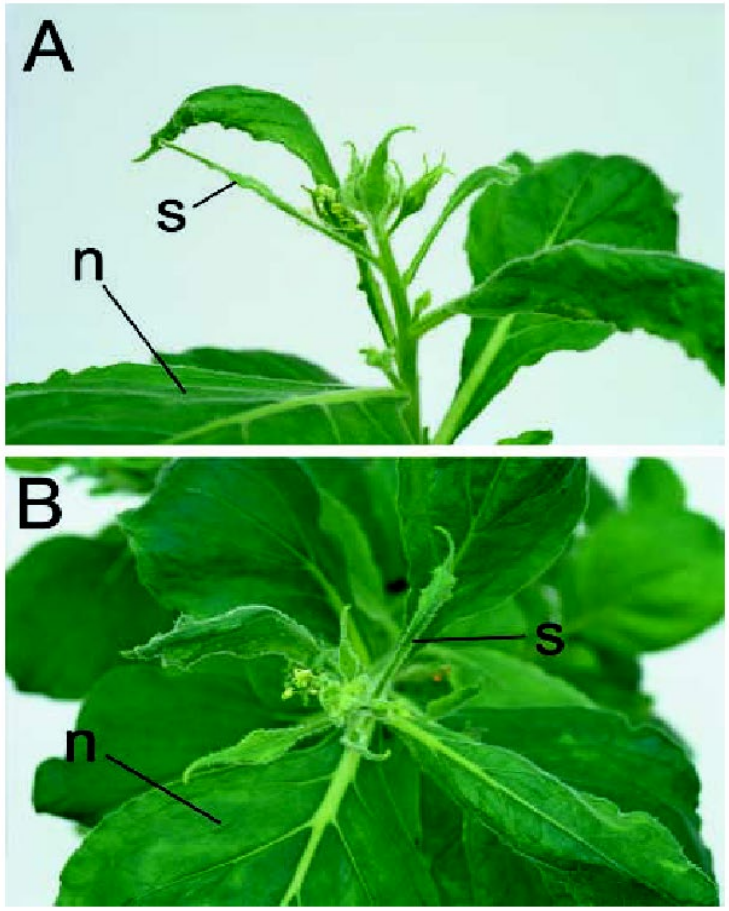
Nucleic Acids Research (2006) 34:6233-46

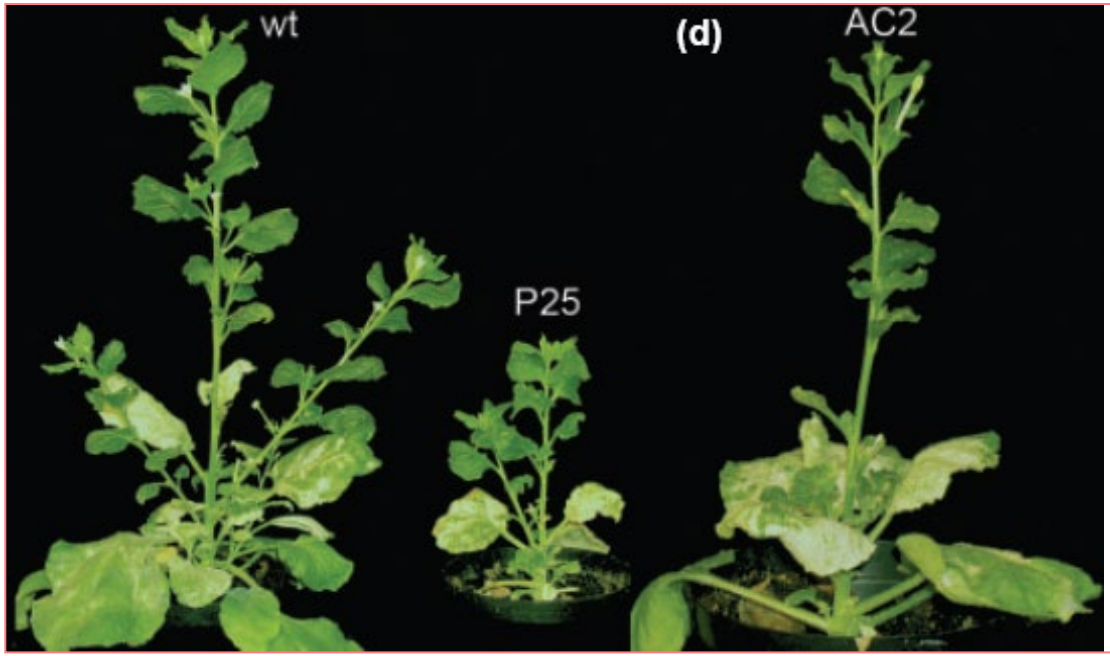
共同抑制是siRNA產生的結果



De Paoli, E., Dorantes-Acosta, A., Zhai, J., Accerbi, M., Jeong, D.-H., Park, S., Meyers, B.C., Jorgensen, R.A., and Green, P.J. (2009). Distinct extremely abundant siRNAs associated with cosuppression in petunia. RNA 15: 1965–1970.

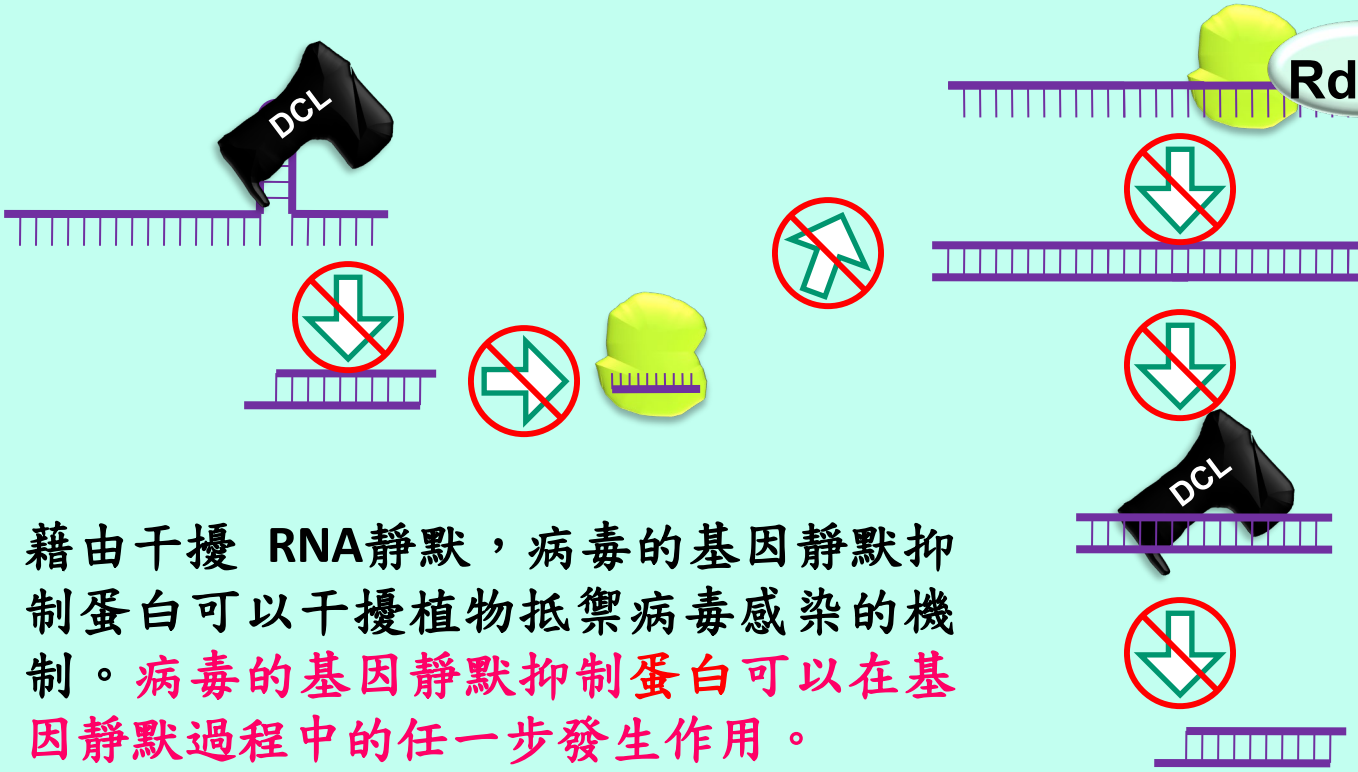
基因靜默抑制蛋白為致病因子





Spikey Phenotype of Transgenic *N. benthamiana* Expressing the WCIMV Movement
, and Reversion of the Phenotype after WCIMV Inoculation.

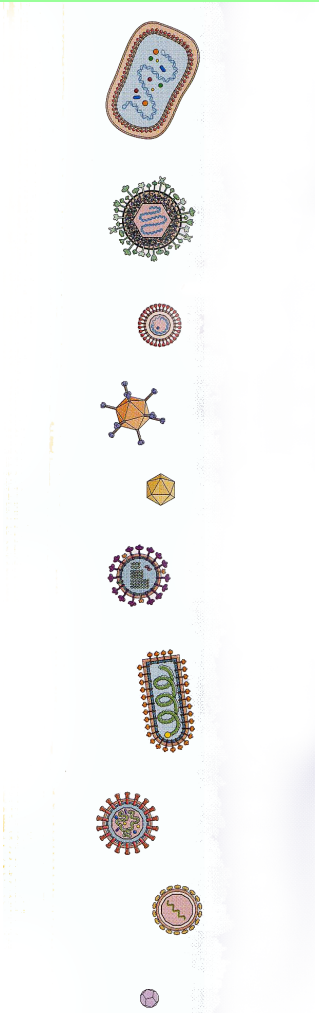
病毒具有干擾基因靜默的蛋白質



藉由干擾 RNA靜默，病毒的基因靜默抑制蛋白可以干擾植物抵禦病毒感染的機制。病毒的基因靜默抑制蛋白可以在基因靜默過程中的任一步發生作用。

➢ 植物中的 RNA 靜默機制—防禦和反制

植物病毒面面觀 ---是敵？是友？



病毒病害防治

抗病毒株的培育

- 1929 McKinney
  交互保護 (cross-protection)

- 1986 Powell et al.
  表現鞘蛋白基因具抗性之轉殖株

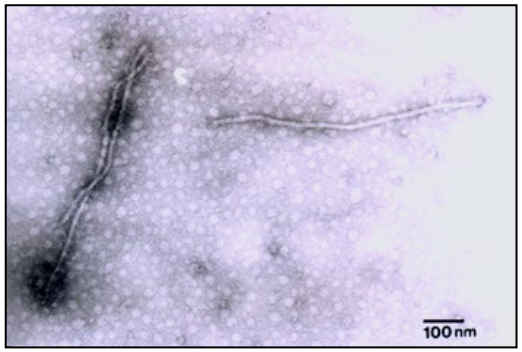
- RNA 引發抗性 (RNA-mediated resistance)

交互保護法（cross protection）

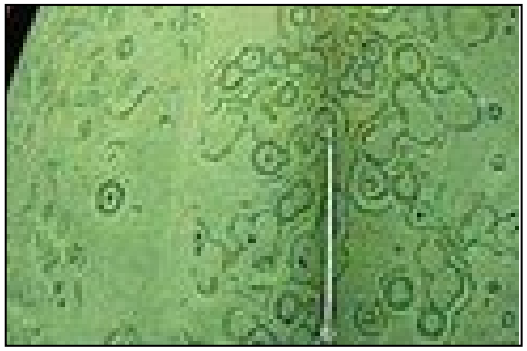
病毒作物先行於苗期在網室或溫室內先接種輕症系統（mild strain），再移至田間種植。

如此將可避免或降低植物在田間被強迫系統的病毒感染，稱為交叉保護（cross protection）。

木瓜輪點病毒 (Papaya ringspot virus, PRSV)



木瓜輪點病毒



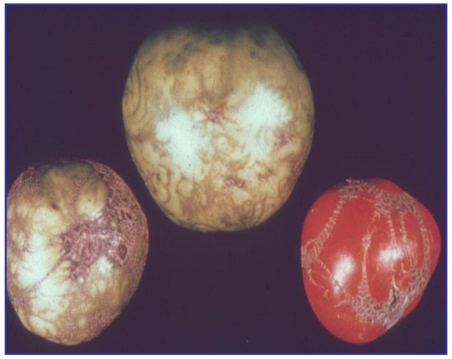
木瓜輪點病之果實病徵

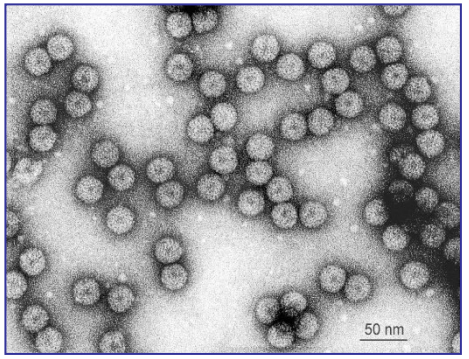
轉基因木瓜於培養瓶中再生 (左)
再生的組培苗移至溫室生長 (右)

轉基因木瓜植株的果實

蕃茄感染Cucumber mosaic virus (CMV)

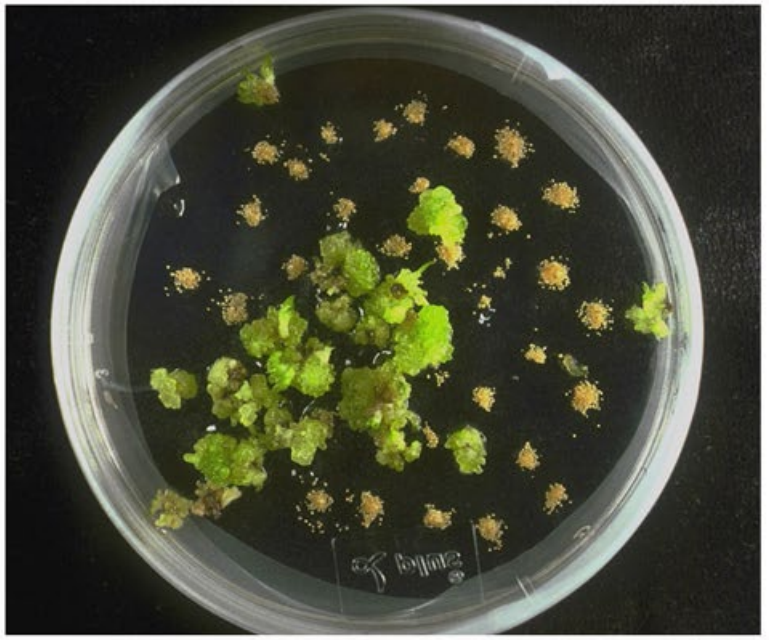
感染CMV的蕃茄植株型態

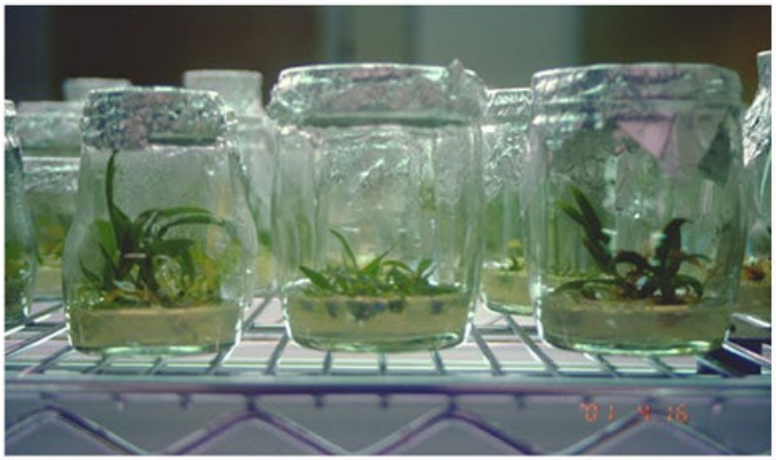




感染CMV的非轉基因(左) 及轉基因蕃茄植株(右)

Transgenic *Dendrobium*
（轉殖石角斗蘭）





植物病毒學研究對生物學的貢獻

- 開啟病毒學之研究
- 建立生物學之基礎研究
- 發現基因靜默 (RNA-silencing)機制
  - siRNA (#1 breakthrough of year 2002 in Science)
  - 2008 Nobel Prize

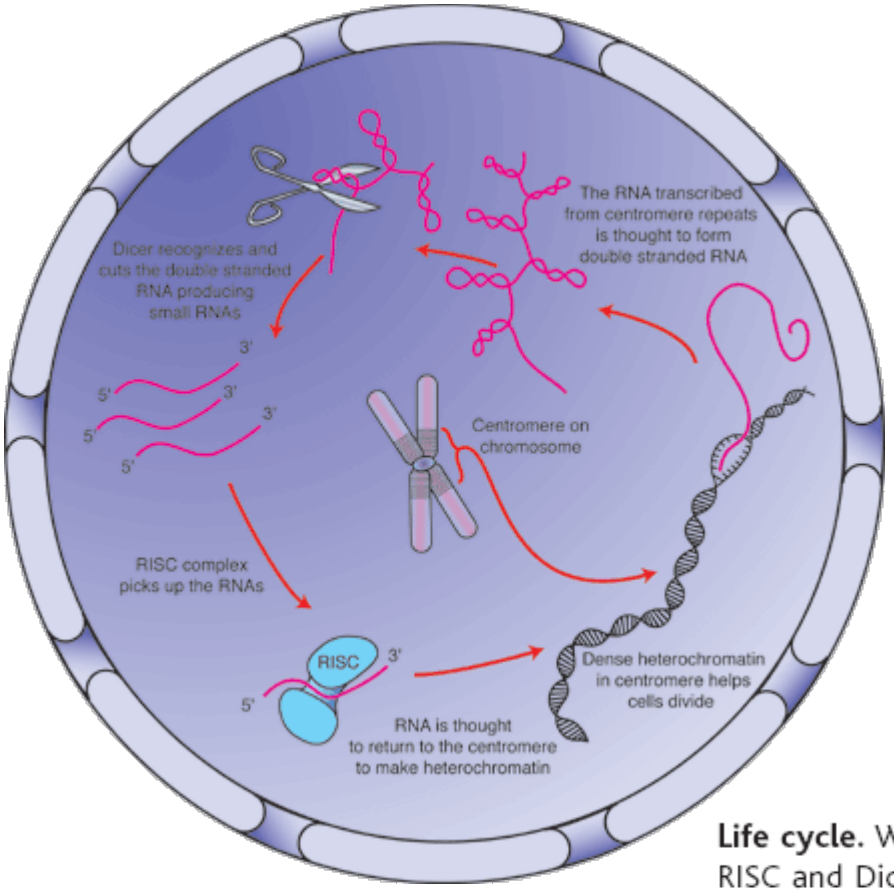
2002

Breakthrough of the Year

#1 **The Winner**

Just when scientists thought they had deciphered the roles played by the cell's leading actors, a familiar performer has turned up in a stunning variety of guises. RNA, long upstaged by its more glamorous sibling, DNA, is turning out to have star qualities of its own.

Small RNAs Make Big Splash



Life cycle. With a helping hand from proteins RISC and Dicer, small RNAs are born. We now know that these molecules keep DNA in line and ensure a cell's good health.

The Nobel Prize in Physiology or Medicine 2006

"for their discovery of RNA interference - gene silencing by double-stranded RNA"

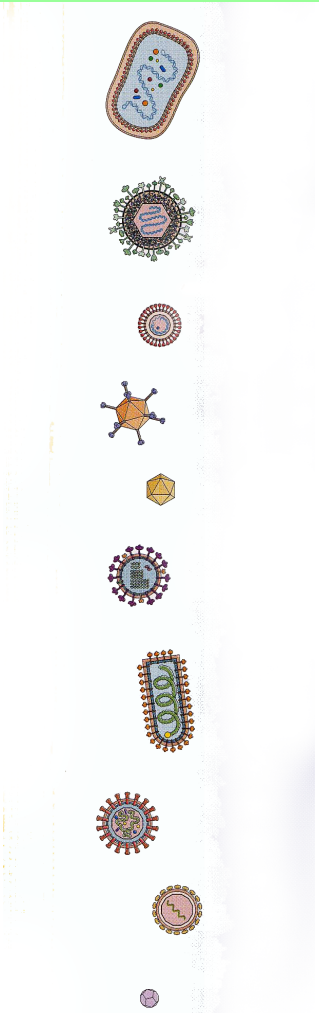
Dr. Andrew Fire

Dr. Craig Mello

- **"They started a revolution with the discovery of RNA interference," Nobel Laureate Philip Sharp**
- **Their landmark *Nature* study in 1998 -- cited in more than 2,500 papers**
- **It launched a new field in RNA research and has had "profound impacts" on the understanding of gene regulation and function**

one of the quickest recognitions of a discovery
To find this in 1998 and get a Nobel Prize in 2006 is remarkable

植物病毒面面觀 ---是敵？是友？



病毒在生物科技之應用

植物病毒在生物技術之應用

- 啟動子，促進子基因表現
- 病毒載體
  - 基因表現
  - 基因靜默
  - 抗原表現生產疫苗
- 作物改良
  - 降低栽培時間
  - 增加抗生物及非生物逆境抗性

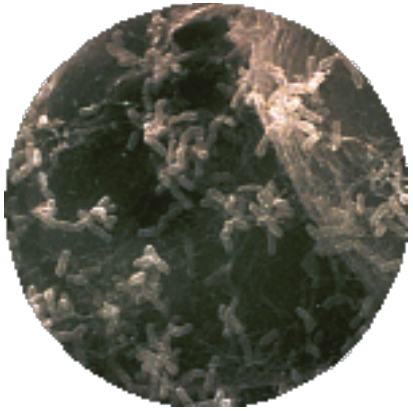
利用轉殖植物表現外源蛋白的優點

- 有經濟效益
- 有真核細胞修飾蛋白的功能
- 不含動物病原的汙染的困擾
- 可以在基因層次可以操控

疫苗大餐

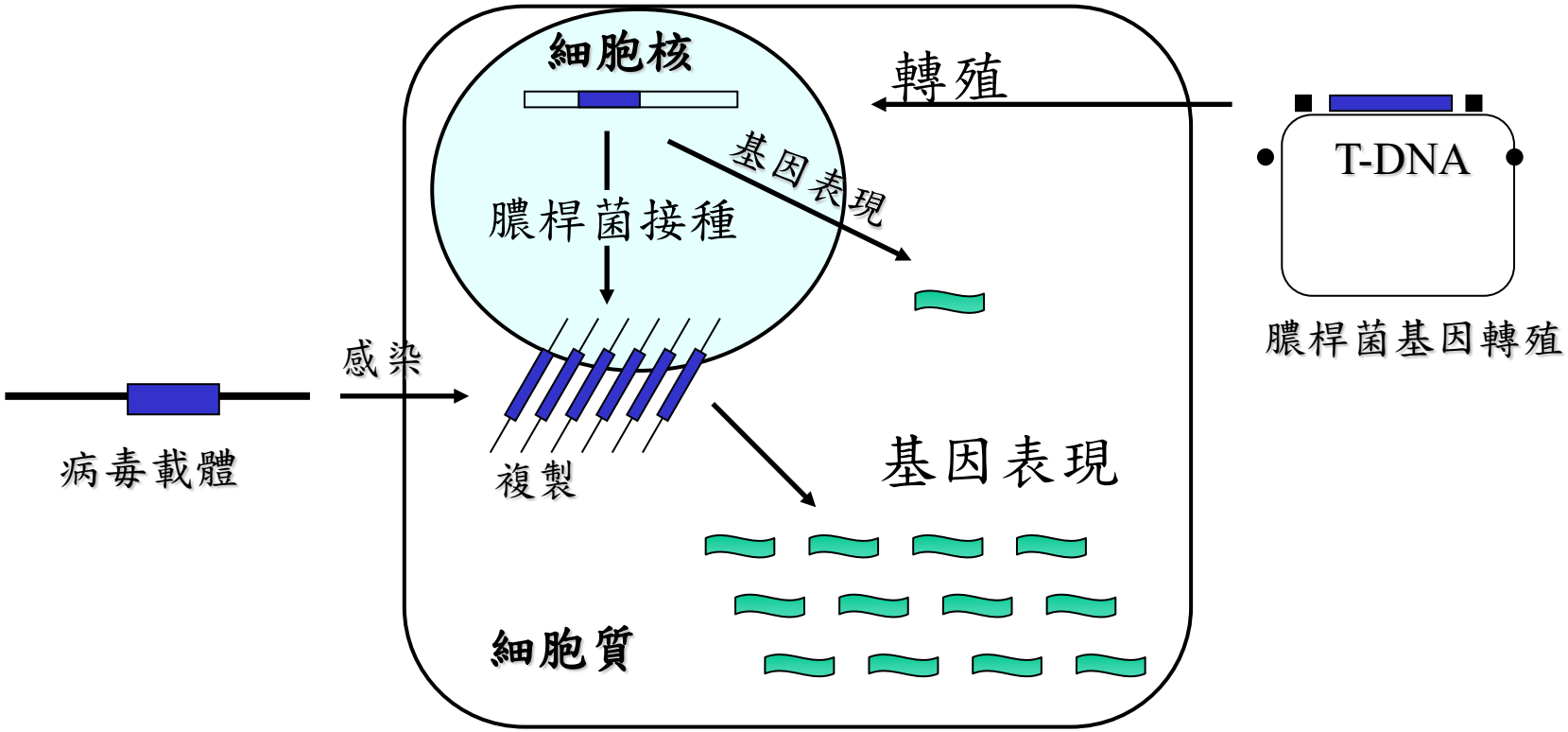
Art: Joseph Tart

Veggie vectors. Children may soon have another reason for eating their fruits and vegetables: to get vaccinated.
Photo Credit: Hugh S. Mason/Boyce Thompson (Top), *Gregory May/Boyce Thompson* (Middle), *Mich Hein/The Scripps Research Institute* (Bottom)



Gene shuttle.
Agrobacterium carry genes for disease proteins into fruit cells.
(Source: Hugh S. Mason)

利用病毒在植物細胞表現外源蛋白



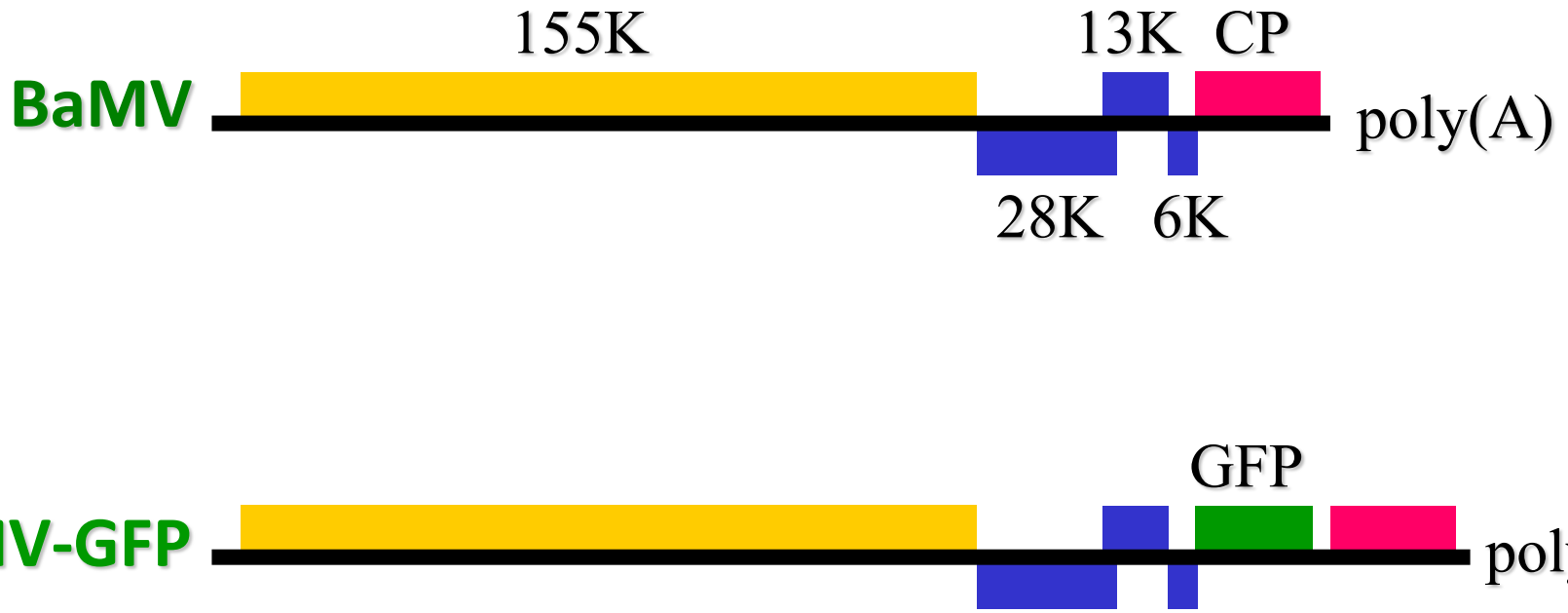
優點:

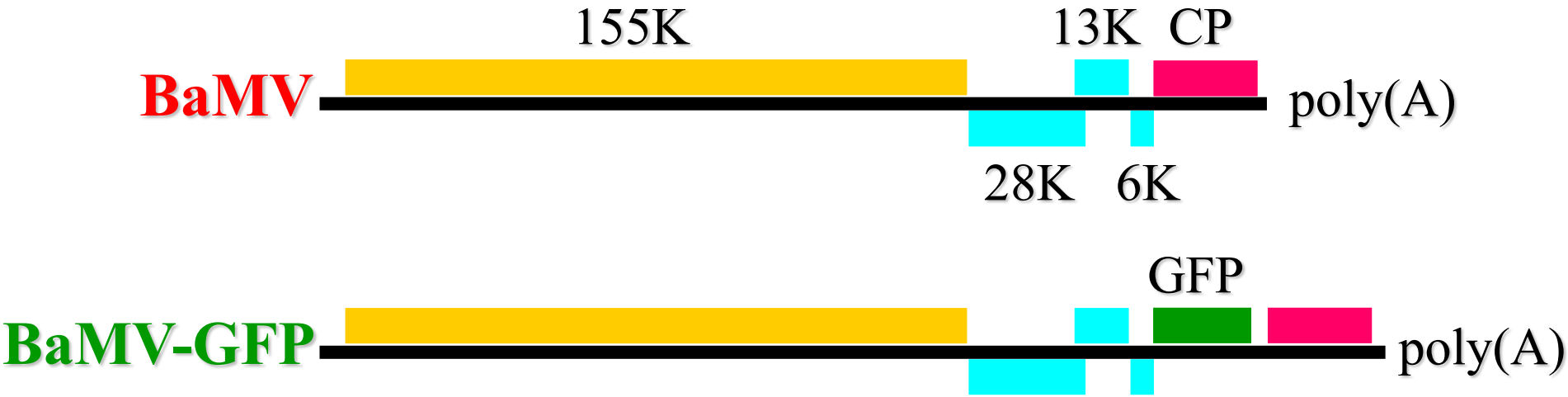
- 高拷貝數
- 短感染期
- 不需植物再生過程
- 可感染多種寄主
- 較無環境生態衝擊

困難度:

- 基因穩定度
- 基因重組現象
- 病毒基因功能的喪失，如：系統係移動、包被等等

竹嵌紋病毒
Bamboo mosaic virus (BaMV)





BaMV-S BaMV-GFP *(Liao, C. T. & Y. H. Hsu)*

以病毒為載體誘發基因靜默
(Virus-induced Gene Silencing)

cDNA

Kumagai et al., 1995. Cytoplasmic Inhibition of Carotenoid Biosynthesis with Virus-Derived RNA. PNAS (USA) 92: 1679-1683

以病毒為載體誘發基因靜默之功能性分析

TRV-PDS (phytoene desaturase)

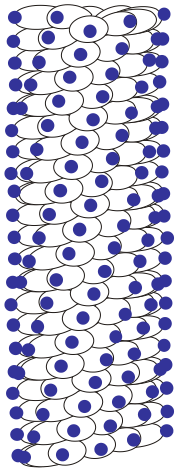
10 DPI

14 DPI

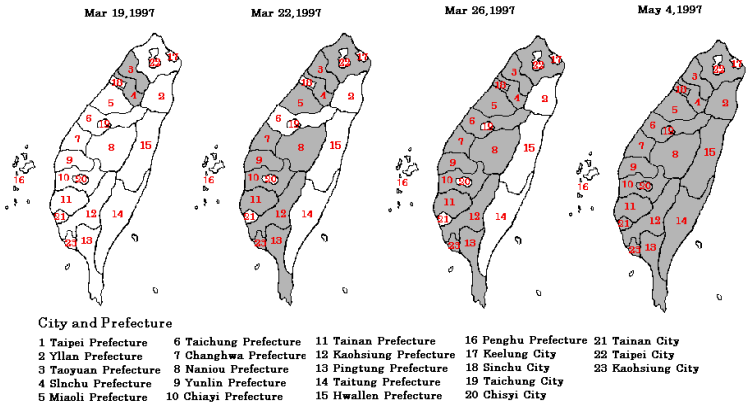
植物病毒在生物技術之應用

- 啟動子，促進子基因表現
- 病毒載體
  - 基因表現
  - 基因靜默
  - 抗原表現生產疫苗
- 作物改良
  - 降低栽培時間
  - 增加抗生物及非生物逆境抗性

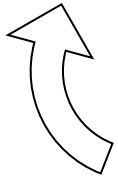
表達抗原基（以雜交病毒方式）



1997

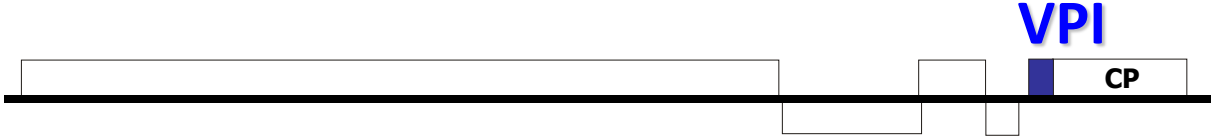


約20萬頭豬死亡影響牽豬農
共200億台幣損失

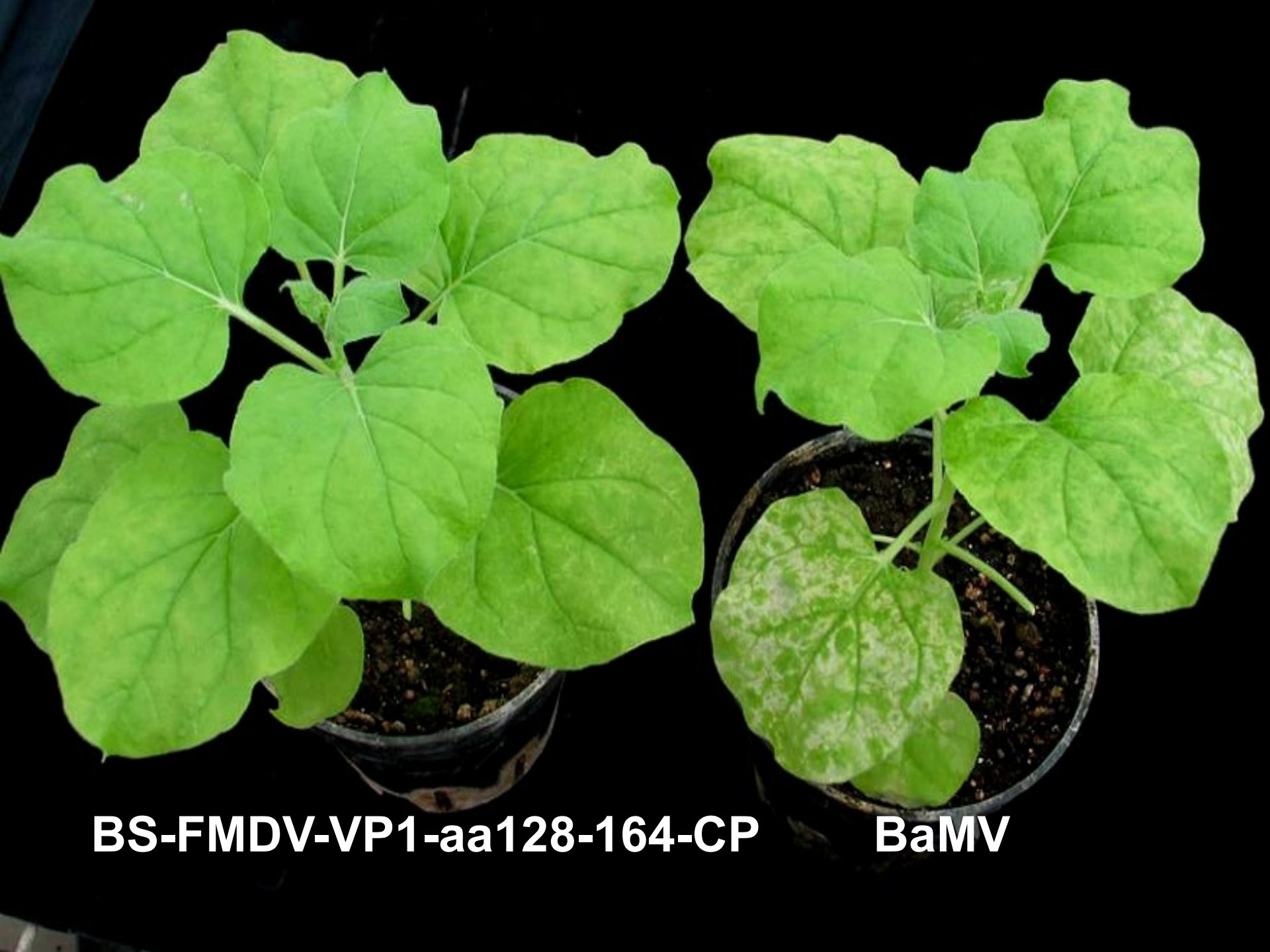


豬口蹄疫病毒

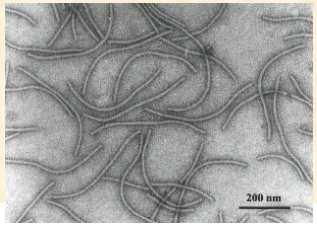
(Food and mouth disease virus)



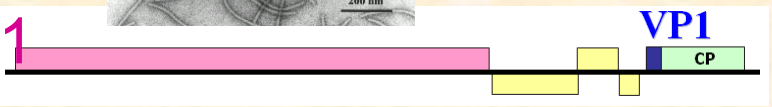
(Hsu & Liao)


BS-FMDV-VP1-aa128-164-CP
BaMV

用植物病毒載體表現胜肽疫苗



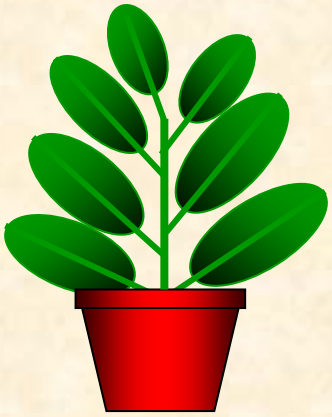
pBVP1

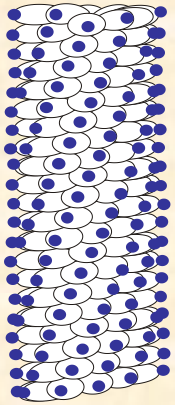


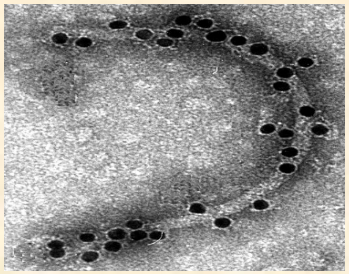

Yau-Heiu Hsu


Na-Sheng Lin


Su-May Liang







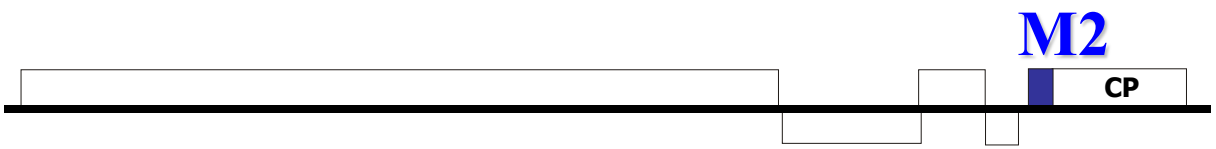
100% protection

Challenge with FMDV

竹嵌紋病毒載體之應用

傳染華氏囊病毒（Infectious bursal disease virus, Virus Res. 2012)
日本腦炎病毒（Japanese encephalitis virus, Front Microbiol. 2017)
人干擾素(human IFNγ protein (Viruses 2019)

流感病毒 (Influenza virus)
禽流感病毒 (Avian influenza virus)



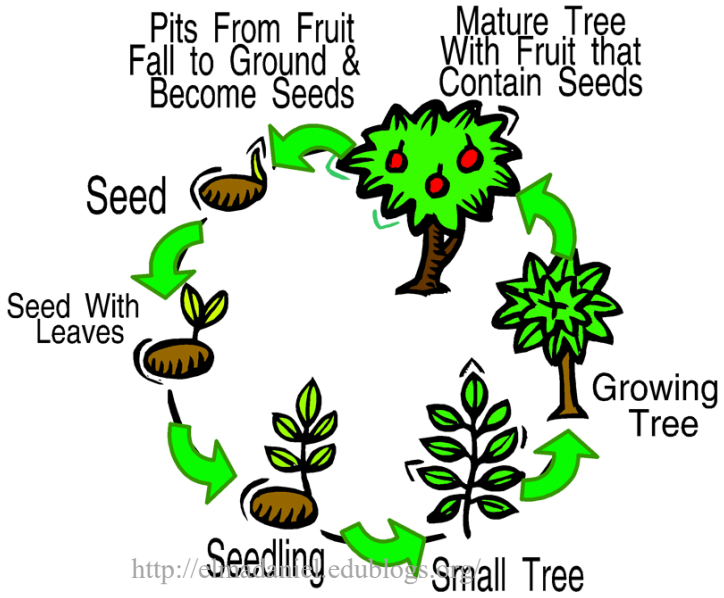
植物病毒在生物技術之應用

- 啟動子，促進子基因表現
- 病毒載體
  - 基因表現
  - 基因靜默
  - 抗原表現生產疫苗
- 作物改良
  - 降低栽培時間
  - 增加抗生物及非生物逆境抗性

利用植物病毒載體可大幅減少蘋果苗生活史

-新的植物育種技術

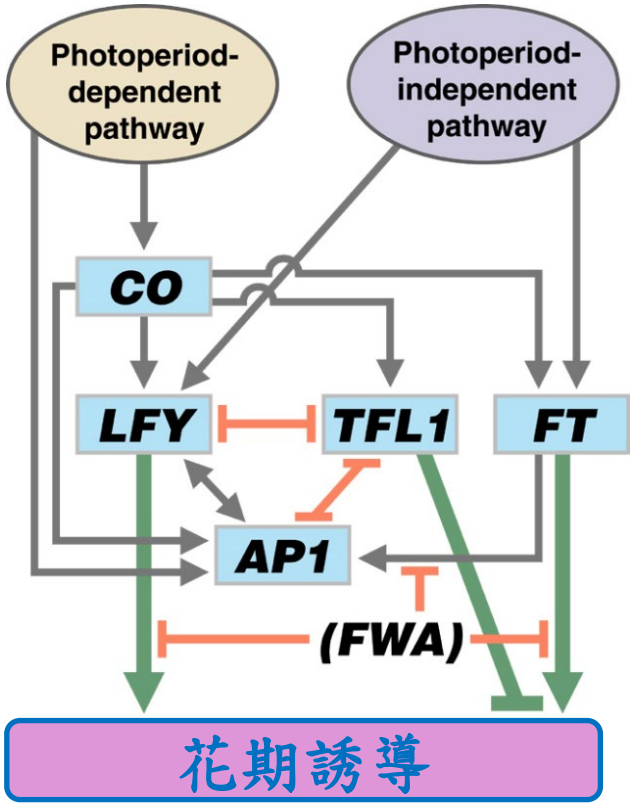
- 蘋果有很長的幼苗期約5-12年
- 目前有一些農業生物技術促進蘋果生長期改變，但仍需幾年才能開花



Yamagishi et al., 2014. Plant Biotechnology J. 12:60-68.

FLOWERING LOCUS T (AtFT)可促進植物開花
TERMINAL FLOWER 1 (TFL1)抑制植物開花

- 在阿拉伯芥FT可以和轉錄因子FD之啟動子結合促進開花.
- TFL1 和 FT有很相似之保守胺基酸序列但是有不同功能.

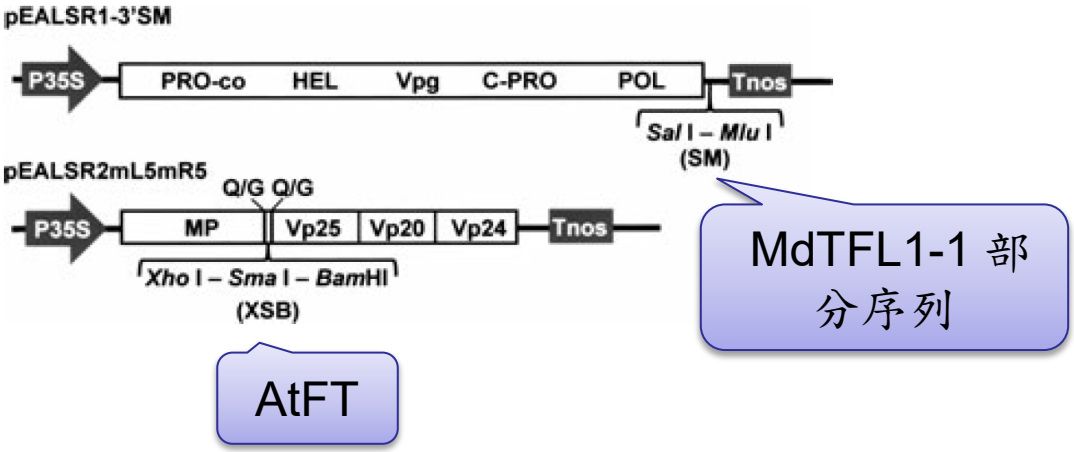


(Shigeru Hanano , 2011)

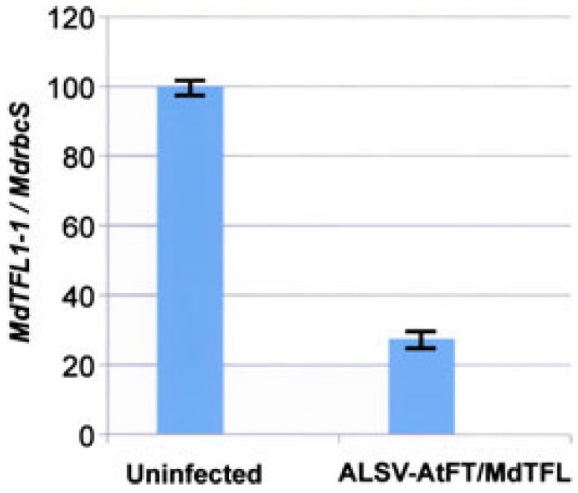
FT促進花期誘導，但是TFL1抑制它

(Kobayashi, 1999)

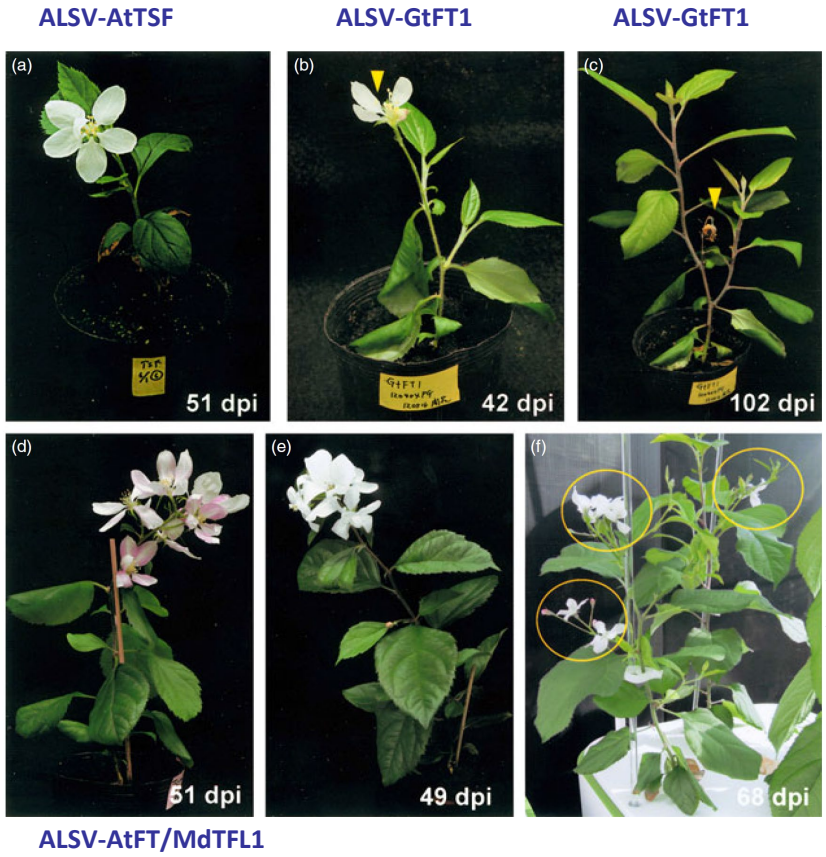
利用蘋果潛在球形病毒 (*Apple latent spherial virus*) (*ALSV*) 載體可以降低*MdTFL1-1* 表現



- RT-qPCR 偵測 ALSV-AtFT/MdTFL1 感染之蘋果 MdTFL1-mRNA之量降低很多



蘋果苗感染 ALSV-AtFT/MdTFL1可提早並連續開花

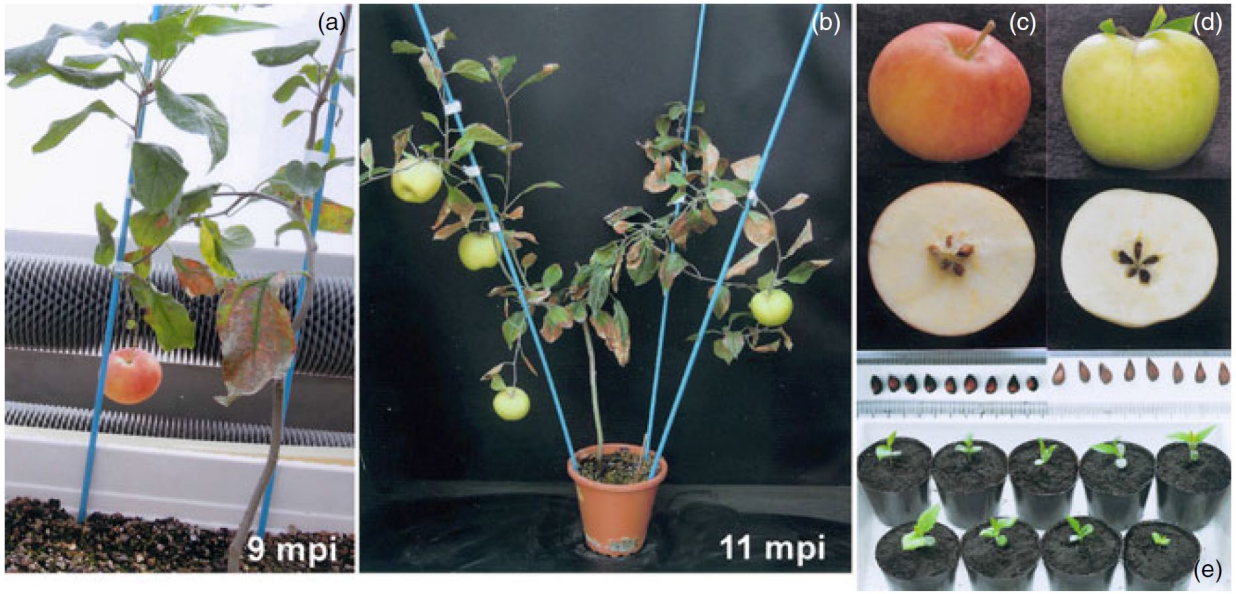


- 蘋果感染 ALSV-AtTFL, 或 ALSV-GtFT1 通常只開一朵花
- 但是感染ALSV-AtFT/MdTFL1可連續開花

AtTSF: ortholog of FT (TWIN SISTER OF FY)

利用蘋果潛在球形病毒誘導蘋果提早開花技術成功的縮短結果生活史至一年左右

提早開花之蘋果苗可利用人工授粉，5個月後產出正常蘋果果實



5 months after pollination

植物病毒在生物技術之應用

- 啟動子，促進子基因表現
- 病毒載體
  - 基因表現
  - 基因靜默
  - 抗原表現生產疫苗
- 作物改良
  - 降低栽培時間
  - 增加抗生物及非生物逆境抗性

黄石公園

Dichanthelium lanuginosum

植物寄生的真菌（Curvularia protuberata）

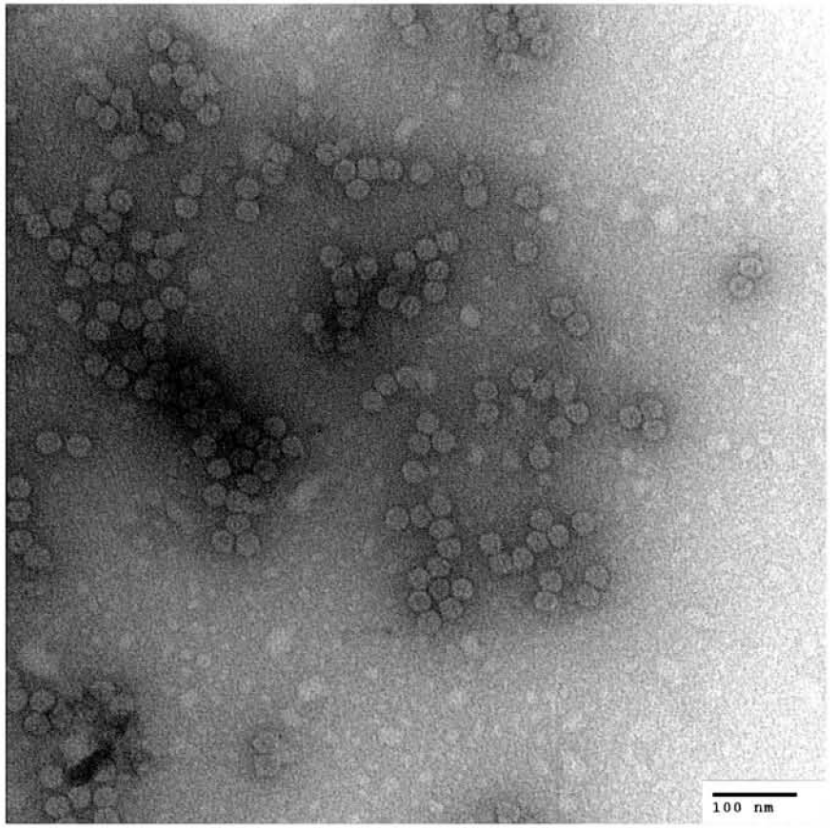
有真菌共生的植物可在高溫存活

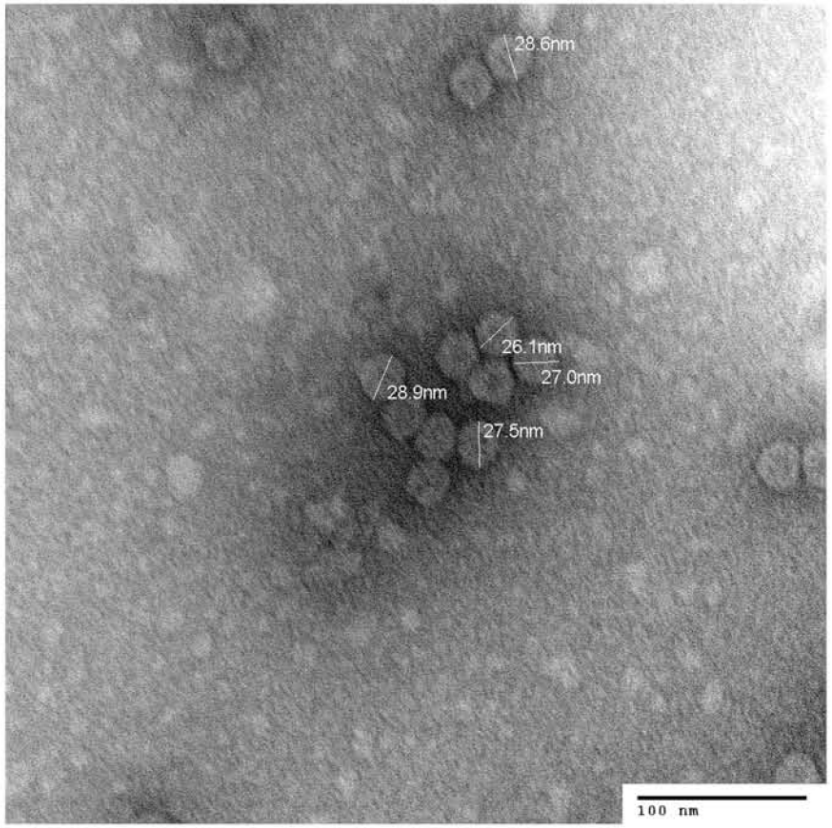
無真菌共生的植物在高溫死亡

65^0C

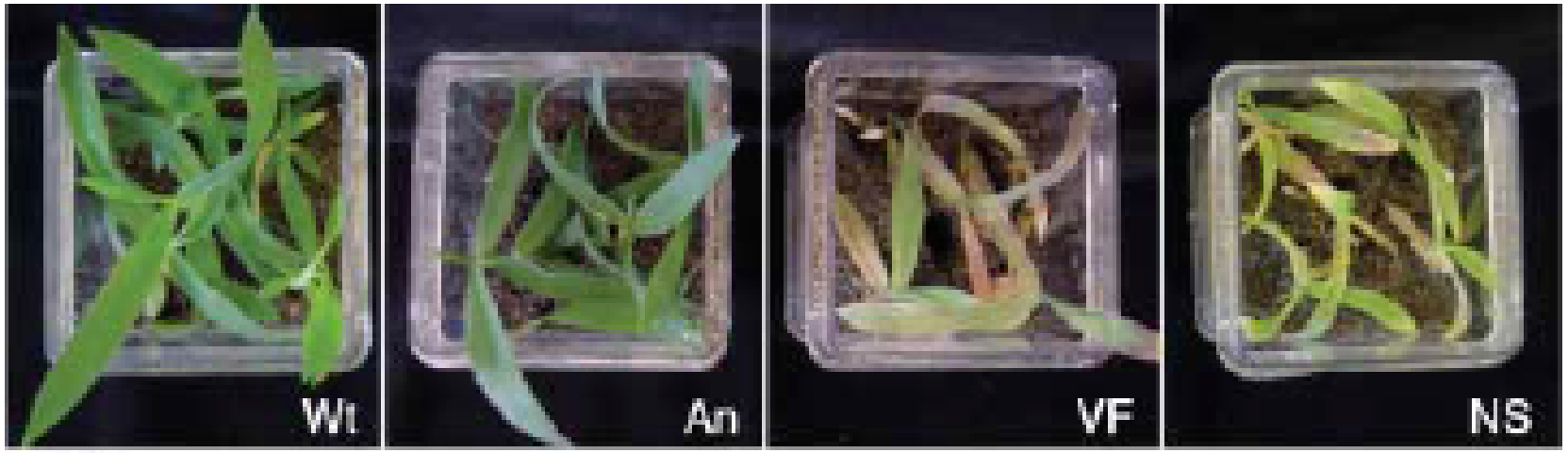
Curvularia thermal tolerance virus (CThTV)

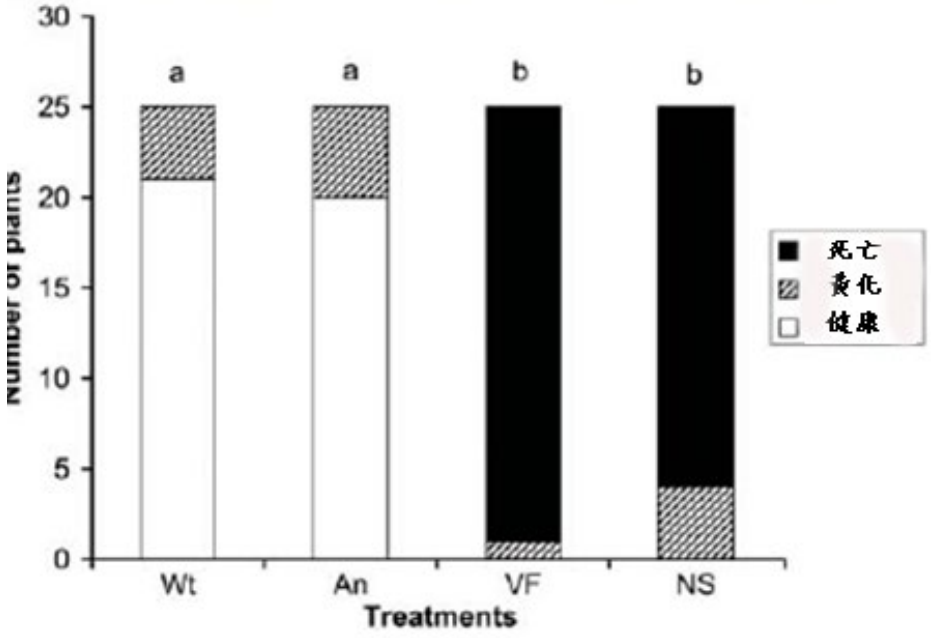
Science 315:513, 2007





高溫處理:每天65℃10小時, 37℃14小時，連續在溫室處理14天

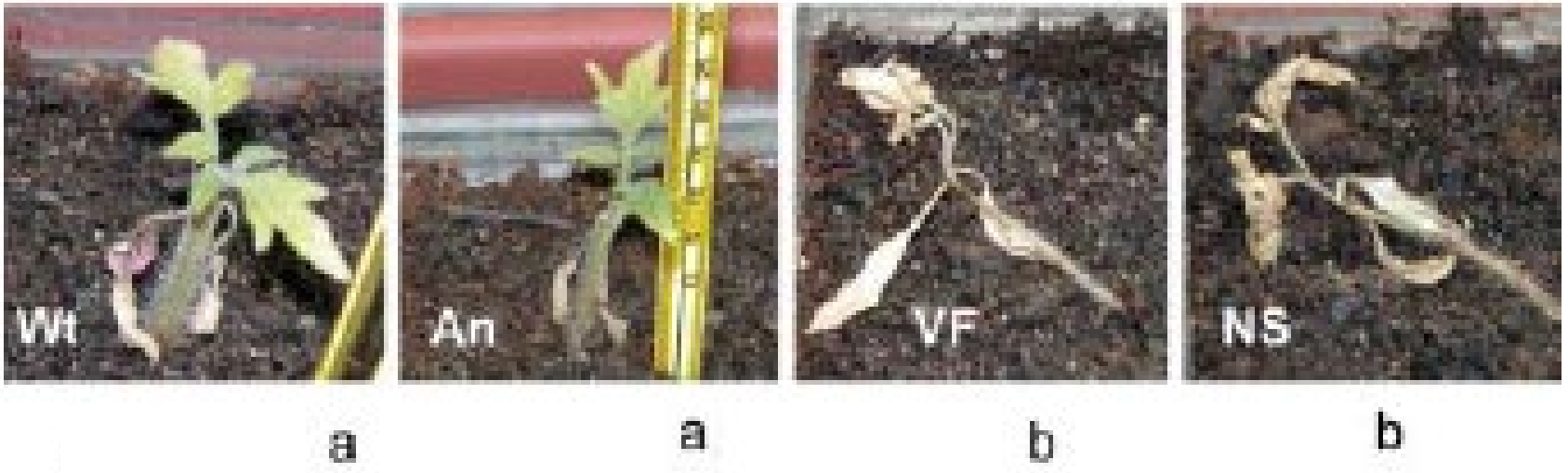


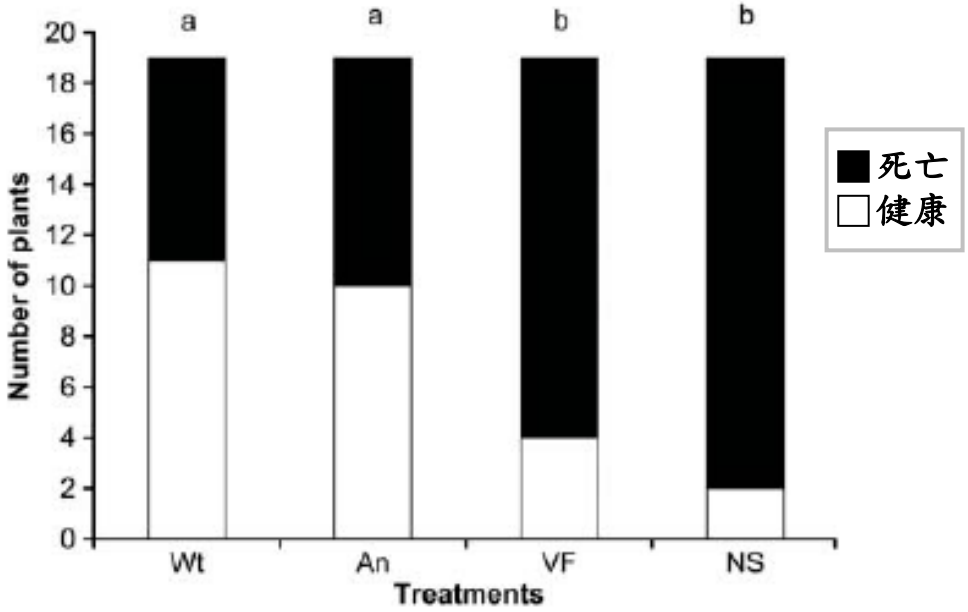


Wt:有真菌共生,病毒感染的植物
An: 人工改造真菌,含有抗生素病毒感染的植物
VF: 人工改造真菌無病毒感染的植物
NS: 沒有真菌共生的植物

Marquez et al., 2007. Science 315:513.

番茄在溫室處理14天:每天65℃10小時, 26℃14小時



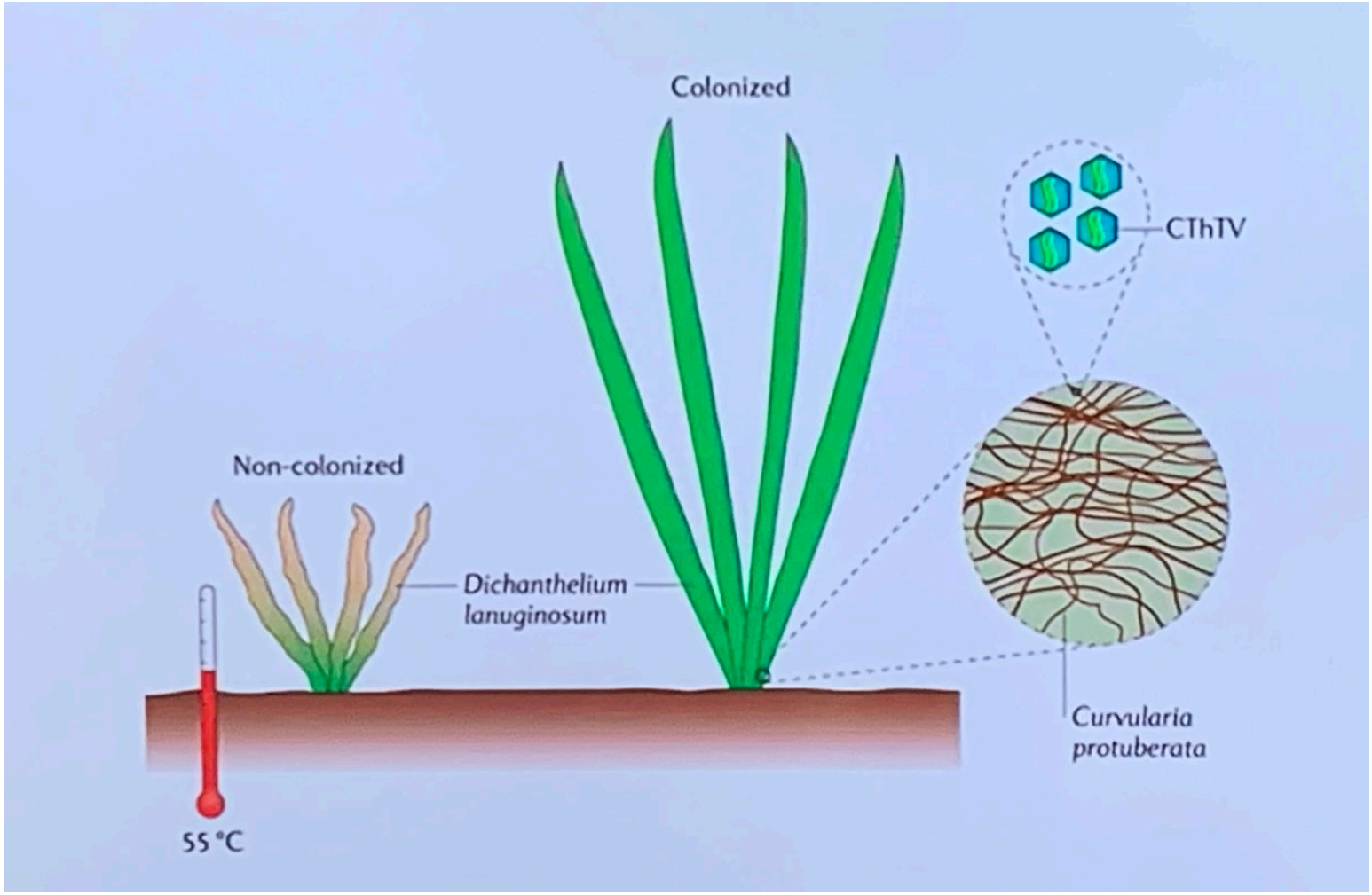


Wt: 真菌共生,病毒感染的番茄
An: 人工改造真菌,含有病毒感染的番茄
VF: 人工改造真菌,無病毒感染的番茄
NS: 沒有真菌共生的番茄

Marquez et al., 2007. Science 315:513.

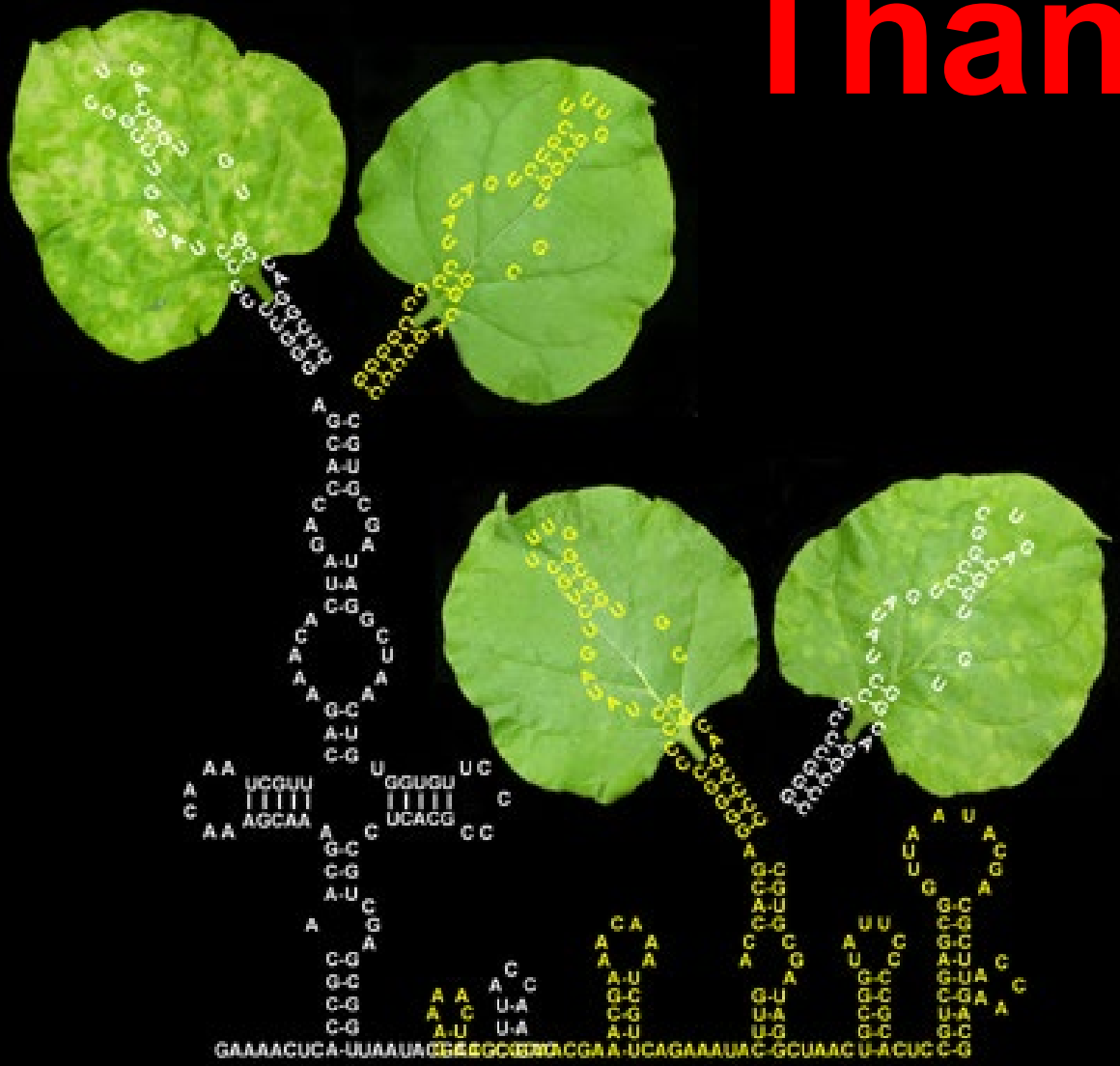
A Virus in a Fungus in a Plant

Three-Way Symposis required for termal tolerance



Science 315:513, 2007

Thank You



5’ UTRs of satBaMV