高中生命科學研究人才培育計畫

認知單元

微生物學特論--植物病毒

113年04月27日（星期六）10:40～12:00
中研院農科大樓一樓A134

授課大綱

- 病毒基本定義
- 植物病毒的常見病徵與經濟重要性
- 植物病毒的種類
- 植物病毒的基本結構
- 植物病毒的移動與傳播
- 植物病毒的基本防治策略
- 植物病毒在生物科技上的應用

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何謂病毒?

-- “濾過性” 病毒???

-- 病毒的發現史:

1714: 勞倫斯 (Lawrence): 茉莉花嵌紋病可經由嫁接傳染。

1886: 梅爾(Mayer): 菸草嵌紋病(Tobacco mosaic virus, TMV)，病葉的汁液具有傳染力，但沒有正確結論病原為何。

1892: 伊凡諾夫斯基 (Ivanowski), 過濾實驗，但仍未有正確結論。

1898: 班傑林克(Beijerink), “filterable living fluid”，首次將病毒定義為”可以通過陶瓷濾器的具有活性的液體” 。

-- 當時已知最小的病原體

Pasteur Chamberland filter
巴斯德-張伯倫 過濾器

可以濾除當
時已知最小
的細菌

https://www.nlm.nih.gov/exhibition/fromdnatobeer/exhibition-interactive/pasteur-chamberland-filter/pasteur-chamberland-filter-alt.html

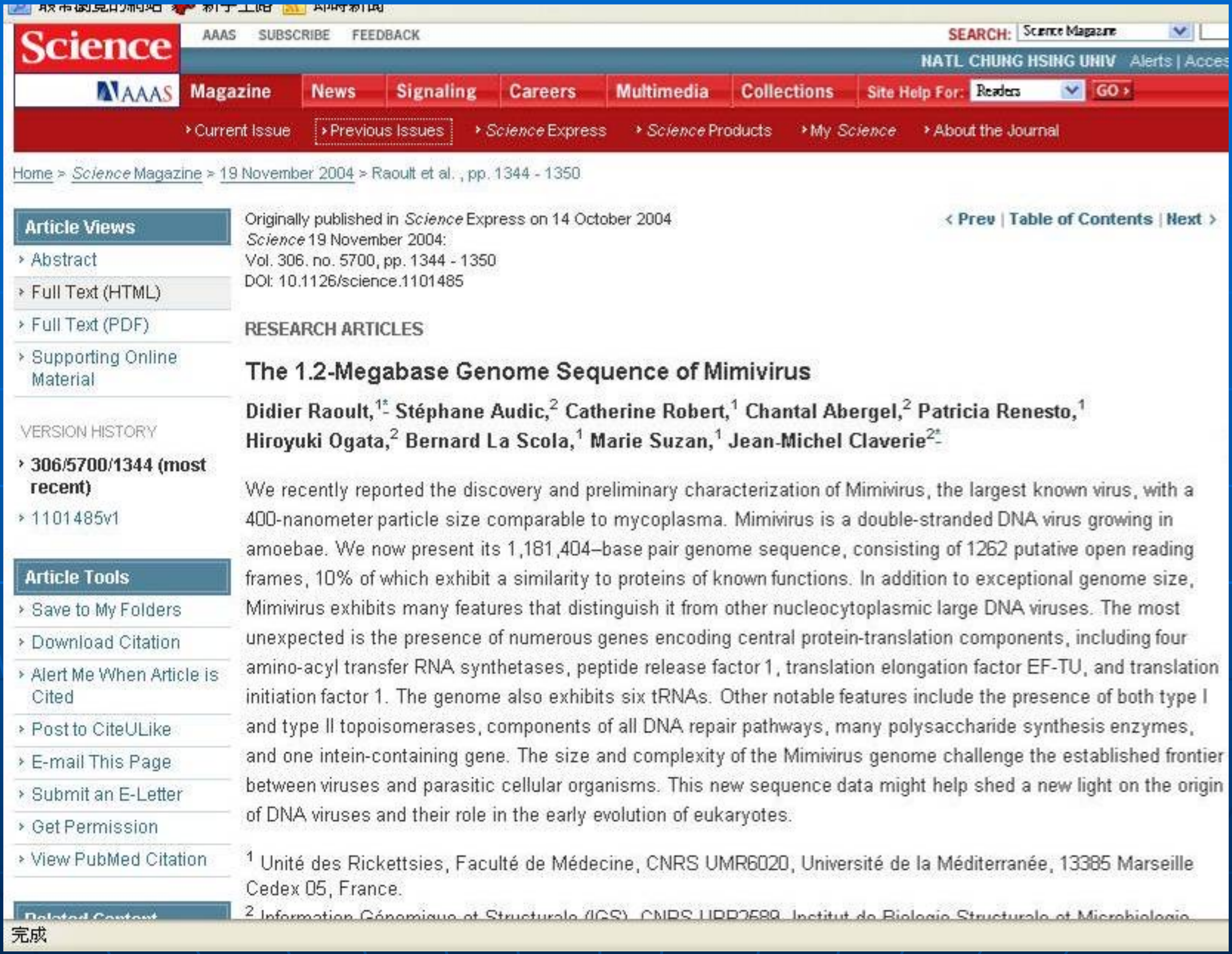
何謂病毒? (續)

-- 不能以是否能通過細菌過濾器來定義病毒!!

-- 目前已知有些病毒顆粒與基因體皆大於大腸桿菌

-- 例如Mimivirus, Mamavirus, Pithovirus, Pandoravirus, Tupanvirus 等 (如下圖所示)

在 2013年以前所知道最大的病毒



Originally published in *Science* Express on 14 October 2004
Science 19 November 2004:
Vol. 306. no. 5700, pp. 1344 - 1350
DOI: 10.1126/science.1101485

RESEARCH ARTICLES

The 1.2-Megabase Genome Sequence of Mimivirus

Didier Raoult,[1*] Stéphane Audic,[2] Catherine Robert,[1] Chantal Abergel,[2] Patricia Renesto,[1] Hiroyuki Ogata,[2] Bernard La Scola,[1] Marie Suzan,[1] Jean-Michel Claverie[2*]

We recently reported the discovery and preliminary characterization of Mimivirus, the largest known virus, with a 400-nanometer particle size comparable to mycoplasma. Mimivirus is a double-stranded DNA virus growing in amoebae. We now present its 1,181,404–base pair genome sequence, consisting of 1262 putative open reading frames, 10% of which exhibit a similarity to proteins of known functions. In addition to exceptional genome size, Mimivirus exhibits many features that distinguish it from other nucleocytoplasmic large DNA viruses. The most unexpected is the presence of numerous genes encoding central protein-translation components, including four amino-acyl transfer RNA synthetases, peptide release factor 1, translation elongation factor EF-TU, and translation initiation factor 1. The genome also exhibits six tRNAs. Other notable features include the presence of both type I and type II topoisomerases, components of all DNA repair pathways, many polysaccharide synthesis enzymes, and one intein-containing gene. The size and complexity of the Mimivirus genome challenge the established frontier between viruses and parasitic cellular organisms. This new sequence data might help shed a new light on the origin of DNA viruses and their role in the early evolution of eukaryotes.

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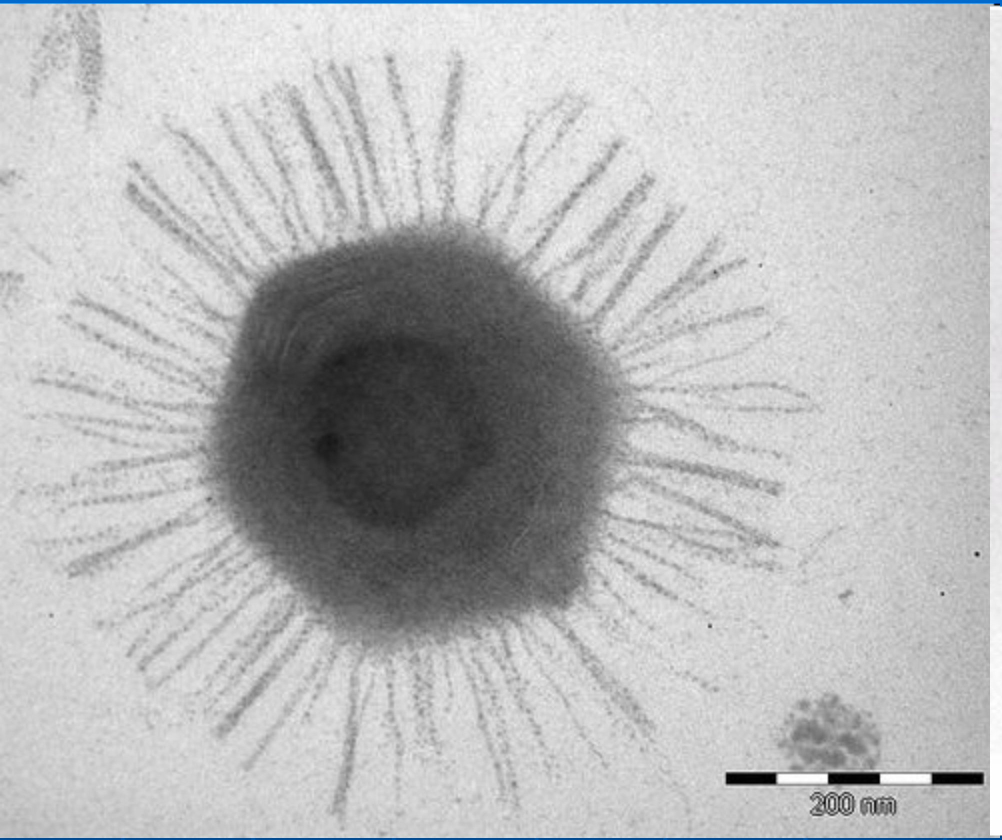
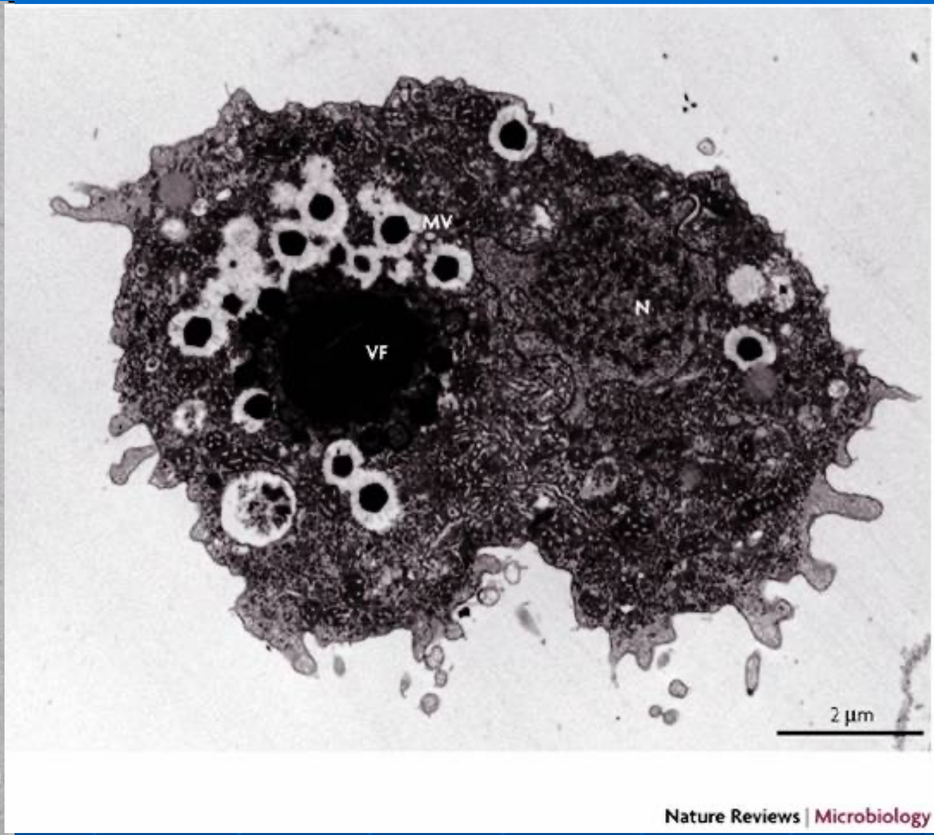
[2] Information Génomique et Structurale (IGS), CNRS UPR2589, Institut de Biologie Structurale et Microbiologie





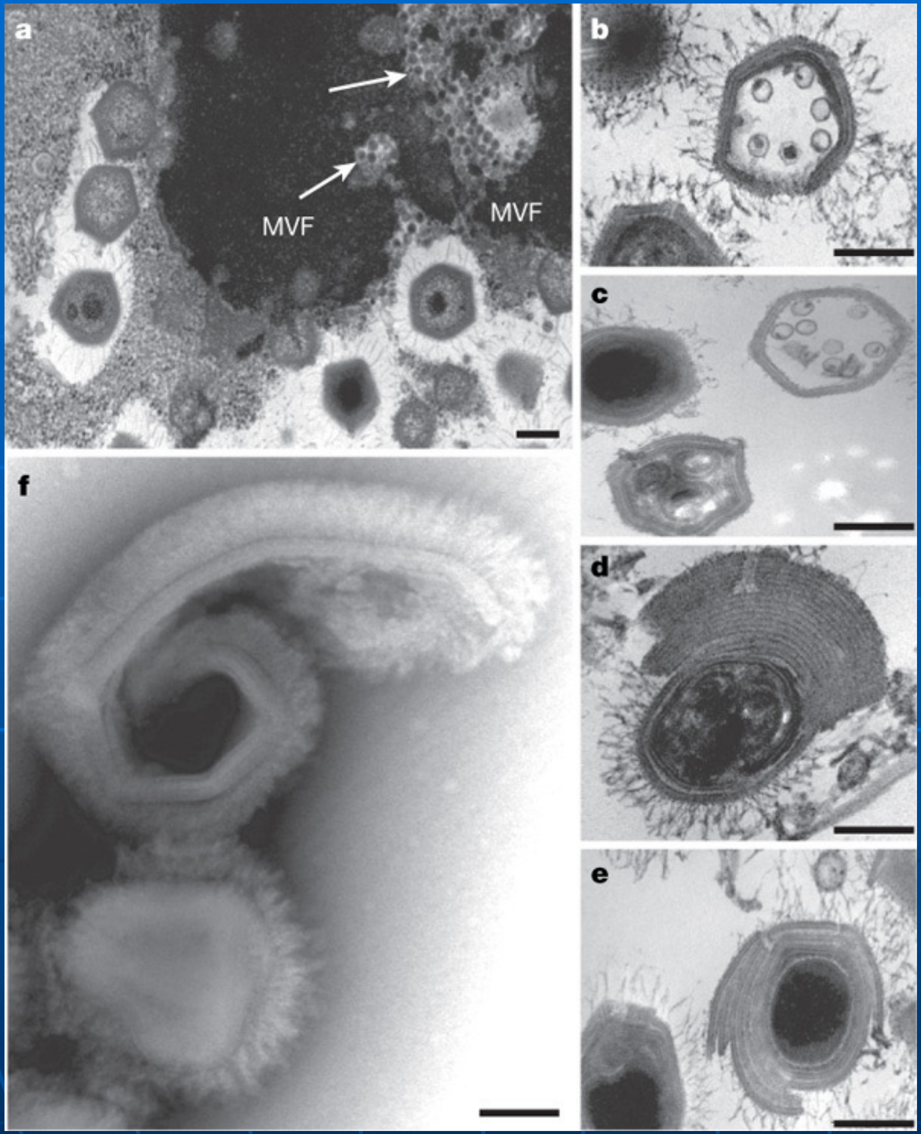
Nature Reviews | Microbiology

www.stanford.edu/group/virus/mimi/2005/index.htm

www.nature.com/.../fig_tab/nrmicro1858_F1.html

Mamavirus 被Sputnik virophage (史普尼克噬病毒體)感染後出現的不同型態

病毒也會生病，因此病毒是活的!!!



BL Scola *et al.* *Nature* **000**, 1-5 (2008) doi:10.1038/nature07218

2014年之前已知最大病毒: 潘朵拉病毒Pandoravirus

http://www.sciencemag.org/content/341/6143/281.full

Science 19 July 2013:
Vol. 341 no. 6143 pp. 281-286
DOI: 10.1126/science.1239181

REPORT

Pandoraviruses: Amoeba Viruses with Genomes Up to 2.5 Mb Reaching That of Parasitic Eukaryotes

Nadège Philippe[1,2,*], Matthieu Legendre[1,*], Gabriel Doutre[1], Yohann Couté[3], Olivier Poirot[1], Magali Lescot[1], Defne Arslan[1], Virginie Seltzer[1], Lionel Bertaux[1], Christophe Bruley[3], Jérome Garin[3], Jean-Michel Claverie[1,†], Chantal Abergel[1,†]

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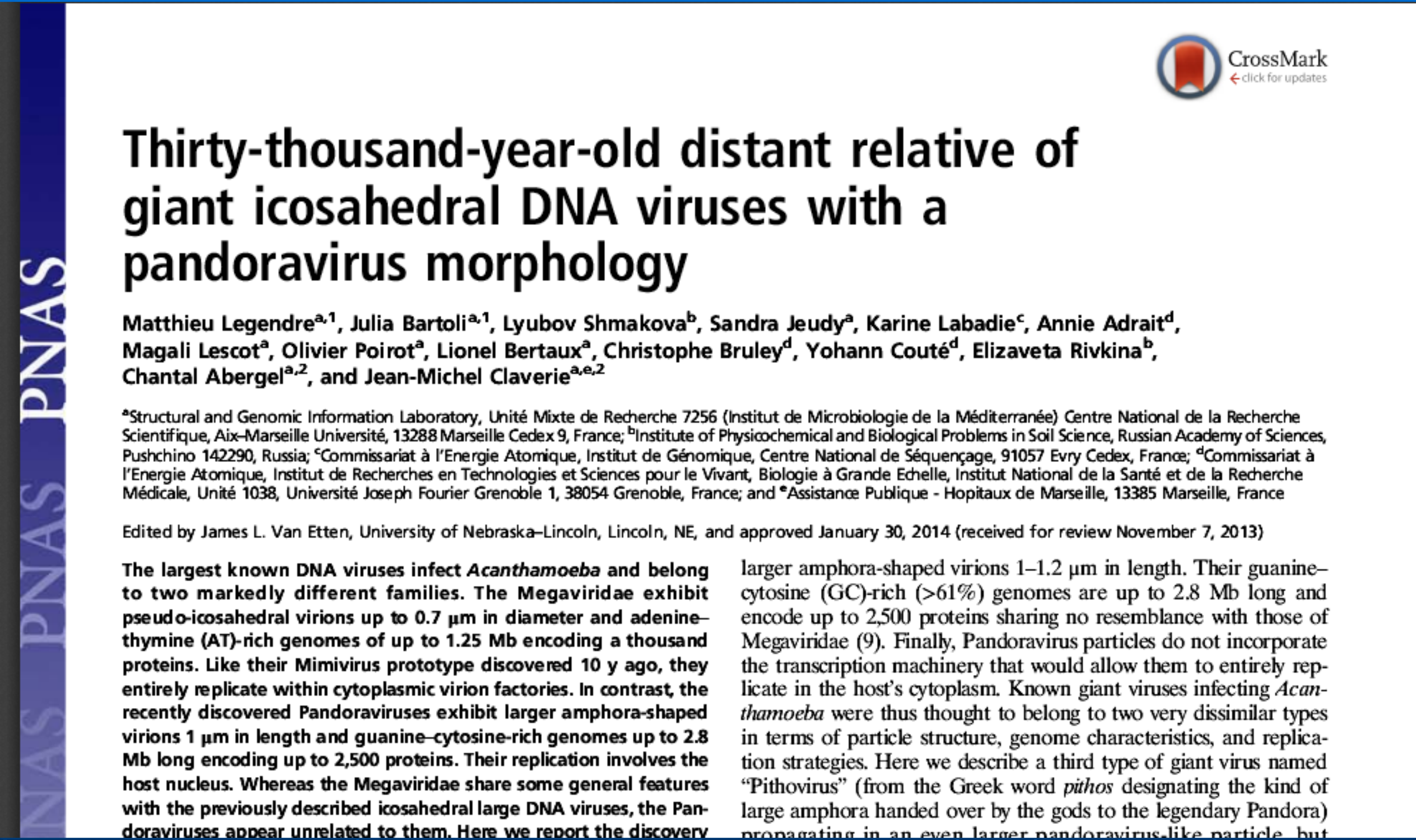
[*] These authors contributed equally to this work.

ABSTRACT | EDITOR'S SUMMARY

Ten years ago, the discovery of Mimivirus, a virus infecting Acanthamoeba, initiated a reappraisal of the upper limits of the viral world, both in terms of particle size (>0.7 micrometers) and genome complexity (>1000 genes), dimensions typical of parasitic bacteria. The diversity of these giant viruses (the Megaviridae) was assessed by sampling a variety of aquatic environments and their associated sediments worldwide. We report the isolation of two giant viruses, one off the coast of central Chile, the other from a freshwater pond near Melbourne (Australia), without morphological or genomic resemblance to any previously defined virus families. Their micrometer-sized ovoid particles contain DNA genomes of at least 2.5 and 1.9 megabases, respectively. These viruses are the first

目前已知最大病毒:闊口罐病毒Pithovirus
由三萬年前的冰層中復活



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PNAS PNAS PNAS

Thirty-thousand-year-old distant relative of giant icosahedral DNA viruses with a pandoravirus morphology

Matthieu Legendre[a,1], Julia Bartoli[a,1], Lyubov Shmakova[b], Sandra Jeudy[a], Karine Labadie[c], Annie Adrait[d], Magali Lescot[a], Olivier Poirot[a], Lionel Bertaux[a], Christophe Bruley[d], Yohann Couté[d], Elizaveta Rivkina[b], Chantal Abergel[a,2], and Jean-Michel Claverie[a,e,2]

[a]Structural and Genomic Information Laboratory, Unité Mixte de Recherche 7256 (Institut de Microbiologie de la Méditerranée) Centre National de la Recherche Scientifique, Aix-Marseille Université, 13288 Marseille Cedex 9, France; [b]Institute of Physicochemical and Biological Problems in Soil Science, Russian Academy of Sciences, Pushchino 142290, Russia; [c]Commissariat à l'Energie Atomique, Institut de Génomique, Centre National de Séquençage, 91057 Evry Cedex, France; [d]Commissariat à l'Energie Atomique, Institut de Recherches en Technologies et Sciences pour le Vivant, Biologie à Grande Echelle, Institut National de la Santé et de la Recherche Médicale, Unité 1038, Université Joseph Fourier Grenoble 1, 38054 Grenoble, France; and [e]Assistance Publique - Hopitaux de Marseille, 13385 Marseille, France

Edited by James L. Van Etten, University of Nebraska–Lincoln, Lincoln, NE, and approved January 30, 2014 (received for review November 7, 2013)

The largest known DNA viruses infect *Acanthamoeba* and belong to two markedly different families. The Megaviridae exhibit pseudo-icosahedral virions up to 0.7 μm in diameter and adenine–thymine (AT)-rich genomes of up to 1.25 Mb encoding a thousand proteins. Like their Mimivirus prototype discovered 10 y ago, they entirely replicate within cytoplasmic virion factories. In contrast, the recently discovered Pandoraviruses exhibit larger amphora-shaped virions 1 μm in length and guanine–cytosine-rich genomes up to 2.8 Mb long encoding up to 2,500 proteins. Their replication involves the host nucleus. Whereas the Megaviridae share some general features with the previously described icosahedral large DNA viruses, the Pandoraviruses appear unrelated to them. Here we report the discovery

larger amphora-shaped virions 1–1.2 μm in length. Their guanine–cytosine (GC)-rich (>61%) genomes are up to 2.8 Mb long and encode up to 2,500 proteins sharing no resemblance with those of Megaviridae (9). Finally, Pandoravirus particles do not incorporate the transcription machinery that would allow them to entirely replicate in the host's cytoplasm. Known giant viruses infecting *Acanthamoeba* were thus thought to belong to two very dissimilar types in terms of particle structure, genome characteristics, and replication strategies. Here we describe a third type of giant virus named "Pithovirus" (from the Greek word *pithos* designating the kind of large amphora handed over by the gods to the legendary Pandora) propagating in an even larger pandoravirus-like particle, but

http://www.pnas.org/content/111/11/4274.full.pdf

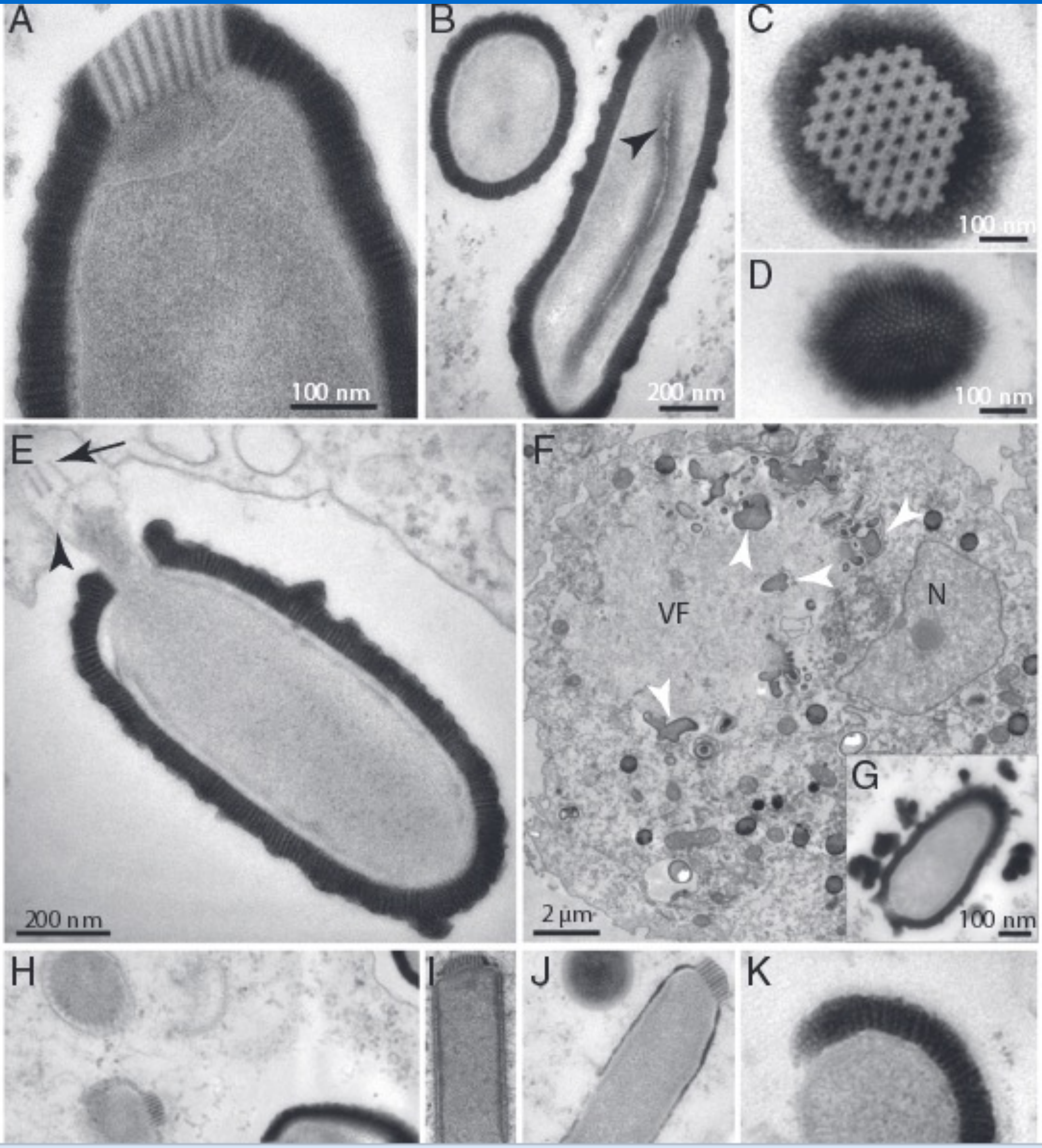
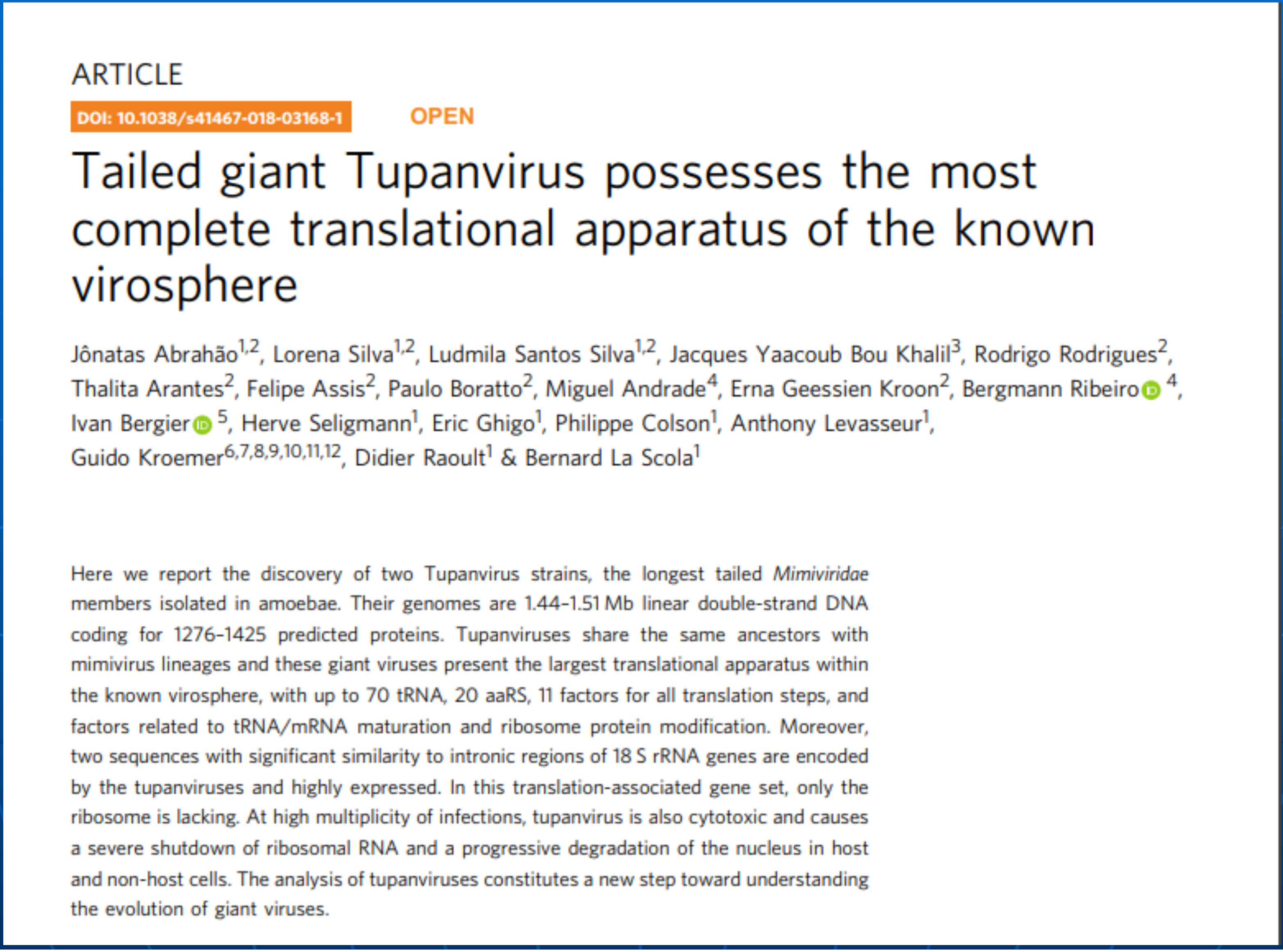


Fig. 1. Electron microscopy imaging of the Pithovirus replication cycle in *A. castellanii*. (*A*) Apex of the Pithovirus particle showing its unique cork made of 15 nm-spaced stripes, rolled membranes underneath, and the internal membrane. (*B*) Two perpendicular views of the Pithovirus particles (cross- and longitudinal sections). The particles are wrapped into a 60 nm-thick envelope made of 10 nm-spaced parallel stripes. A lipid membrane is enclosing a homogeneous interior where a tubular structure is seen episodically, but in a reproducible fashion (arrowhead). (*C*) Top view of the cork revealing a hexagonal honeycomb-like array. (*D*) Bottom view of the particle showing the striated organization of the envelope. (*E*) An opened Pithovirus particle in the host vacuole. Parts of the expelled cork are visible (black arrows) and the internal membrane of the particle (black arrowhead) appears ready to fuse with the vacuole membrane. (*F*) Maturing virions at a late stage of infection. Structures made of stripes, pieces of cork, and dense material accumulate (white arrowhead) in the periphery of the virion factory (VF). These structures may contain preassembled particle building blocks (Fig. S1). The cell nucleus (N) is visible. (*G*) *Inset* highlighting a late stage of virion maturation with globular striated structures accumulating at the virion periphery. (*H*) Various stages of particle assembly in the same cell. (*I*) Incompletely assembled rectangular particle lacking its thick envelope. The striated cork is already visible. (*J*) At a later stage,

http://www.pnas.org/content/111/11/4274.full.pdf

目前已知製造蛋白功能最完整的病毒：雷神病毒



ARTICLE

DOI: 10.1038/s41467-018-03168-1

OPEN

Tailed giant Tupanvirus possesses the most complete translational apparatus of the known virosphere

Jônatas Abrahão[1,2], Lorena Silva[1,2], Ludmila Santos Silva[1,2], Jacques Yaacoub Bou Khalil[3], Rodrigo Rodrigues[2], Thalita Arantes[2], Felipe Assis[2], Paulo Boratto[2], Miguel Andrade[4], Erna Geessien Kroon[2], Bergmann Ribeiro[4], Ivan Bergier[5], Herve Seligmann[1], Eric Ghigo[1], Philippe Colson[1], Anthony Levasseur[1], Guido Kroemer[6,7,8,9,10,11,12], Didier Raoult[1] & Bernard La Scola[1]

Here we report the discovery of two Tupanvirus strains, the longest tailed *Mimiviridae* members isolated in amoebae. Their genomes are 1.44–1.51 Mb linear double-strand DNA coding for 1276–1425 predicted proteins. Tupanviruses share the same ancestors with mimivirus lineages and these giant viruses present the largest translational apparatus within the known virosphere, with up to 70 tRNA, 20 aaRS, 11 factors for all translation steps, and factors related to tRNA/mRNA maturation and ribosome protein modification. Moreover, two sequences with significant similarity to intronic regions of 18 S rRNA genes are encoded by the tupanviruses and highly expressed. In this translation-associated gene set, only the ribosome is lacking. At high multiplicity of infections, tupanvirus is also cytotoxic and causes a severe shutdown of ribosomal RNA and a progressive degradation of the nucleus in host and non-host cells. The analysis of tupanviruses constitutes a new step toward understanding the evolution of giant viruses.

NATURE COMMUNICATIONS | (2018)9:749 | DOI: 10.1038/s41467-018-03168-1 |

Tupanviruses 雷神病毒

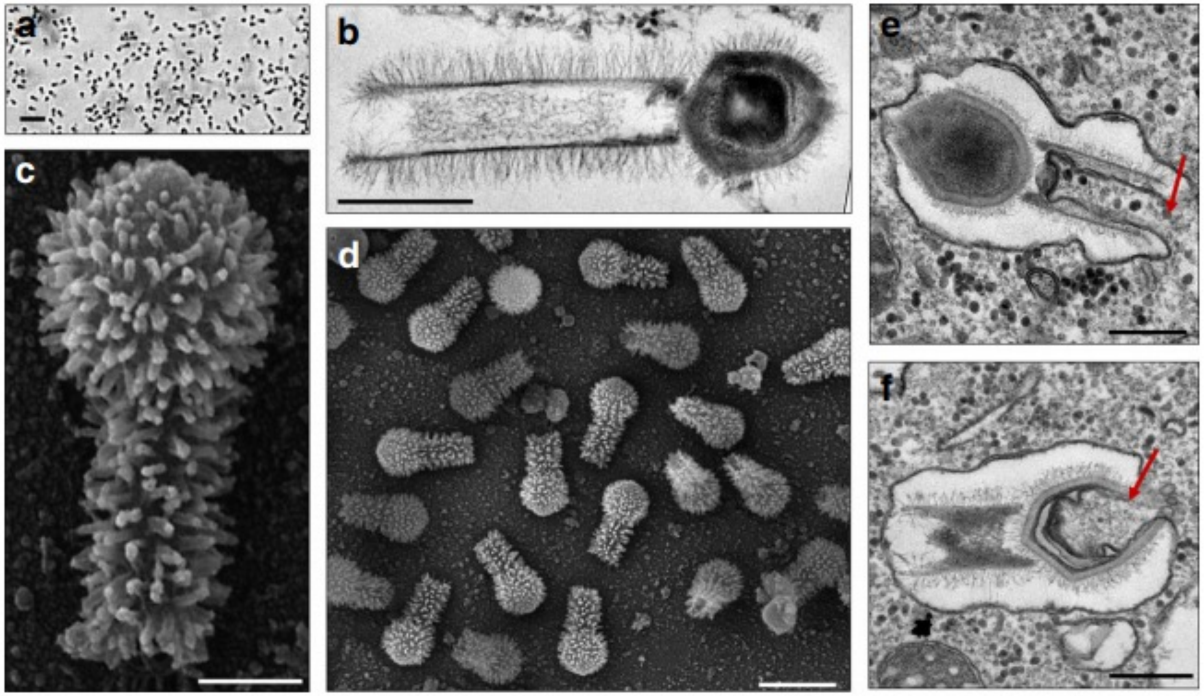


Fig. 1 Tupanvirus soda lake particles and cycle. **a** Optical microscopy of Tupanvirus particles after haemacolour staining (1000 ×). Scale bar, 2 μm. **b** Super particle (>1000 nm) observed by transmission electron microscopy (TEM). Scale bar, 500 nm. **c**, **d** Scanning electron microscopy (SEM) of Tupanvirus particles. Scale bars 250 nm and 1 μm, respectively. **e**, **f** The initial steps of infection in *A. castellanii* involve the release of both capsid (**e**) and tail (**f**) content into the amoeba cytoplasm (red arrows). Scale bars, 350 nm and 450 nm, respectively

NATURE COMMUNICATIONS | (2018)9:749 | DOI: 10.1038/s41467-018-03168-1 |

GiantVirus.org

巨大病毒資料網站

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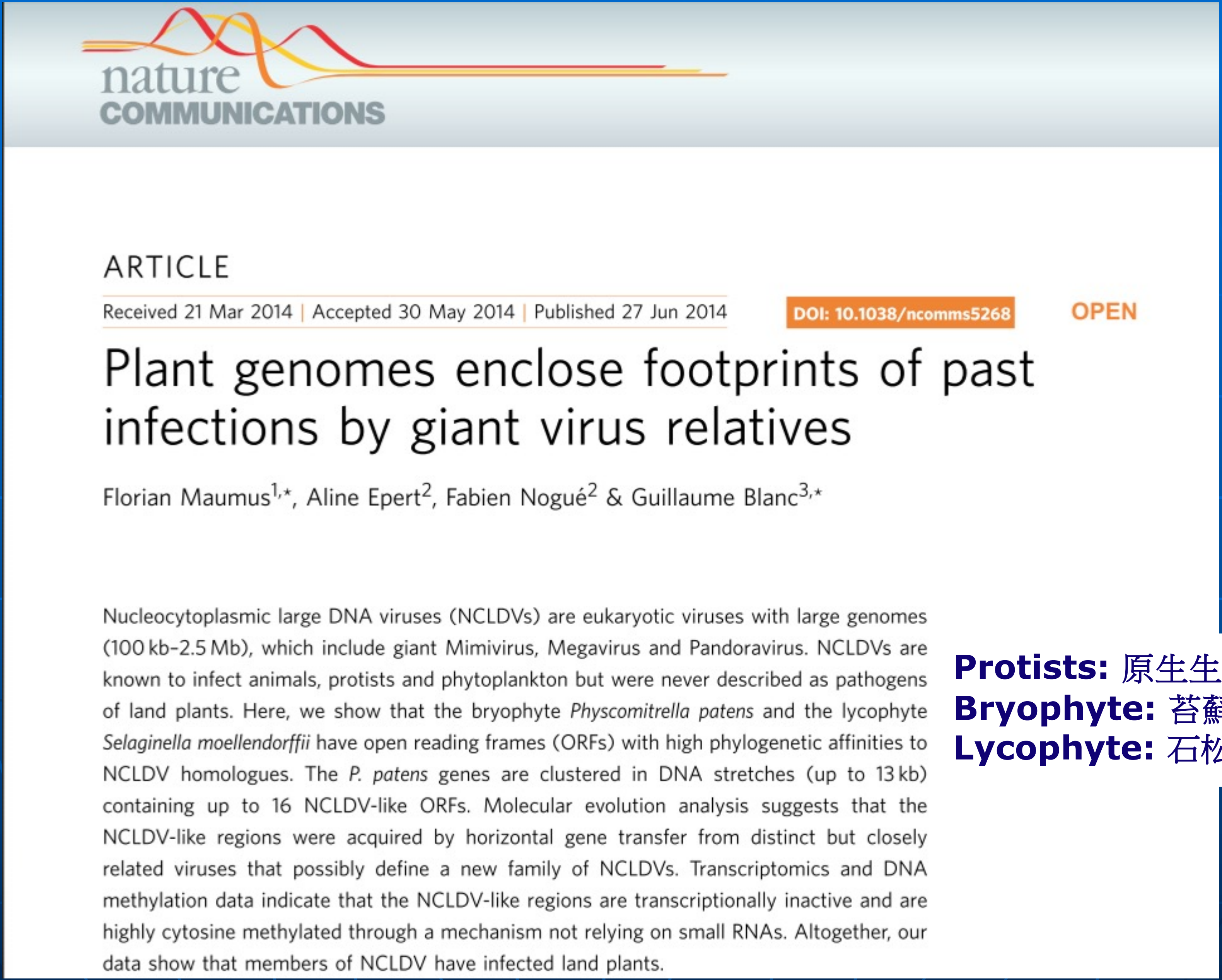
The largest known viral genomes (completely sequenced, > 170 kb)

	Virus	Length	Segm	Protein	NC Id
1	Pandoravirus salinus (Pandoraviridae)	2473870	1	2541	NC_022098.1
2	Pandoravirus dulcis	1908524	1	1487	NC_021858
3	Megavirus chilensis (Megaviridae)	1259197	1	1120	NC_016072
4	Mamavirus	1191693	1	1023	JF801956
5	Mimivirus	1181549	1	979	NC_014649
6	Moumouvirus	1021348	1	894	NC_020104
7	Mimivirus, isolate M4	981813	1	≈ 620	JN036606
8	Cafeteria roenbergensis virus BV-PW1	617453	1	544	NC_014637
9	Cotesia congregata bracovirus (Polydnaviridae)	567670	30	155	NC_6633-6662
10	Bacillus megaterium phage G (Caudovirales)	497513	1	*675*	JN638751
11	Phaeocystis globosa virus (Phycodnaviridae)	460000	1	460	HQ634147
12	Emiliania huxleyi virus 86	407339	1	472	NC_007346
13	Paramecium bursaria Chlorella virus NY2A	368683	1	*886*	NC_009898
14	Marseillevirus	368454	1	428	NC_013756
15	Canarypox virus (Poxviridae)	359853	1	328	NC_005309
16	Lausannevirus, isolate 7715	346754	1	444	NC_015326
17	Paramecium bursaria Chlorella virus AR158	344691	1	*814*	NC_009899
18	Ectocarpus siliculosus virus	335593	1	240	NC_002687
19	Paramecium bursaria Chlorella virus 1	330611	1	*802*	NC_000852
20	Paramecium bursaria Chlorella virus FR483	321240	1	335	NC_008603
21	Pseudomonas phage 201phi2-1	316674	1	*461*	NC_010821
22	Paramecium bursaria chlorella virus MT325	314335	1	331	DQ491001
23	Shrimp white spot syndrome virus (Nimaviridae)	305107	1	*531*	NC_003225
24	Cyprinid herpesvirus 3 (Herpesvirales)	295146	1	163	NC_009127
25	Glypta fumiferanae ichnovirus	291597	105	103	NC_008837-008941

Legend: The largest known viral genomes. For each virus family, the largest representative is highlighted in green. Probably inflated counts for predicted protein-coding genes are indicated in red. Fragmented genomes are highlighted in yellow. Genome larger or close to 300 kb are now known for 8 families: *Megaviridae, Polydnaviridae, Caudoviridae, Phycodnaviridae, Marseilleviruses, Poxviridae, Nimaviridae* and *Herpesvirales*. Notre that the smallest genomes of parasitic microorganisms considered "cellular" in nature are less than 170 kb in size.

http://www.giantvirus.org/top.html

植物基因體中保存著巨型病毒基因體的遺跡



nature
COMMUNICATIONS

ARTICLE

Received 21 Mar 2014 | Accepted 30 May 2014 | Published 27 Jun 2014

DOI: 10.1038/ncomms5268

OPEN

Plant genomes enclose footprints of past infections by giant virus relatives

Florian Maumus[1,*], Aline Epert[2], Fabien Nogué[2] & Guillaume Blanc[3,*]

Nucleocytoplasmic large DNA viruses (NCLDVs) are eukaryotic viruses with large genomes (100 kb–2.5 Mb), which include giant Mimivirus, Megavirus and Pandoravirus. NCLDVs are known to infect animals, protists and phytoplankton but were never described as pathogens of land plants. Here, we show that the bryophyte *Physcomitrella patens* and the lycophyte *Selaginella moellendorffii* have open reading frames (ORFs) with high phylogenetic affinities to NCLDV homologues. The *P. patens* genes are clustered in DNA stretches (up to 13 kb) containing up to 16 NCLDV-like ORFs. Molecular evolution analysis suggests that the NCLDV-like regions were acquired by horizontal gene transfer from distinct but closely related viruses that possibly define a new family of NCLDVs. Transcriptomics and DNA methylation data indicate that the NCLDV-like regions are transcriptionally inactive and are highly cytosine methylated through a mechanism not relying on small RNAs. Altogether, our data show that members of NCLDV have infected land plants.

Protists: 原生生物
Bryophyte: 苔蘚類植物
Lycophyte: 石松類植物

所有病毒的相同處：(病毒的定義)

-- 濾過性病毒??? (不能以“可通過陶瓷過濾器”作為定義!)

-- a set of one or more nucleic acid molecules encased in protective protein coat(s). (一組核酸包裹在蛋白質外殼中)

-- multiply by ASSEMBLY from pools of required materials (nucleic acids, proteins, and lipids), not by binary fission (以”組合”方式進行複製，而非”二裂法”生殖)

-- does not possess independent energy production systems (沒有獨立的能量合成系統)

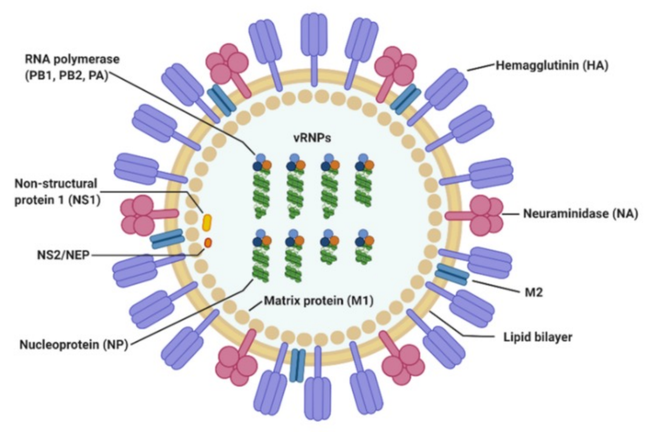
-- does not possess independent protein synthesis systems (沒有獨立的蛋白質合成系統)

-- does not GROW (不能長大)

植物與動物寄主具有下列之主要差異

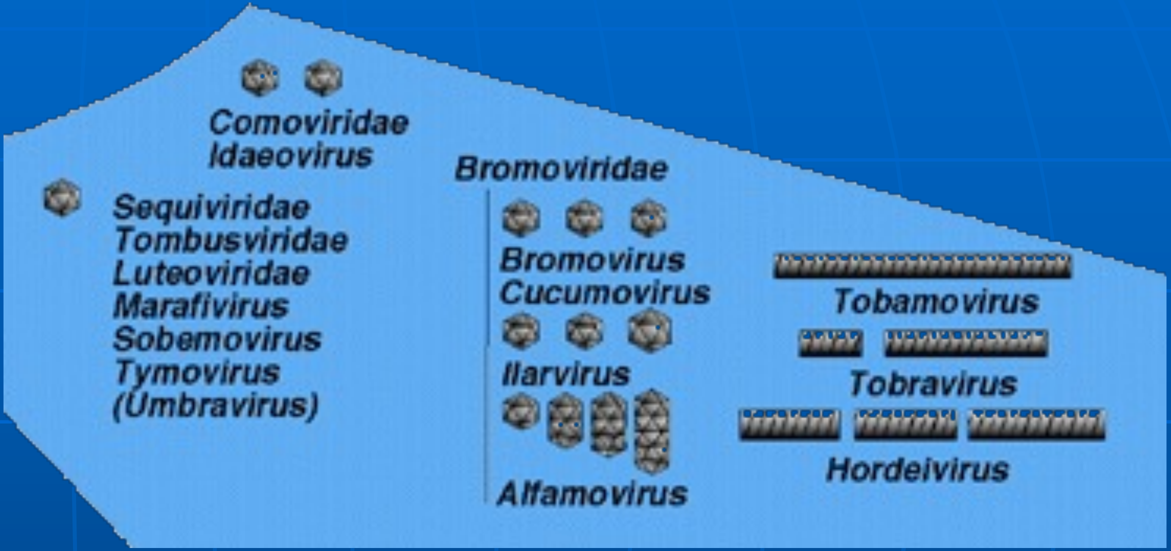
- 植物細胞僅在形成層與頂芽分生組織等極少數組織進行分裂與分化。成熟的植物細胞已經不再進入細胞週期的S-phase (DNA合成期)。(病毒複製與基因表現方式)
- 植物整體可視為互相連通的單一細胞。(病毒移動與基因體包被方式: “汽車”與”火車”的差異)
- 植物細胞具有細胞壁，葉表面具有臘質，病毒入侵寄主途徑主要依賴傷口。(病毒入侵方式)
- 植物經常可經由扦插、嫁接、分芽等方式進行營養繁殖。(病毒傳播方式)
- 植物沒有動物的免疫系統。(病毒累積量與檢測方式)

動物病毒與植物病毒基因體包裝方式不同



https://en.wikipedia.org/wiki/File:Viruses-12-00504-g001.webp

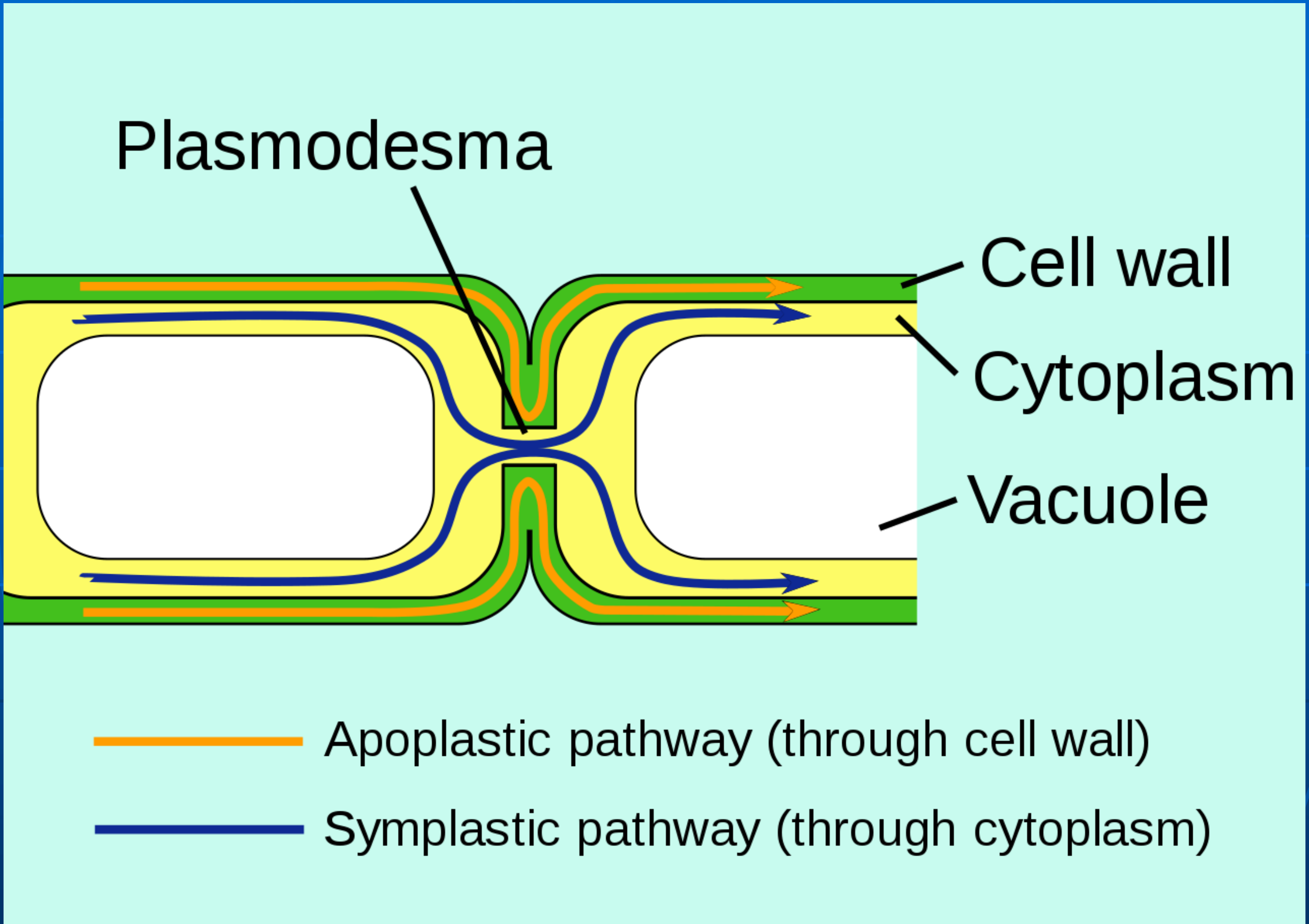
動物病毒



https://www.molmed.nl/uploads/abstracts/1599/Marion%20Koopmans.pdf

植物病毒

Plasmodesmata原生質聯絡絲 示意圖



https://zh.wikipedia.org/wiki/%E5%8E%9F%E7%94%9F%E8%B3%AA%E7%B5%B2#/media/File:Apoplast_and_symplast_pathways.svg

- 植物病毒與動物病毒之異同
- 植物病毒病徵及其經濟重要性
- 植物病毒的種類
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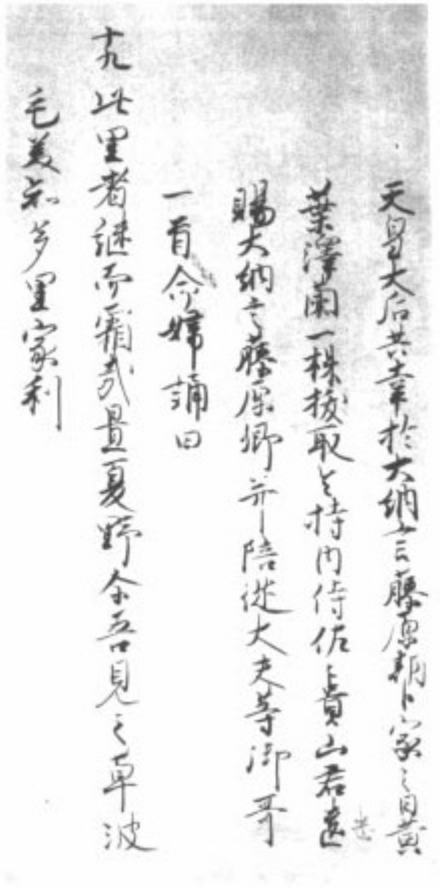
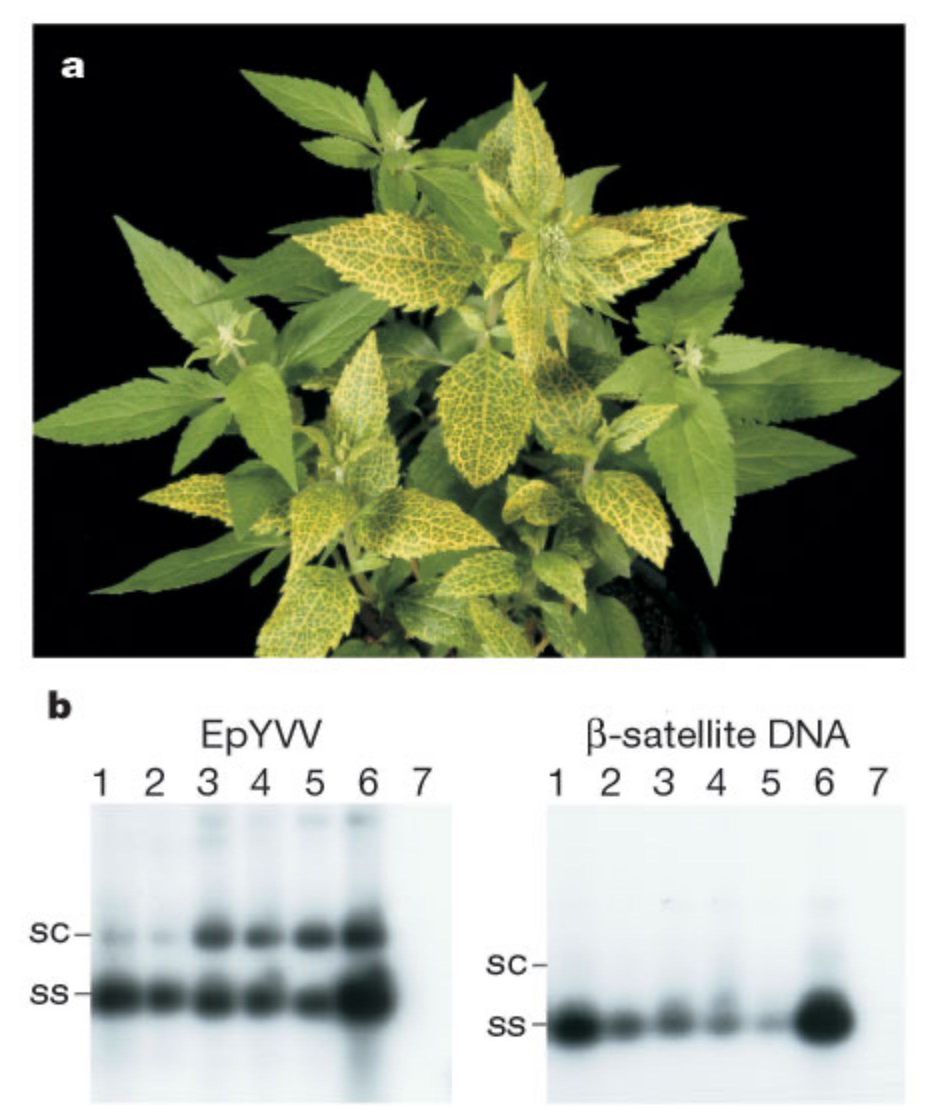
- 植物病毒在生物科技上的應用

最早文字記載的植物病毒病害：

752 AD



天皇大后共幸於大納言藤原朝臣家之日黄
葉澤蘭一株拔取令持内侍佐々貴山君遣
賜大納言藤原卿并陪従大夫等御歌
一首　命婦誦曰
此里者継而霜哉置夏野尓吾見之草波
毛美知多里家利



One poem, attributed to the Empress Koken (孝謙天皇大后) and written in the summer of 752 AD (Fig. 1), describes the autumnal appearance of eupatorium plants in summer and is reputedly the earliest written record of the symptoms of a plant virus disease.

Aetiology: The earliest recorded plant virus disease
Saunders, K., Bedford, I. D., Yahara, T., and Stanley, J. Nature 422, 831 (24 April 2003)

病毒造成之果實表面變形與皺縮

台灣洋香瓜種植面積

動態查詢

日期：☑ 年資料 97年 ▼ ~ 107年 ▼

日期

蔬菜別

蔬菜收穫面積（公頃）

項目別	洋香瓜
97年	3,886.29
98年	3,066.96
99年	3,096.65
100年	3,184.45
101年	2,766.68
102年	3,066.52
103年	2,998.54
104年	2,948.40
105年	2,796.90
106年	2,522.94
107年	2,518.58

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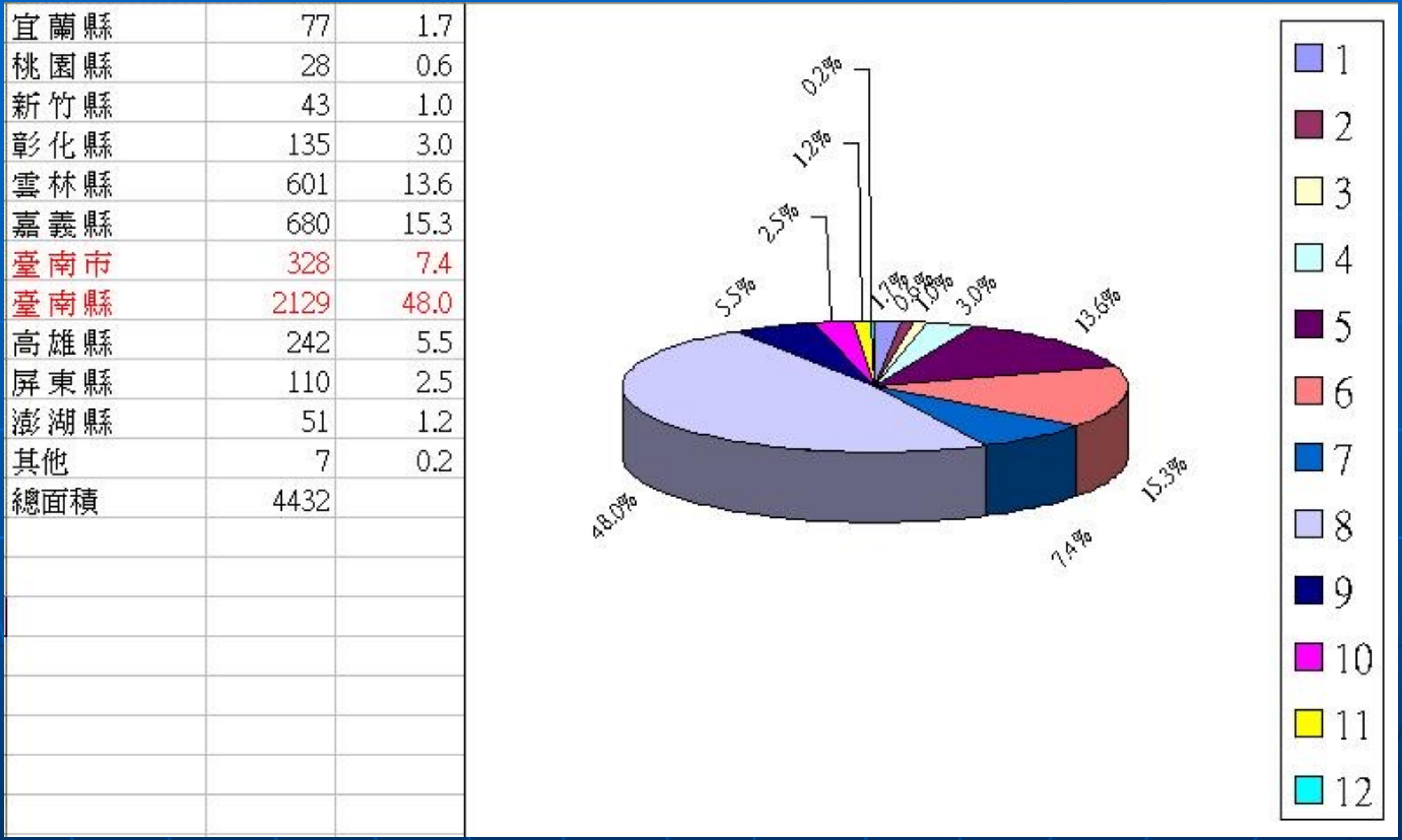
台灣洋香瓜種植面積



宜蘭縣	77	1.7
桃園縣	28	0.6
新竹縣	43	1.0
彰化縣	135	3.0
雲林縣	601	13.6
嘉義縣	680	15.3
臺南市	328	7.4
臺南縣	2129	48.0
高雄縣	242	5.5
屏東縣	110	2.5
澎湖縣	51	1.2
其他	7	0.2
總面積	4432	

- 佳里區農會栽培面積約70公頃，年供貨約1,200公噸，產值約3,600萬元
- 台灣地區近十年栽種面積約2500 ~ 3000公頃，產值約3,600萬元 X 30 ~ 40倍

台南地區秋冬季適合洋香瓜生長，2009年秋作自9月開始陸續種植，栽培面積約355公頃，其中舊制台南市約180公頃、舊制台南縣約175公頃，今年多數產區受南瓜捲葉病毒、南瓜黃化嵌紋病毒及黑點根腐病危害，台南市受害情形較嚴重，罹病率約50-90％，而台南縣罹病率約20%-50%。（農糧署作物生產組蔬菜花卉科）

- 植物病毒與動物病毒之異同
- 植物病毒病徵及其經濟重要性
- 植物病毒的種類
- 植物病毒的基本結構
- 植物病毒的移動與傳播
- 植物病毒的基本防治策略
- 植物病毒在生物科技上的應用

國際病毒分類委員會

International Committee on Taxonomy of Viruses
ICTV

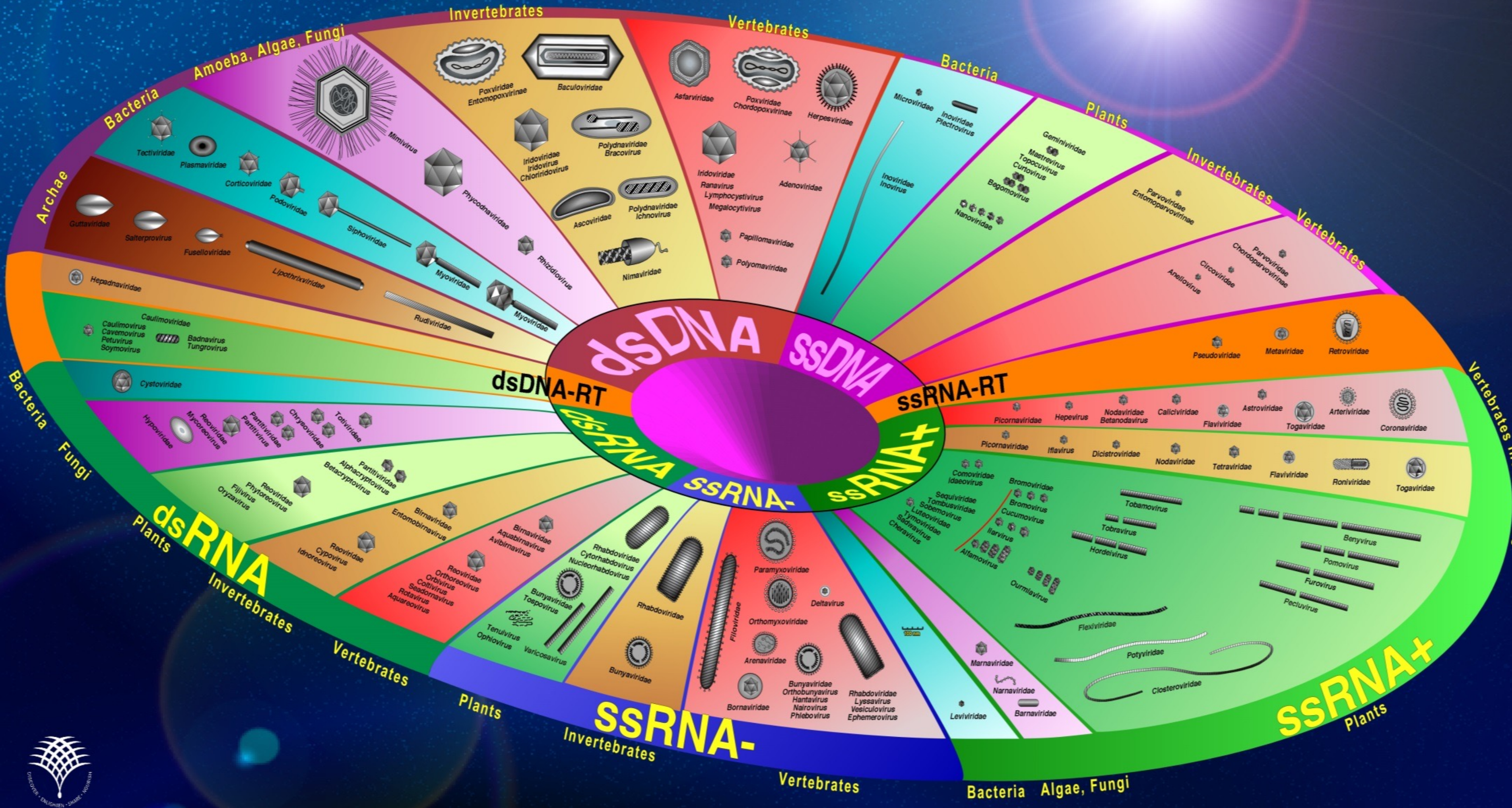
NEW! 2018b Taxonomy Release: MSL#34

The ICTV Report

Online (10th) Report on Virus Taxonomy: The Classification and Nomenclature of Viruses

Virosphere 2005

病毒圈

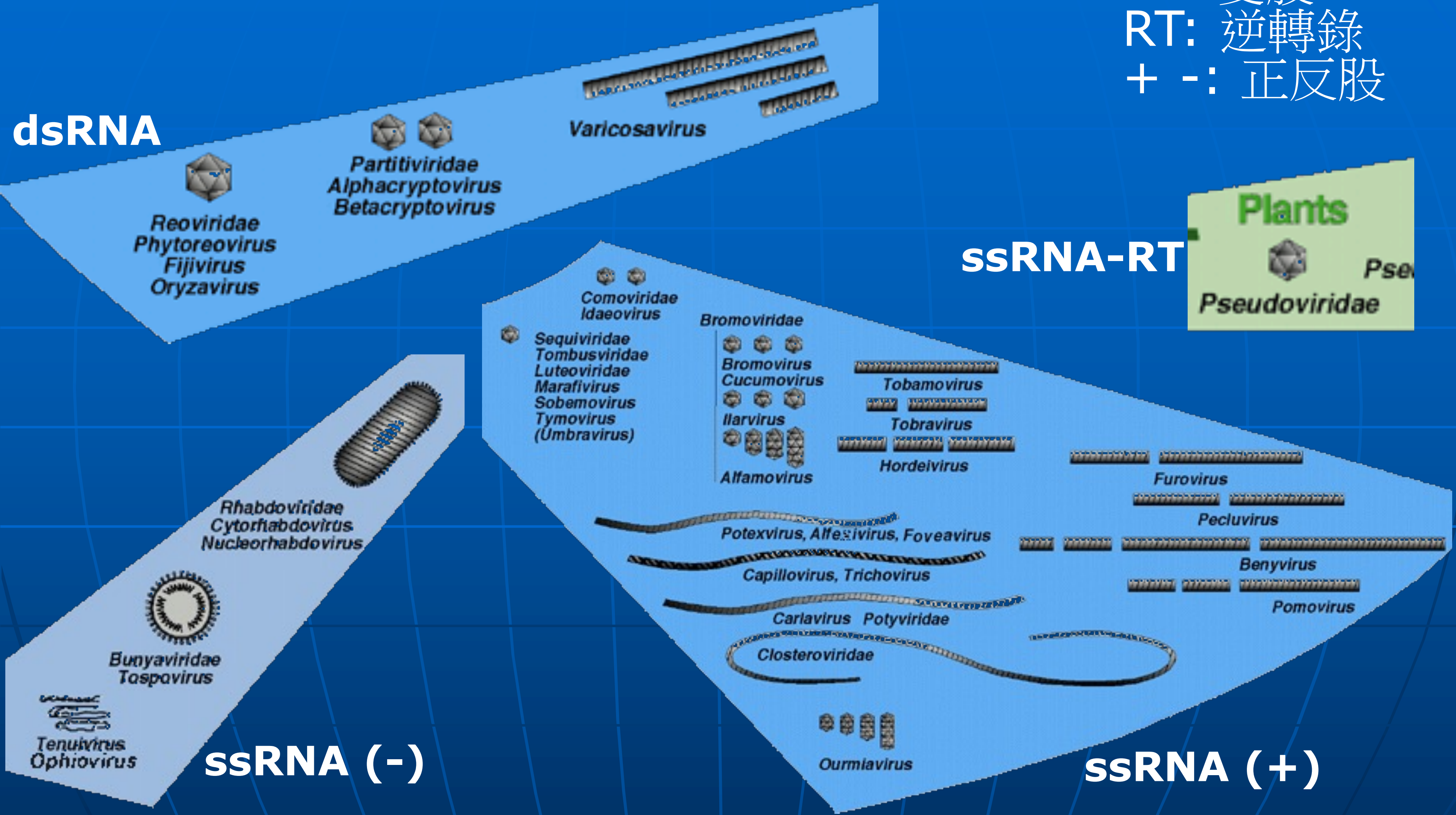


ONALD DANFORTH
ANT SCIENCE CENTER

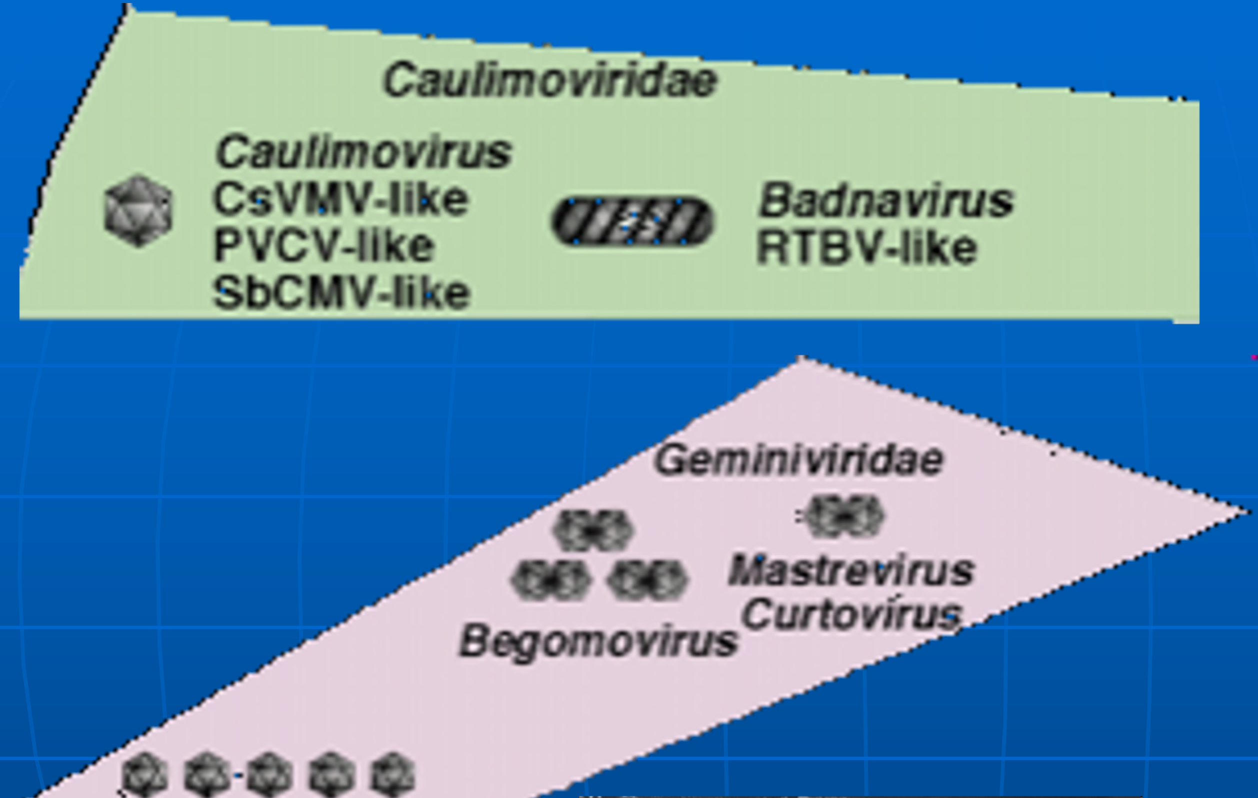
International Committee on Taxonomy of Viruses

植物RNA病毒的種類

ss: 單股
ds: 雙股
RT: 逆轉錄
+ -: 正反股

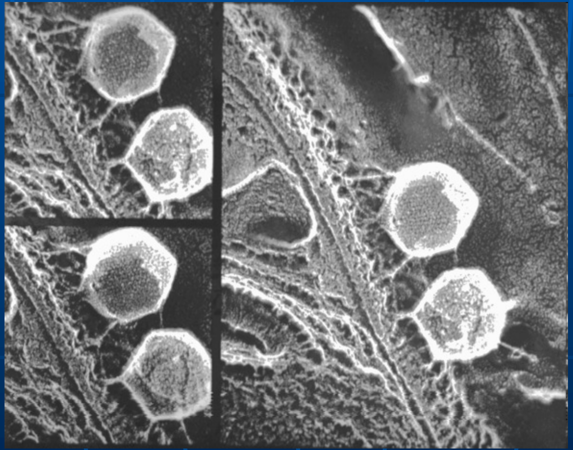


植物 DNA 病毒



dsDNA RT

sscDNA

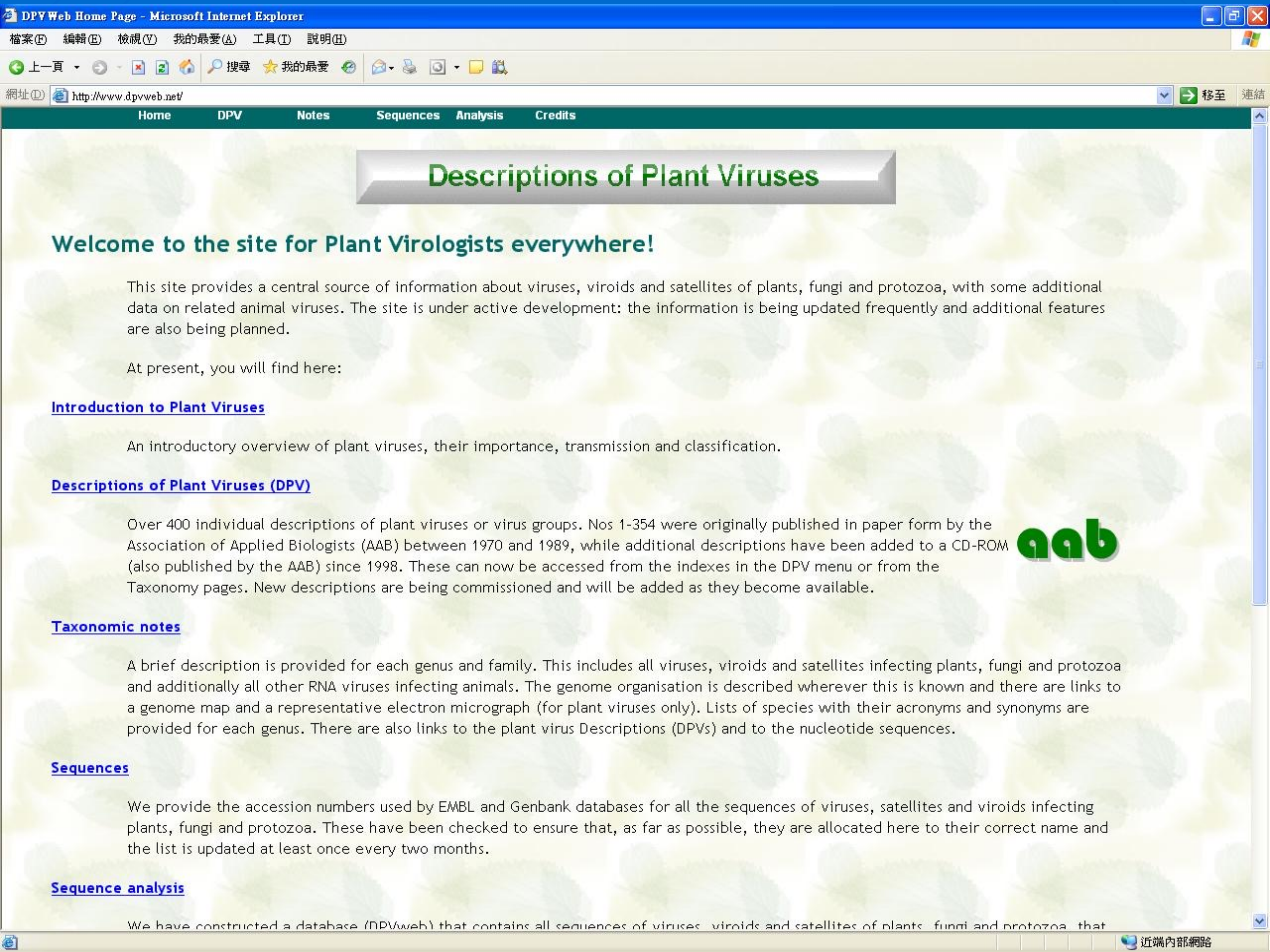


dsDNA

(Phycodnaviridae)

130-200 nm

http://microbewiki.kenyon.edu/index.php/File:Phycodna.gif



Descriptions of Plant Viruses

Welcome to the site for Plant Virologists everywhere!

This site provides a central source of information about viruses, viroids and satellites of plants, fungi and protozoa, with some additional data on related animal viruses. The site is under active development: the information is being updated frequently and additional features are also being planned.

At present, you will find here:

Introduction to Plant Viruses

An introductory overview of plant viruses, their importance, transmission and classification.

Descriptions of Plant Viruses (DPV)

Over 400 individual descriptions of plant viruses or virus groups. Nos 1-354 were originally published in paper form by the Association of Applied Biologists (AAB) between 1970 and 1989, while additional descriptions have been added to a CD-ROM (also published by the AAB) since 1998. These can now be accessed from the indexes in the DPV menu or from the Taxonomy pages. New descriptions are being commissioned and will be added as they become available.

Taxonomic notes

A brief description is provided for each genus and family. This includes all viruses, viroids and satellites infecting plants, fungi and protozoa and additionally all other RNA viruses infecting animals. The genome organisation is described wherever this is known and there are links to a genome map and a representative electron micrograph (for plant viruses only). Lists of species with their acronyms and synonyms are provided for each genus. There are also links to the plant virus Descriptions (DPVs) and to the nucleotide sequences.

Sequences

We provide the accession numbers used by EMBL and Genbank databases for all the sequences of viruses, satellites and viroids infecting plants, fungi and protozoa. These have been checked to ensure that, as far as possible, they are allocated here to their correct name and the list is updated at least once every two months.

Sequence analysis

We have constructed a database (DPVweb) that contains all sequences of viruses, viroids and satellites of plants, fungi and protozoa, that

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- 植物病毒的種類
- **植物病毒的基本結構**
- 植物病毒的移動與傳播
- 植物病毒的基本防治策略
- 植物病毒在生物科技上的應用

1. 螺旋型對稱

決定螺旋形狀的兩個因子:

1. amplitude (diameter)
&
2. pitch (the distance covered by each complete turn of the helix)

Axial rise/ subunit = 0.14 nm
2.3 nm / 16.33333 = 0.14 nm

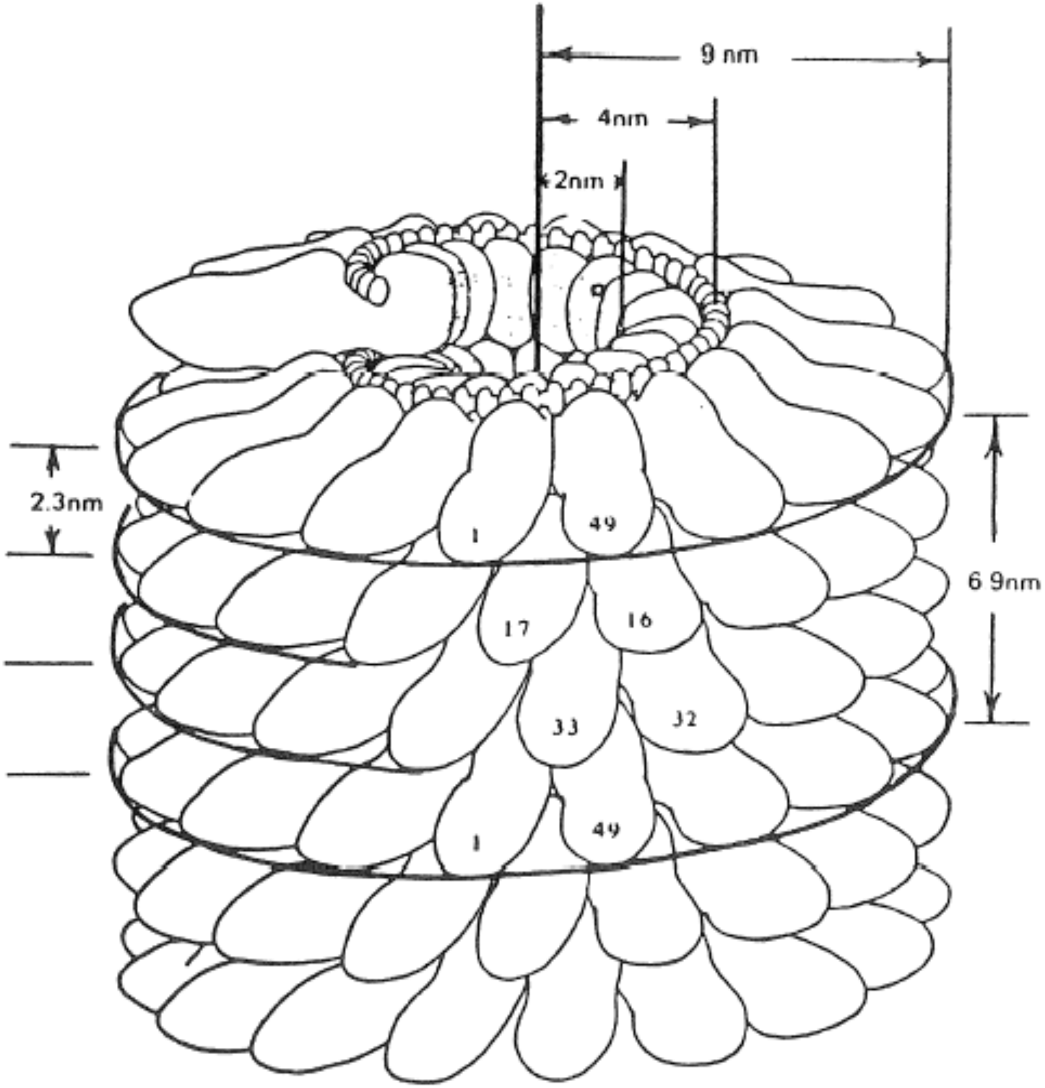
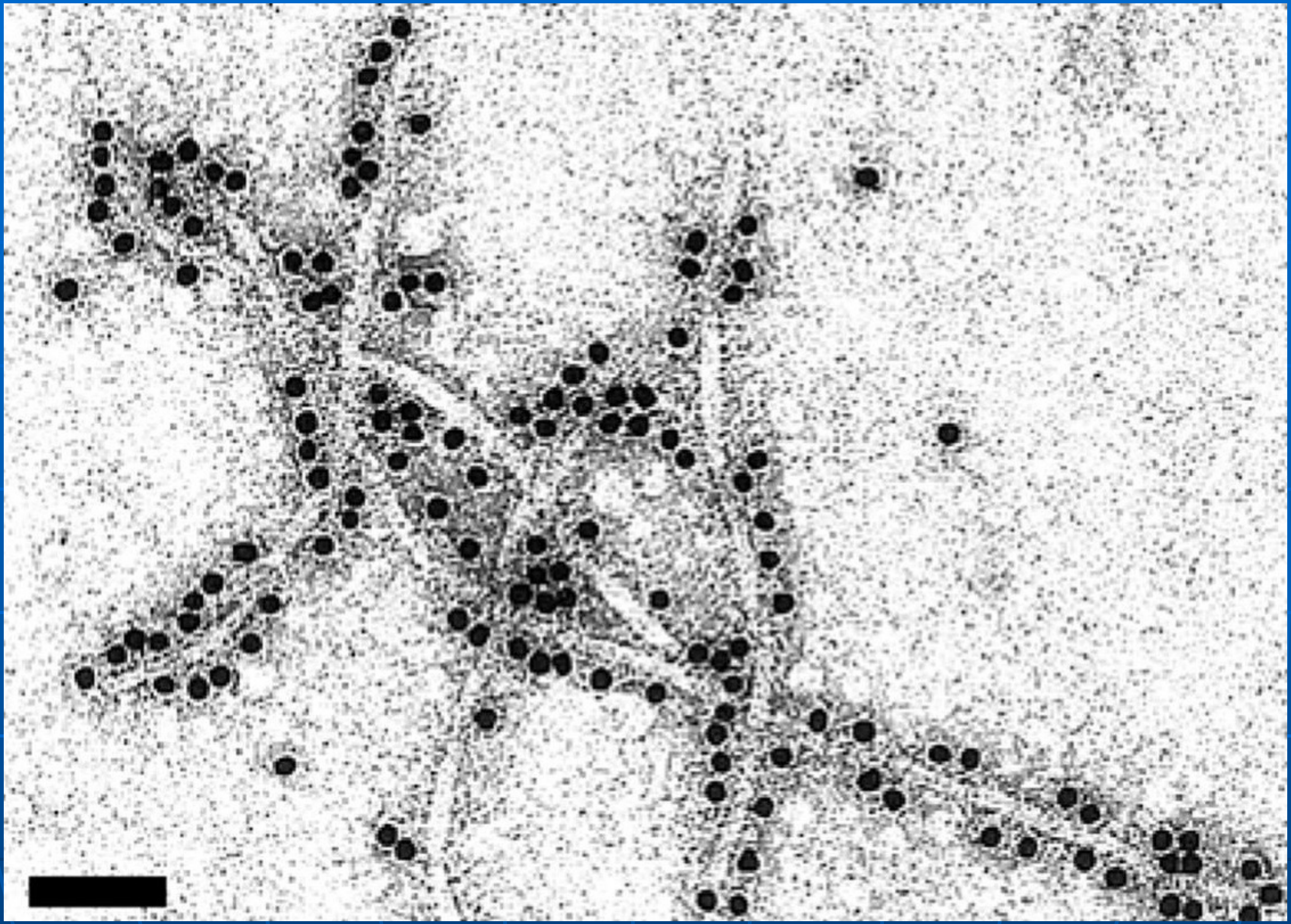


Fig. 1.2 Schematic representation of tobacco mosaic virus structure derived largely from x-ray diffraction studies (modified from Ref. [18]).



長絲狀：竹嵌紋病毒

https://www.frontiersin.org/articles/10.3389/fmicb.2017.00788/full

2. 球型對稱(正二十面體)

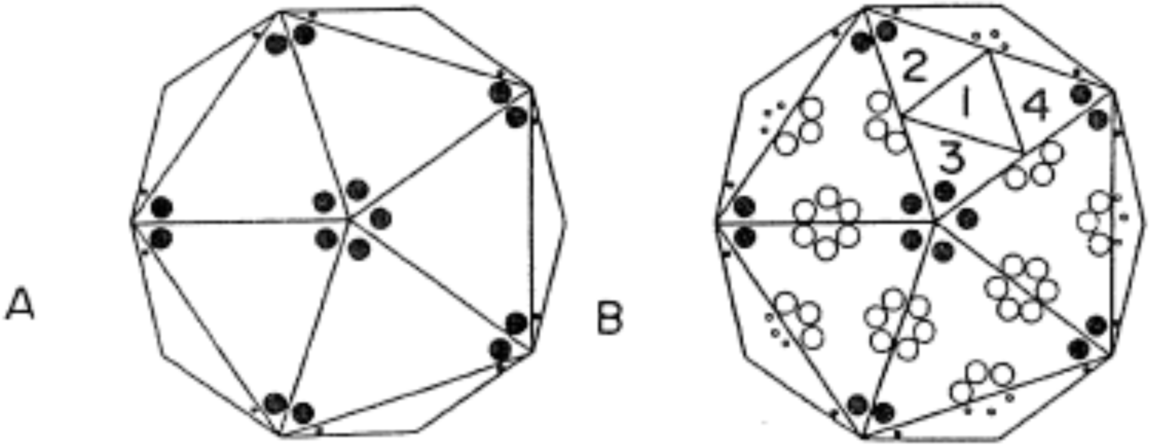
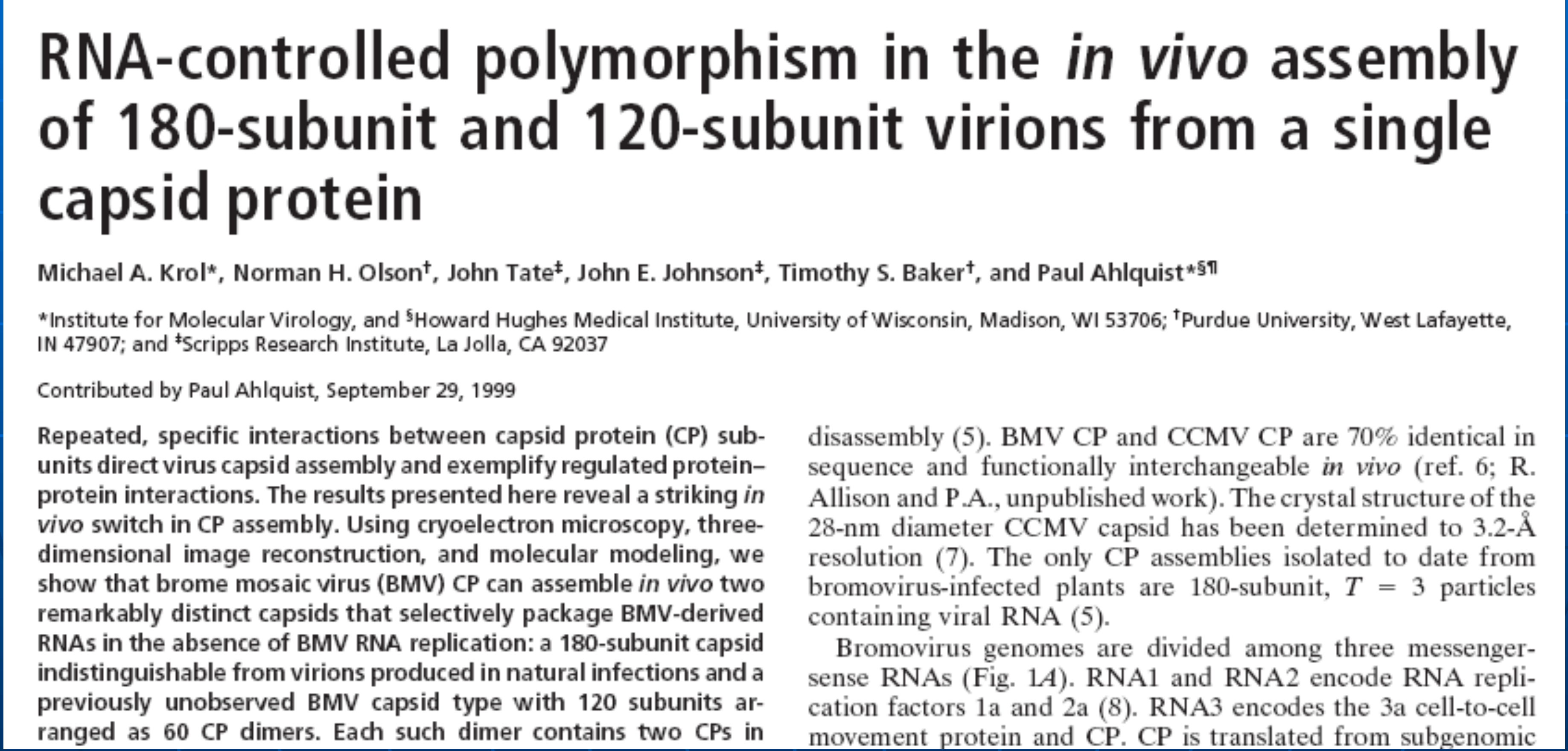


Fig. 3.6. Arrangement of 60n identical sub-units on the surface of an icosahedron. (A) n = 1 and the 60 sub-units are distributed such that there is one sub-unit at the vertices of each triangular facet. Note that each sub-unit has the same arrangement of neighbours and so all the sub-units are equivalently related. (B) n = 4. Each triangular facet is divided into four smaller, but identical equilateral triangles and a sub-unit is again located at each vertex.

Quasi-equivalence, or Quasi-symmetry

Pentamers: filled circles (curved)
Hexamers: open circles (flat)

Virion formation: RNA-controlled polymorphism
由病毒基因體RNA所控制的型態多型性



RNA-controlled polymorphism in the *in vivo* assembly of 180-subunit and 120-subunit virions from a single capsid protein

Michael A. Krol*, Norman H. Olson†, John Tate‡, John E. Johnson‡, Timothy S. Baker†, and Paul Ahlquist*§¶

*Institute for Molecular Virology, and §Howard Hughes Medical Institute, University of Wisconsin, Madison, WI 53706; †Purdue University, West Lafayette, IN 47907; and ‡Scripps Research Institute, La Jolla, CA 92037

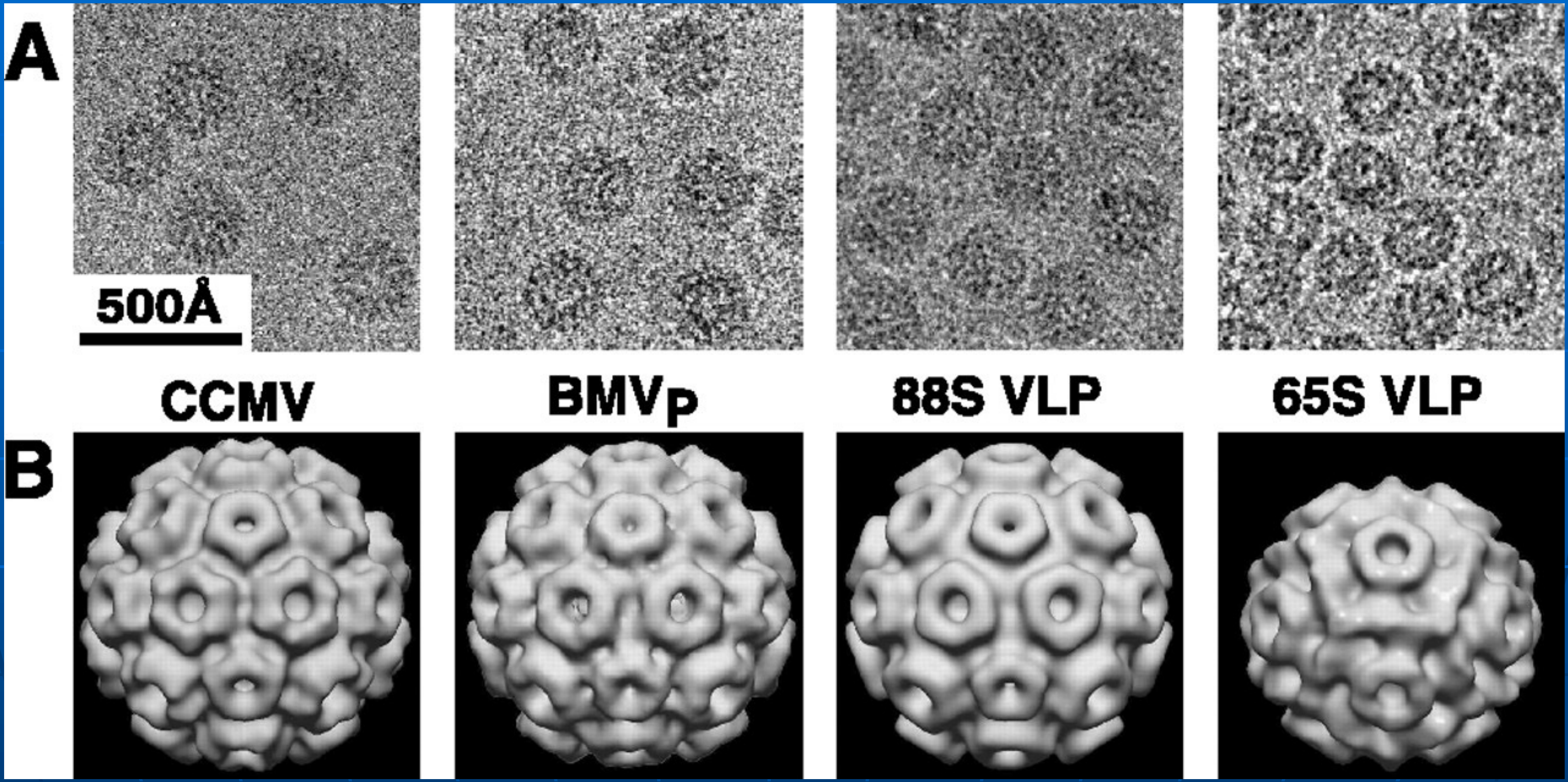
Contributed by Paul Ahlquist, September 29, 1999

Repeated, specific interactions between capsid protein (CP) subunits direct virus capsid assembly and exemplify regulated protein–protein interactions. The results presented here reveal a striking *in vivo* switch in CP assembly. Using cryoelectron microscopy, three-dimensional image reconstruction, and molecular modeling, we show that brome mosaic virus (BMV) CP can assemble *in vivo* two remarkably distinct capsids that selectively package BMV-derived RNAs in the absence of BMV RNA replication: a 180-subunit capsid indistinguishable from virions produced in natural infections and a previously unobserved BMV capsid type with 120 subunits arranged as 60 CP dimers. Each such dimer contains two CPs in

disassembly (5). BMV CP and CCMV CP are 70% identical in sequence and functionally interchangeable *in vivo* (ref. 6; R. Allison and P.A., unpublished work). The crystal structure of the 28-nm diameter CCMV capsid has been determined to 3.2-Å resolution (7). The only CP assemblies isolated to date from bromovirus-infected plants are 180-subunit, $T = 3$ particles containing viral RNA (5).

Bromovirus genomes are divided among three messenger-sense RNAs (Fig. 1*A*). RNA1 and RNA2 encode RNA replication factors 1a and 2a (8). RNA3 encodes the 3a cell-to-cell movement protein and CP. CP is translated from subgenomic

RNA-controlled polymorphism



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植物病毒在植物體中的移動 (一)
Movement and Transmission of Viruses in Plants

-- 病毒在植物中的移動可分為兩種模式，以維管束鞘作為分界:

1. Cell-to-cell movement (細胞到細胞移動)
2. Long distance (systemic) movement. (系統性移動:全株植物)

-- Most plant viruses encode at least one protein, the so-called movement protein (病毒編碼移動蛋白), for movement in plants.

--Additional factors, required for efficient movement, are provided by the host plants(其他因子由植物提供)

-- The direction of virus movement usually follows the direction of nutrients adsorbed or synthesized by the host plants, i.e., from SOURCE leaves to SINK leaves.(病毒移動的方向與營養物質移動的方向一致)

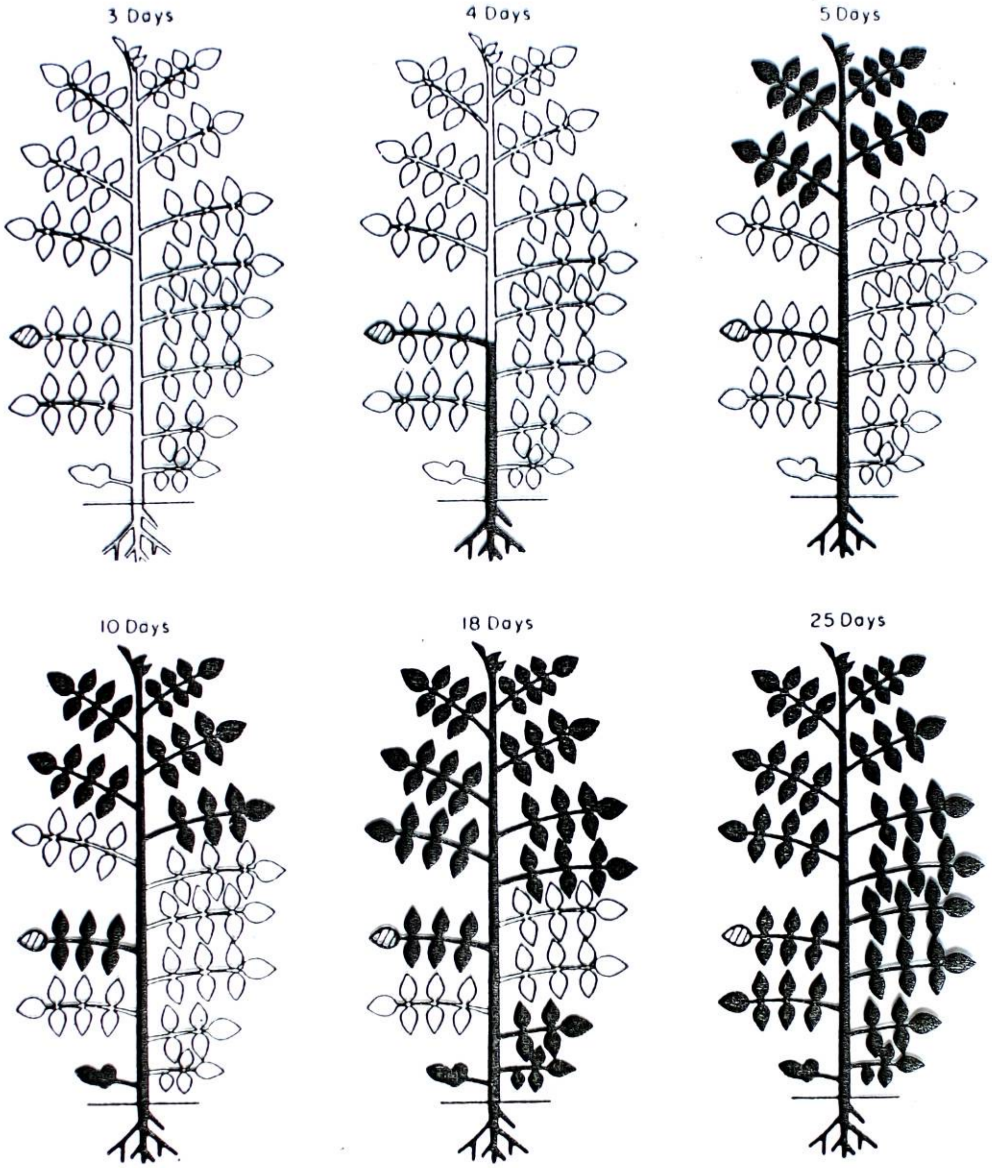


Figure 10.7 Diagram showing the spread of TMV through a medium young tomato plant. The inoculated leaf is shaded, and systematically infected tissues are shown in black. (From Samuel, 1934.)

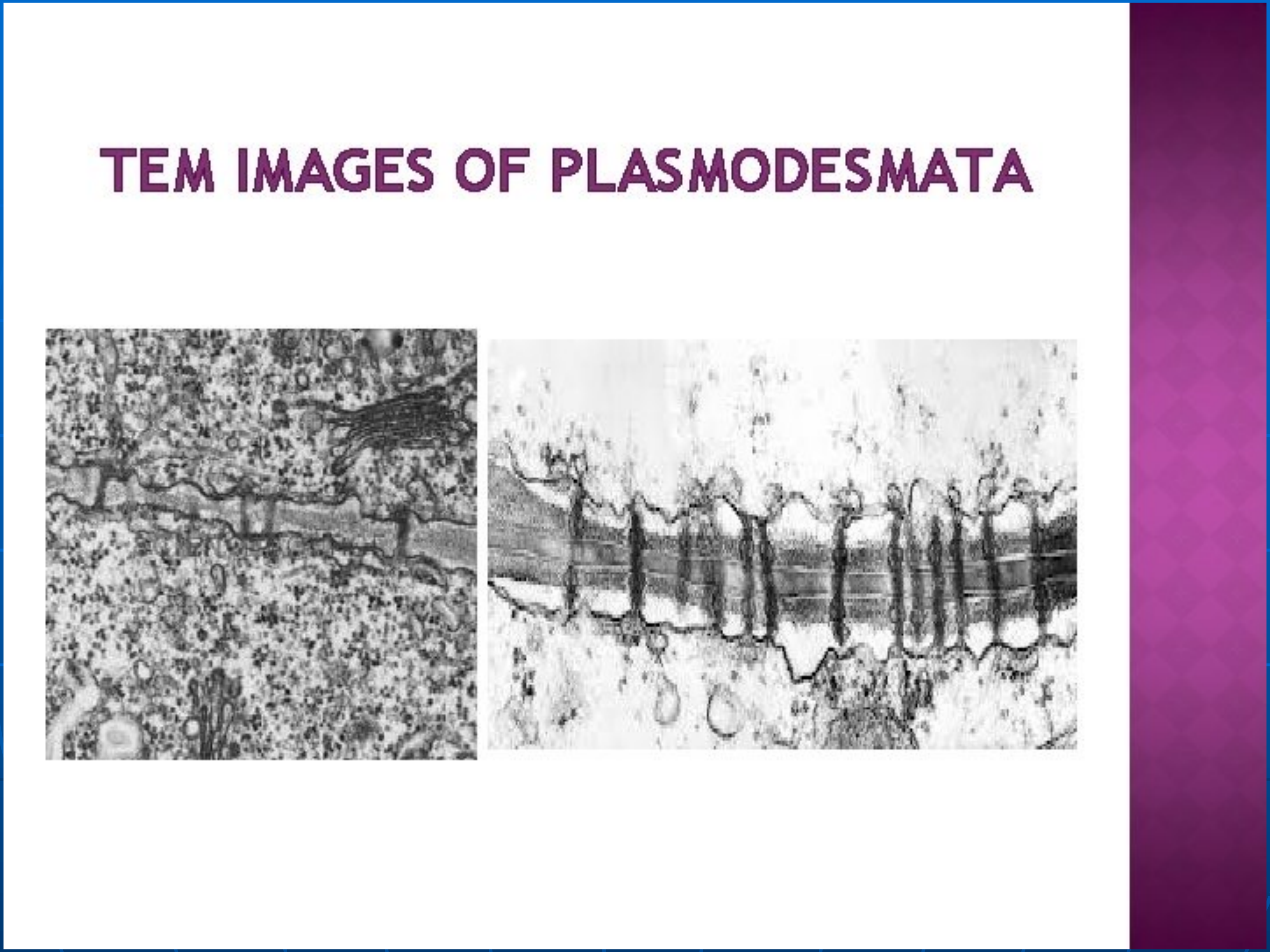
植物病毒在植物體中的移動 (二)

-- 相鄰的植物細胞之間有原生質聯絡絲 (Plasmodesmata, PD)做為溝通的管道

-- 一株植物可以被視為一個大細胞

-- 原生質聯絡絲的重要特性:
(a) 在細胞分裂時形成(初級)或穿透已經存在的細胞壁(次級)
(b) 在結構上發生修飾或在發育過程中進行封閉
(c) 可依據所需要溝通的細胞群之大小而進行移除或重置等調整

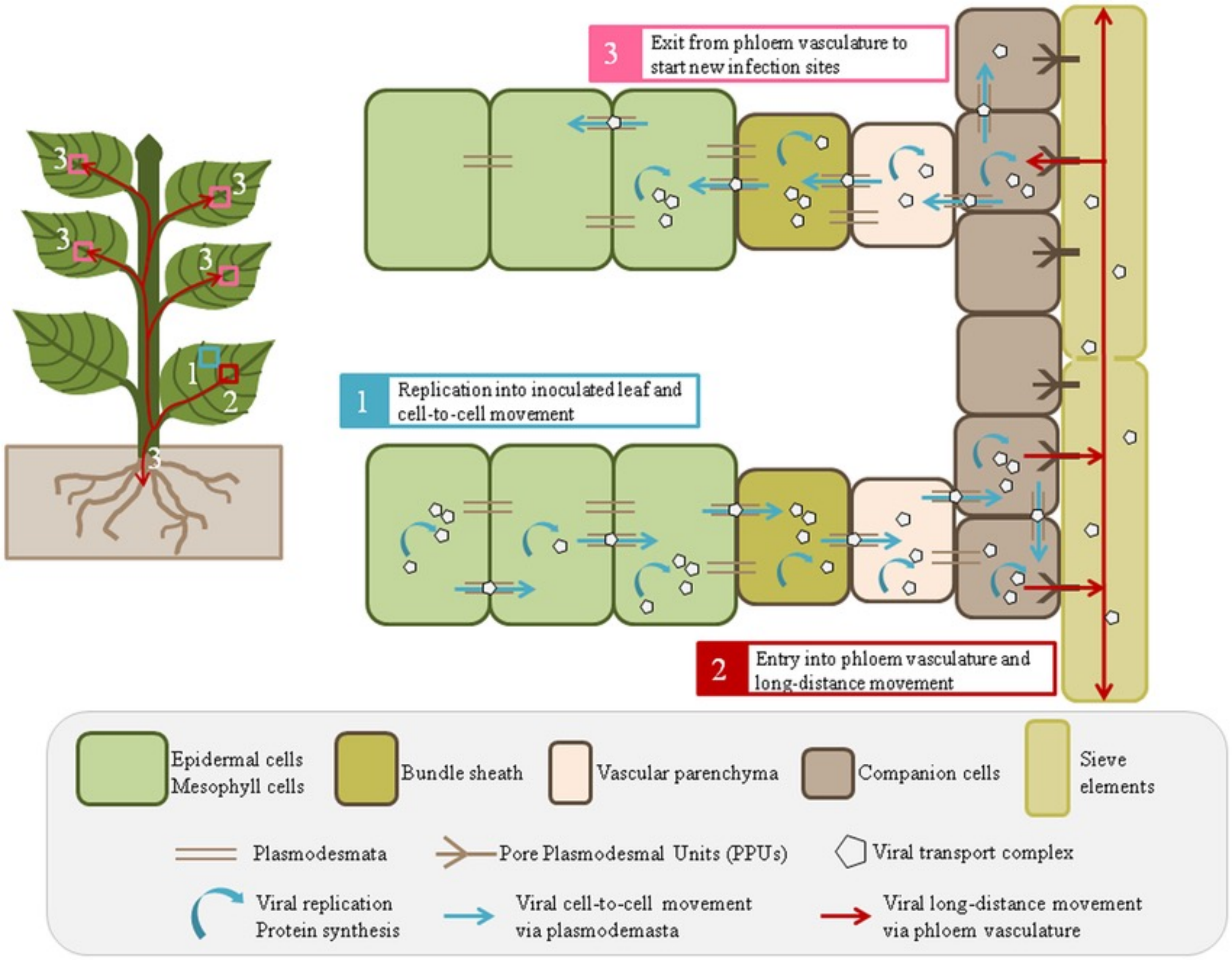
-- 經由初級與次級原生質聯絡絲構成細胞間的通路，主要功能為養分與訊息的流通



TEM IMAGES OF PLASMODESMATA

https://slidetodoc.com/extra-cellular-components-and-connections-extra-cellular-components/

Plant virus movment 植物病毒的移動



Hipper, C.; Brault, V.; Ziegler-Graff, V.; Revers, F., Viral and Cellular Factors Involved in Phloem Transport of Plant Viruses. *Frontiers in plant science* ***2013, 4, 154.***

Classification of Cell-to-Cell Movement Mechanisms of Plant Viruses

I. Viruses that move as virions: (以完整病毒顆粒移動)

A. Comovirus-like mechanism:

-- *Comovirus, Nepovirus, Badnavirus, Tospovirus*

-- Formation of hollow tubules that extend between cells and serve as conduits for transport of virions (形成中空小管)

B. Closterovirus-like mechanism:

-- *Closterovirus* (*Beet yellows virus, Citrus tristeza virus*)

-- Hsp70 homolog MP (Hsp70h), the major capsid protein forms the virion; while the minor capsid protein and Hsp70h form a tail assembly that propels the virion particle into the plasmodesmata. (形成尾部馬達)

-- Hsp70h has ATPase activity

II. Viruses that do not need virion formation for cell-to-cell movement: （不需形成完整病毒顆粒即可移動,但需要”移動蛋白”）

A. TMV 30K-like protein dependent mechanism: TMV, BMV, CMV

B. Triple-Gene-Block proteins (TGBps) dependent mechanism:

1. Hordeivirus-like:

-- *Hordeivirus, Pomovirus, Pecluvirus*

-- CP not required

2. Potexvirus-like:

-- *Potexvirus, Carlavirus, Foveavirus, Allexivirus*

-- CP required

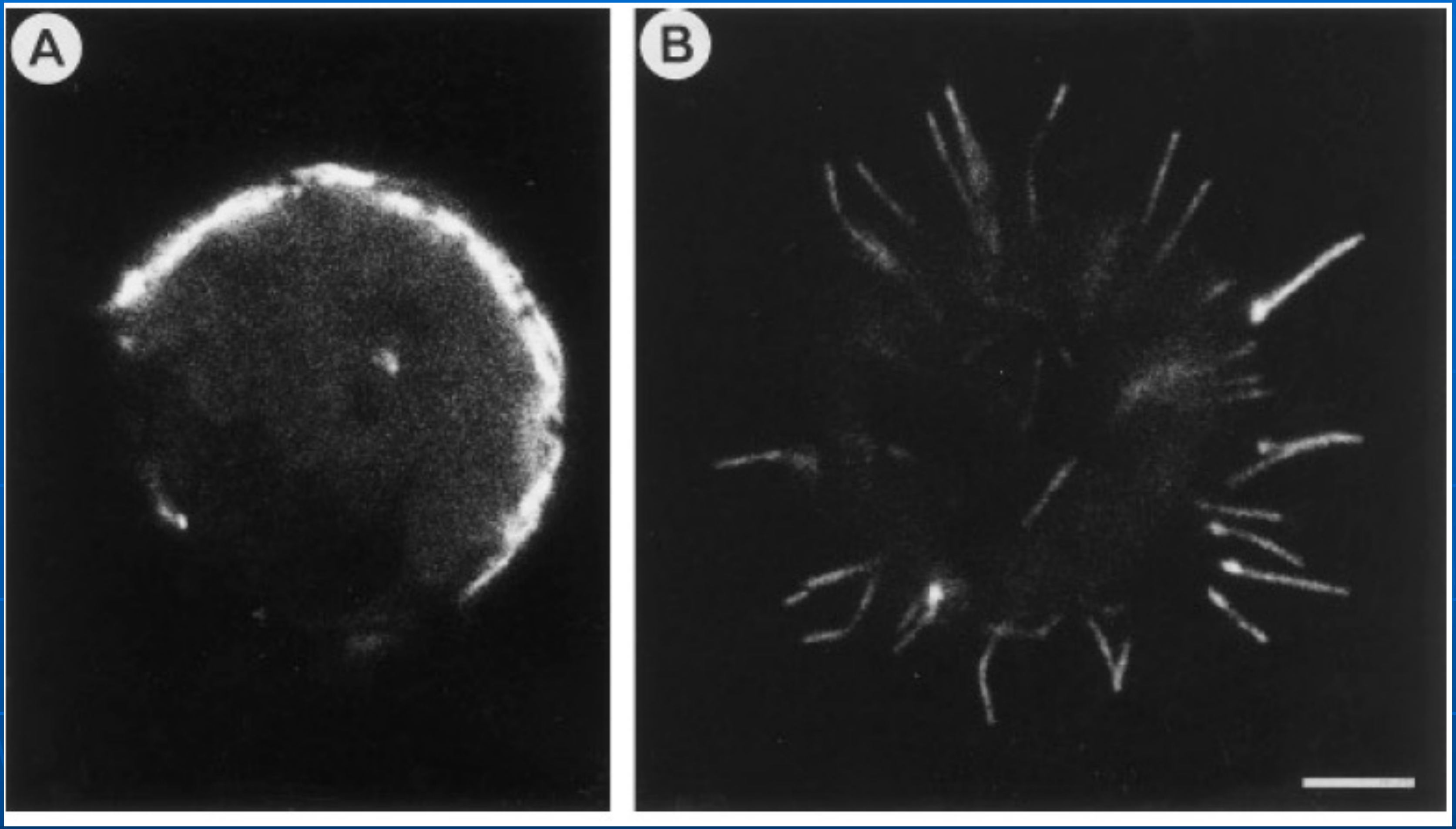
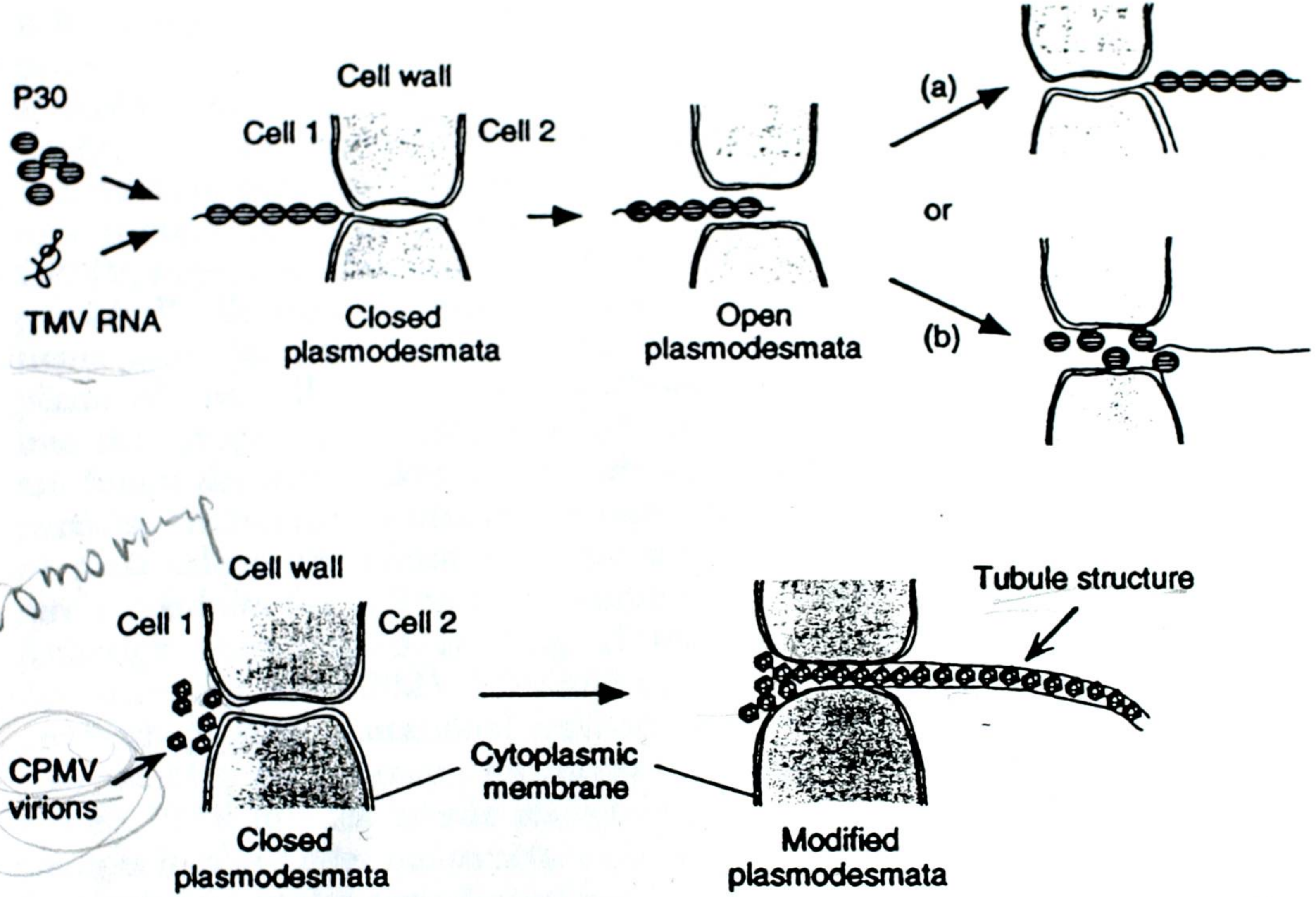


FIG. 1. Immunofluorescent staining of the NSm protein in protoplasts isolated from systemically nfected leaves of *Nicotiana rustica*. (A) Localization of NSm in the cytoplasm of N. rustica protoplasts at 6 days p.i. (B) NSm-containing tubular structures emerging from the protoplast surface at 7 days p.i. Scale bar represents 10 mm. (Storms et al., 1995)



omovirus

Fig. 2. Two mechanisms by which virus-encoded movement proteins mediate cell-to-cell spread of viruses. The TMV-like mechanism **(above)** involves transient modification of the plasmodesmal SEL to allow transport of either (a) the ribonucleoprotein complex or (b) free RNA to the adjacent cell. The CPMV-like mechanism **(below)** involves formation of a tubule through which virions are transported to the adjacent cell; this tubule appears to modify plasmodesmal structure permanently.

植物病毒的蟲媒傳播

Transmission of viruses by arthropods:

Classification by virus-insect interactions:

1. Non-persistent (非永續性)
2. Semi-persistent (半永續性)
3. Persistent (永續性): propagative or non-propagative; circulative or non-circulative

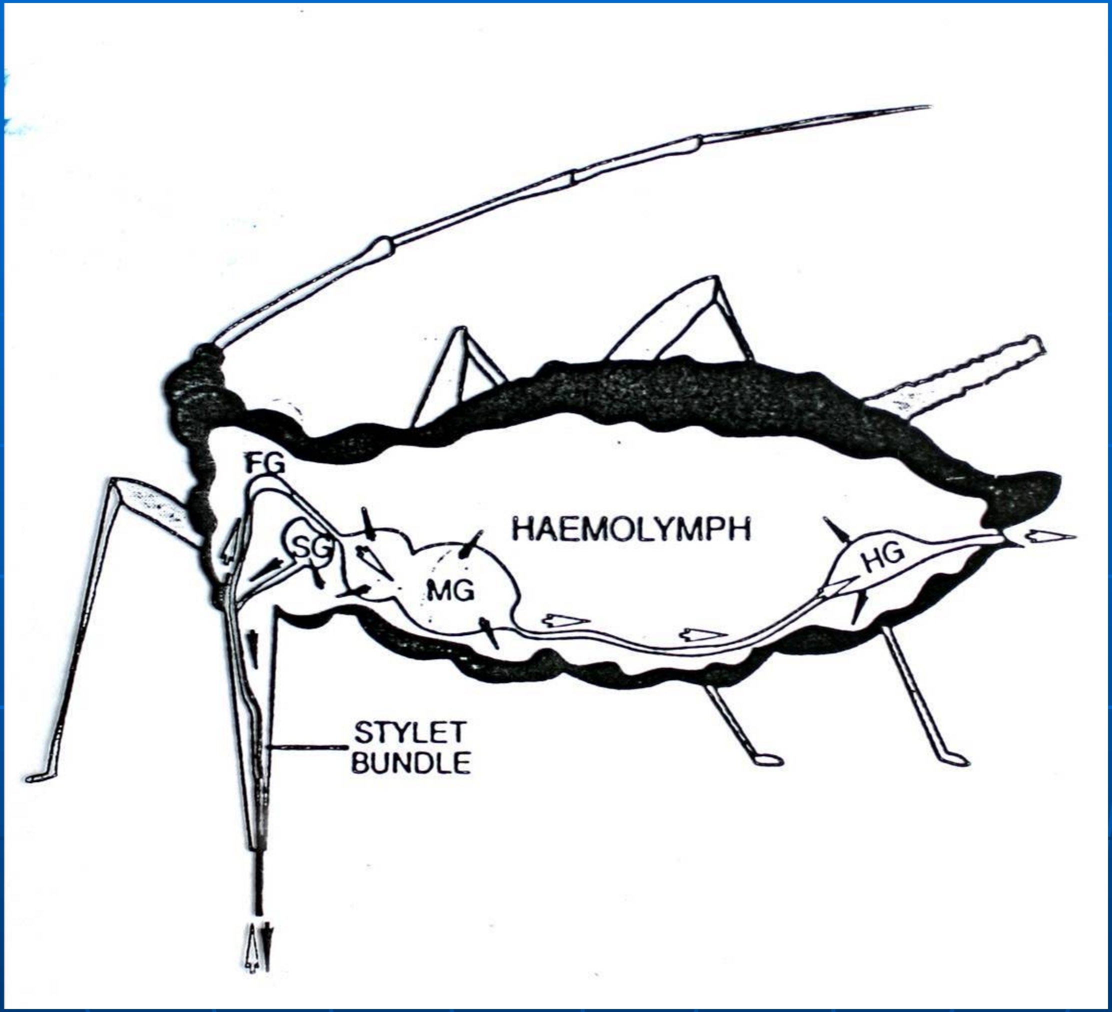
Classification by insect mouth parts:

1. Sucking and piercing stylets (刺吸式口器)
2. Biting mouth parts (咀嚼式口器)

Insects with sucking and piercing stylets:
具有刺吸式口針的昆蟲:

蚜蟲 (Aphids)
粉蝨 (Whiteflies)
浮塵子 (Leafhoppers)
薊馬 (Thrips)

蚜蟲 (Aphid)



Viruses transmitted by beetles: 咀嚼式口器:甲蟲

- *Tymovirus*
- *Comovirus*
- *Bromovirus*
- *Sobemovirus*

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植物病毒的基本防治策略

- Viral diseases of plants are commonly controlled by the following strategies：
  - Chemical control for insect vectors (藥劑控制媒介昆蟲)
  - Virus- free seeds or seedlings (無病毒種苗)
  - Quarantine (海關檢疫、拒疫)
  - Virus resistant cultivars (抗病品種)： natural or transgenic resources
- The use of resistant cultivars presents one of the most effective and economical approaches in viral disease management.
  (抗病品種是最有效與最經濟的病毒病害防治方法之ㄧ)

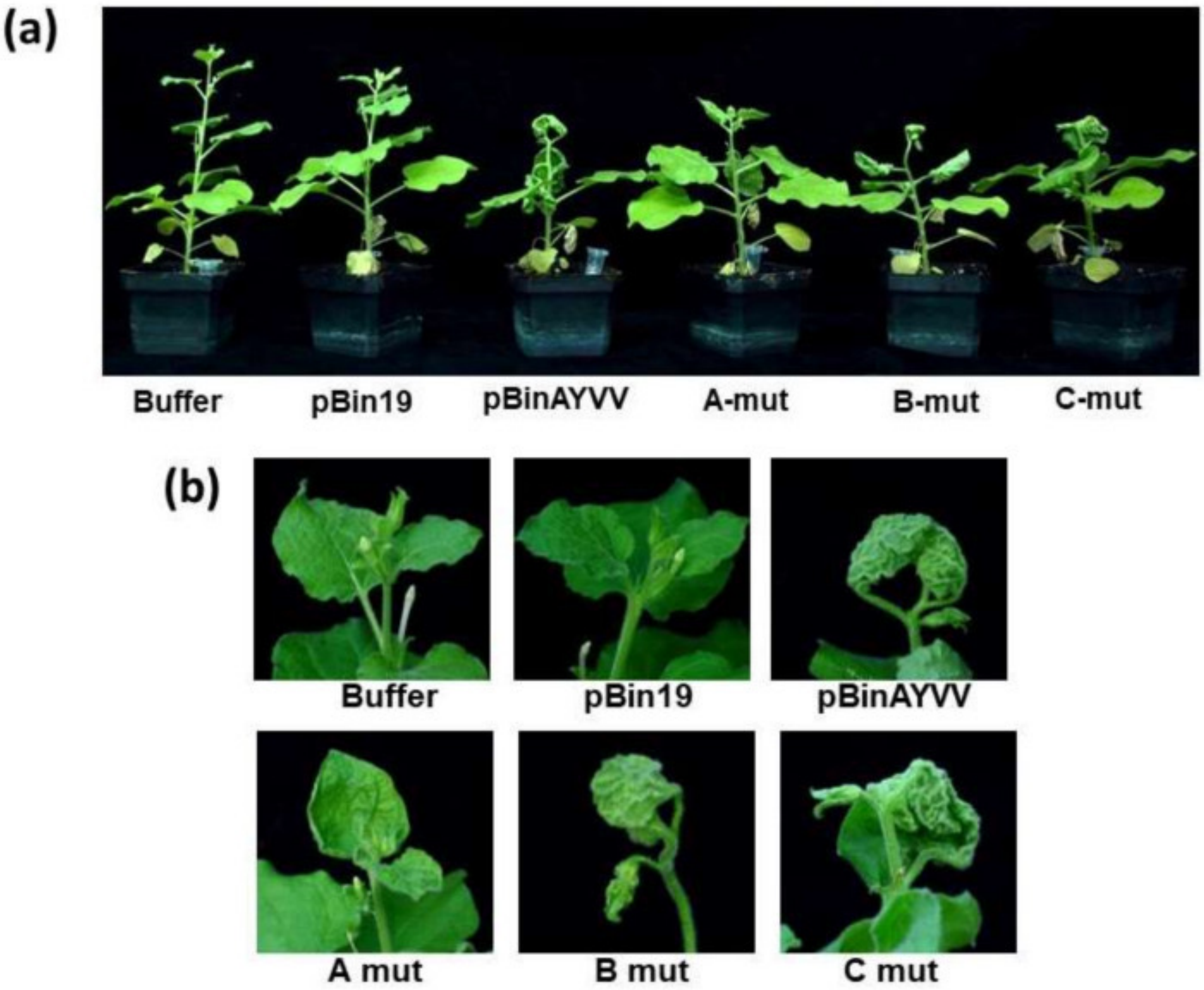
- In TARI, Dr. Deng、Liao and Lin have selected one variety TVI4204 of wax gourd brought from India which showed immunity to PRSV-W and ZYMV. **(農試所鄧博士團隊所測試的抗病毒冬瓜品種TVI4204)**

Resistant TVI 4204 **Susceptible TVI 11577**

(Deng *et al*., unpublished)

植物病毒的”治療”策略: 緩解病徵

Dai et al., 2022



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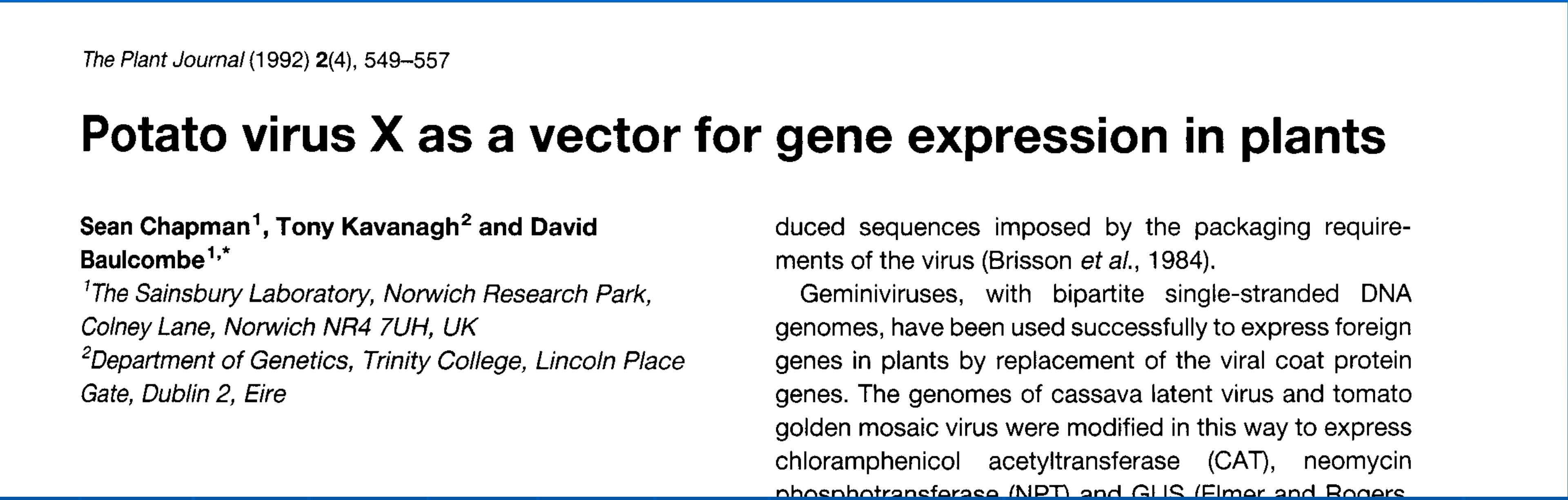
植物病毒在生物科技上的應用

- 植物病毒可作為外源基因表現載體
- 植物病毒可作為基因靜默載體
- 奈米科技：植物病毒作為藥物奈米包裝材料與體內運送工具

植物病毒或衛星核酸作為外源基因表現載體



The Plant Journal (1992) **2**(4), 549–557

Potato virus X as a vector for gene expression in plants

Sean Chapman[1], Tony Kavanagh[2] and David Baulcombe[1,*]
[1]The Sainsbury Laboratory, Norwich Research Park, Colney Lane, Norwich NR4 7UH, UK
[2]Department of Genetics, Trinity College, Lincoln Place Gate, Dublin 2, Eire

duced sequences imposed by the packaging requirements of the virus (Brisson *et al.*, 1984).

Geminiviruses, with bipartite single-stranded DNA genomes, have been used successfully to express foreign genes in plants by replacement of the viral coat protein genes. The genomes of cassava latent virus and tomato golden mosaic virus were modified in this way to express chloramphenicol acetyltransferase (CAT), neomycin phosphotransferase (NPT) and GUS (Elmer and Rogers

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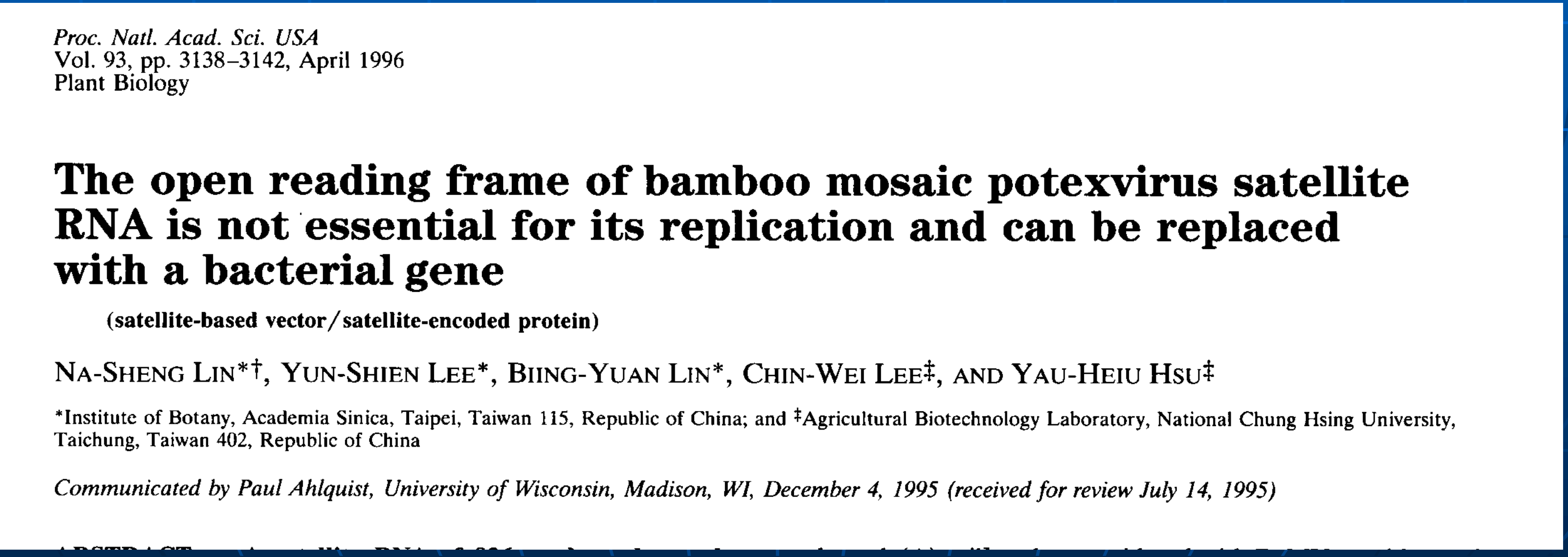


Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 3138–3142, April 1996
Plant Biology

The open reading frame of bamboo mosaic potexvirus satellite RNA is not essential for its replication and can be replaced with a bacterial gene

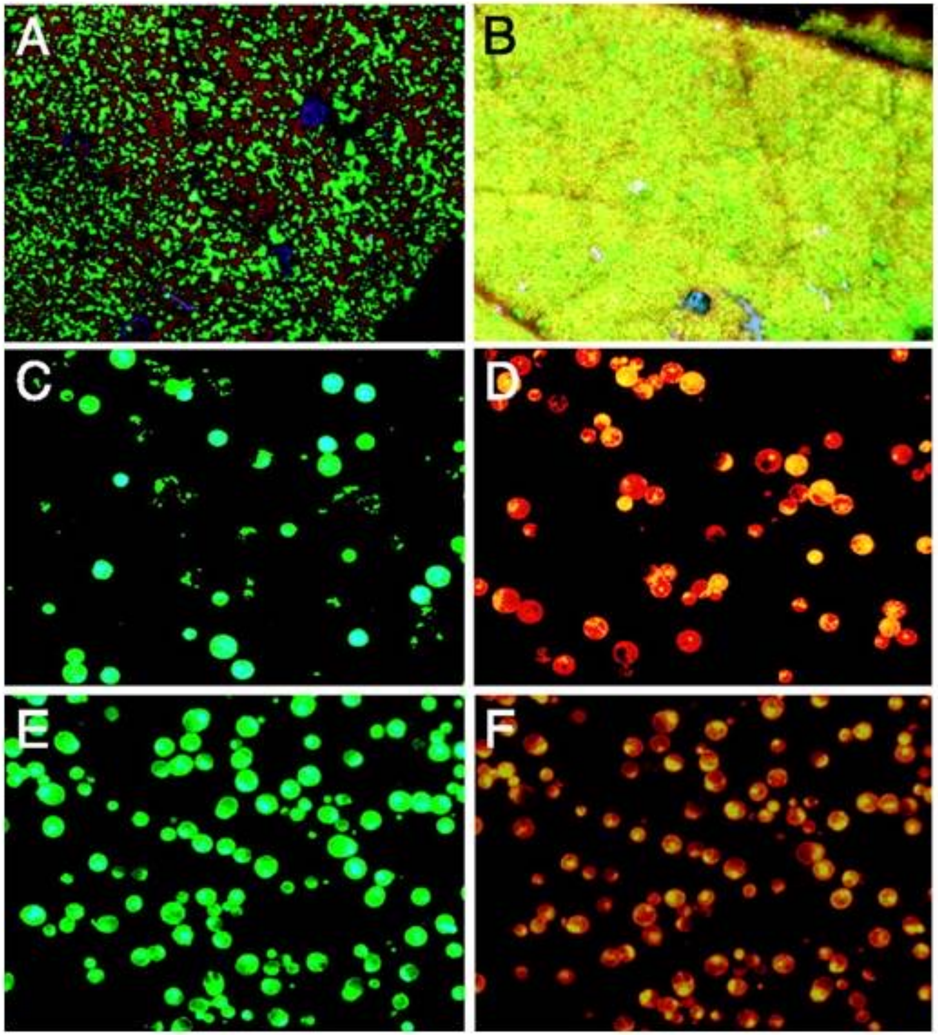
(satellite-based vector/satellite-encoded protein)

NA-SHENG LIN*†, YUN-SHIEN LEE*, BIING-YUAN LIN*, CHIN-WEI LEE‡, AND YAU-HEIU HSU‡

*Institute of Botany, Academia Sinica, Taipei, Taiwan 115, Republic of China; and ‡Agricultural Biotechnology Laboratory, National Chung Hsing University, Taichung, Taiwan 402, Republic of China

Communicated by Paul Ahlquist, University of Wisconsin, Madison, WI, December 4, 1995 (received for review July 14, 1995)

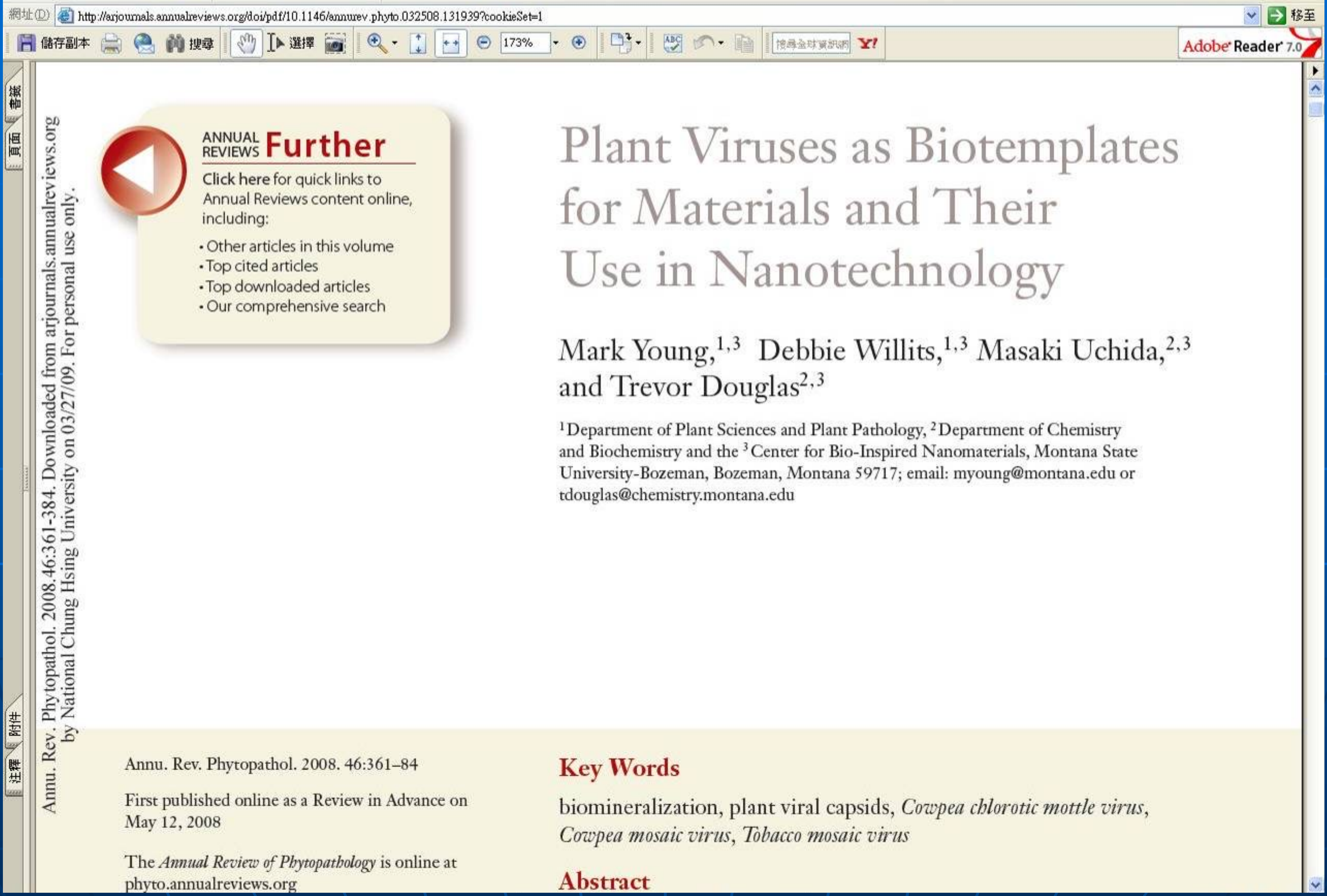
Coexpression of GFP and DsRED in N. benthamiana leaves by using viral vectors (6 dpi).



Anatoli Giritch et al. PNAS 2006;103:14701-14706

PNAS

植物病毒作為奈米科技材料



Plant Viruses as Biotemplates for Materials and Their Use in Nanotechnology

Mark Young,[1,3] Debbie Willits,[1,3] Masaki Uchida,[2,3] and Trevor Douglas[2,3]

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Annu. Rev. Phytopathol. 2008. 46:361–84

First published online as a Review in Advance on May 12, 2008

The *Annual Review of Phytopathology* is online at phyto.annualreviews.org

Key Words

biomineralization, plant viral capsids, *Cowpea chlorotic mottle virus*, *Cowpea mosaic virus*, *Tobacco mosaic virus*

Abstract

病毒顆粒的內、中、外皆可作為奈米生醫材料

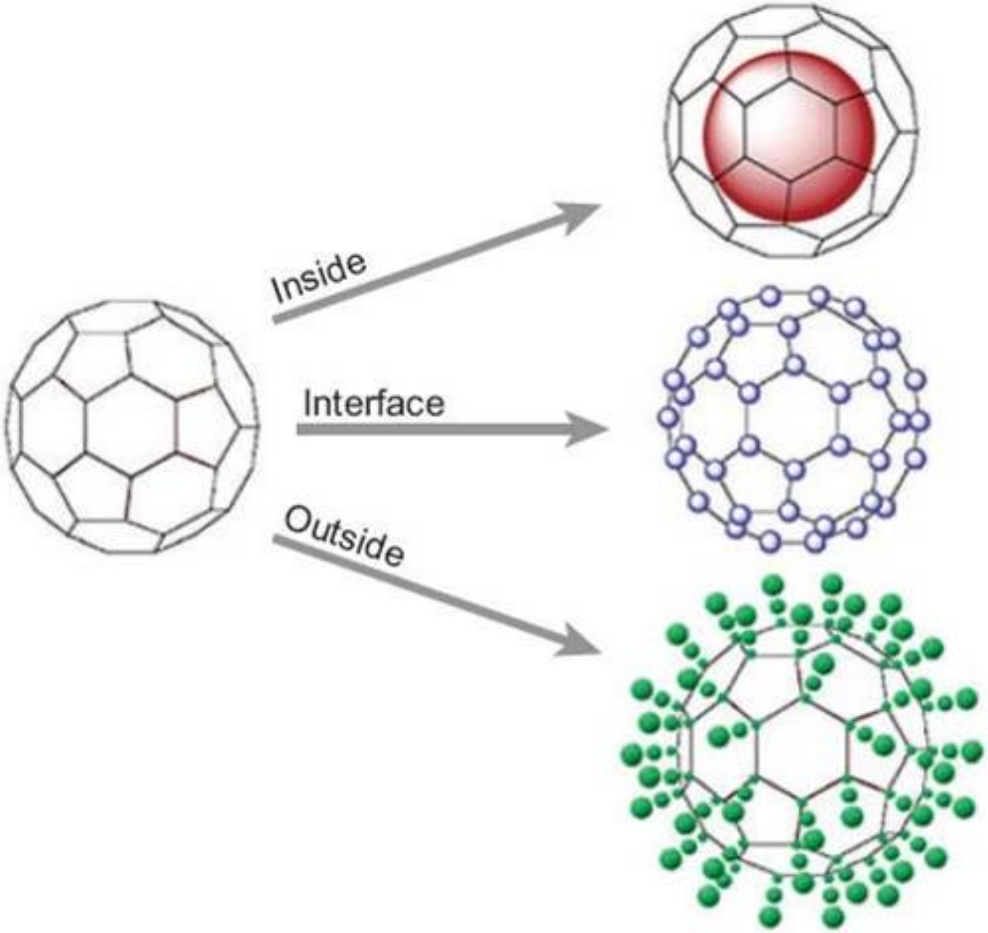


Figure 1

Schematic representation of the three surfaces of plant viral capsid architectures, each of which can be modified to impart function by design [reprinted with permission from (33)].

http://arjournals.annualreviews.org/doi/pdf/10.1146/annurev.phyto.032508.131939?cookieSet=1

以植物病毒顆粒發展奈米材料（如疫苗）

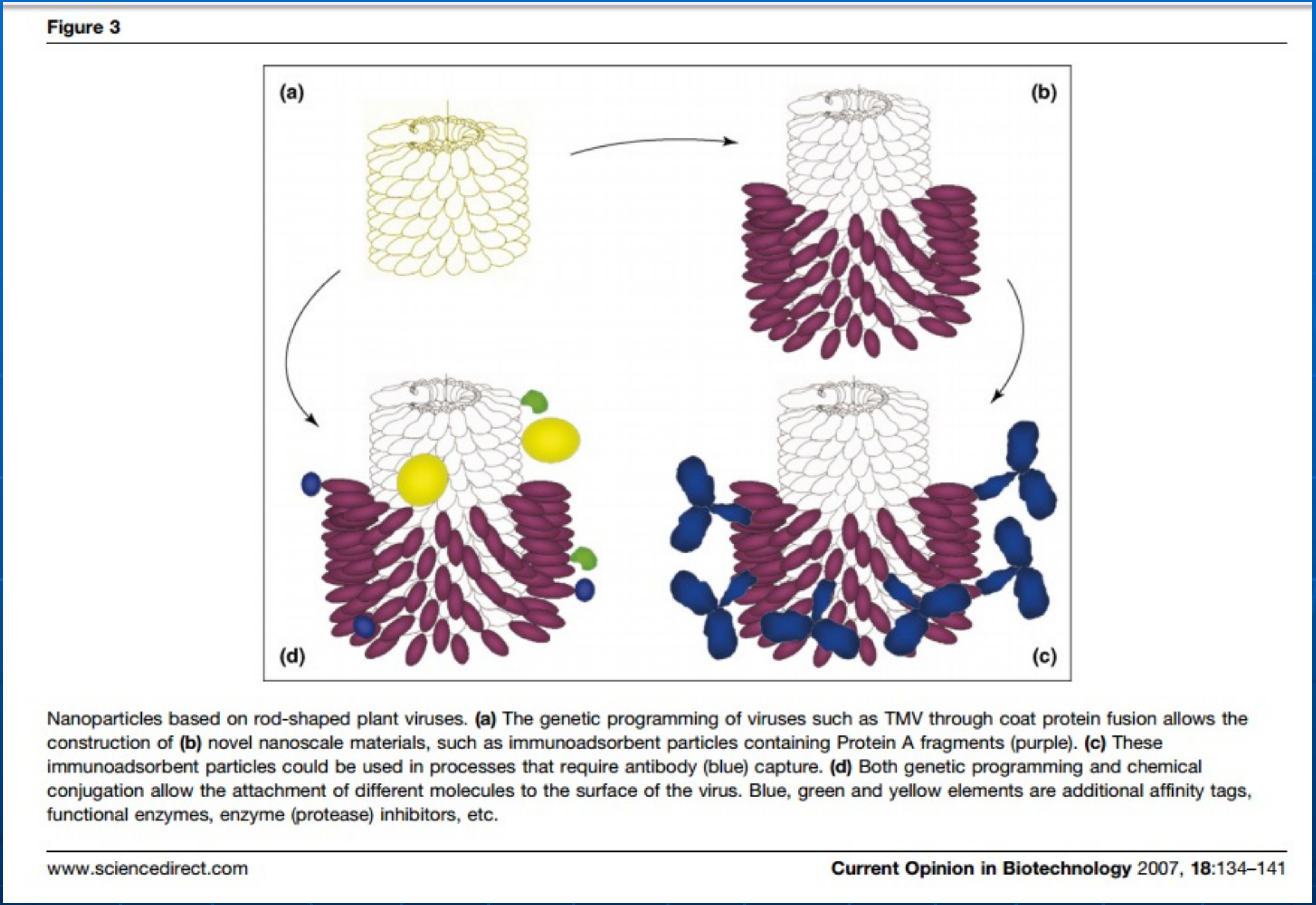
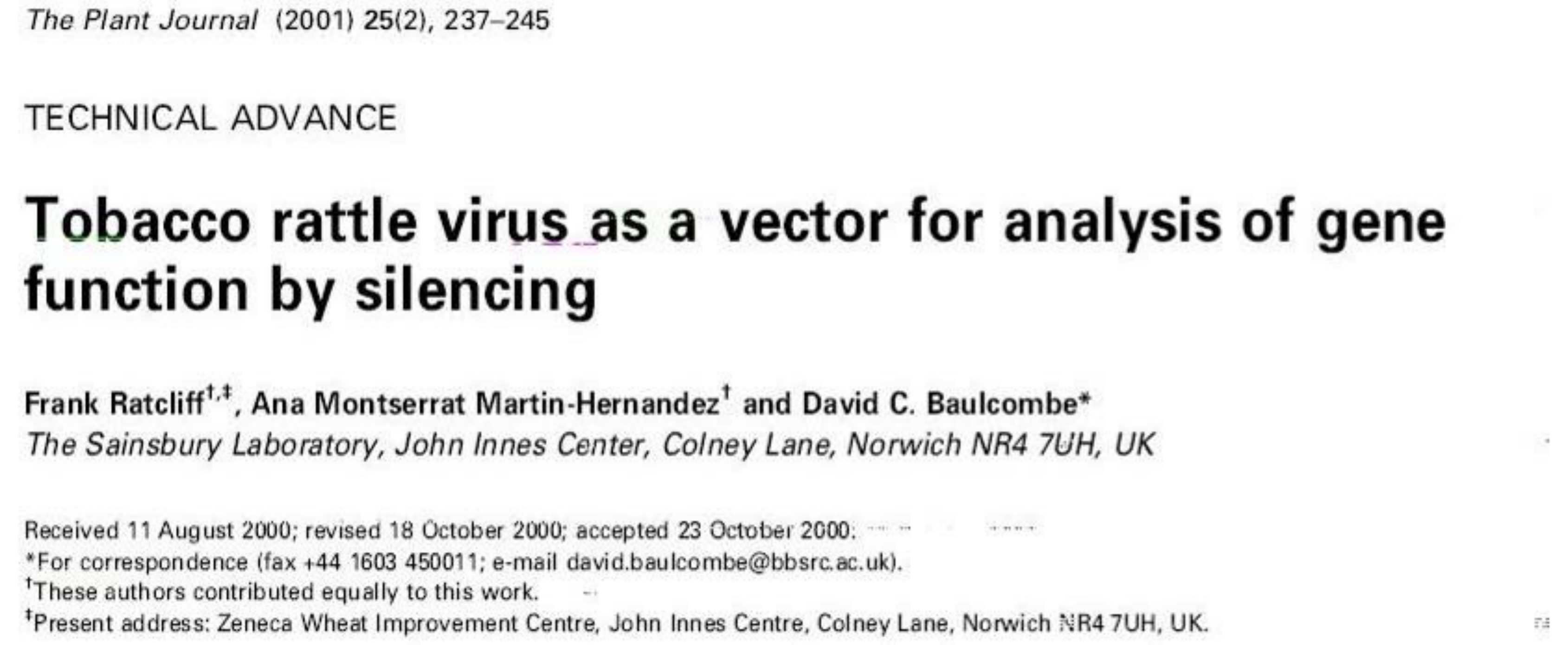


Figure 3

Nanoparticles based on rod-shaped plant viruses. **(a)** The genetic programming of viruses such as TMV through coat protein fusion allows the construction of **(b)** novel nanoscale materials, such as immunoadsorbent particles containing Protein A fragments (purple). **(c)** These immunoadsorbent particles could be used in processes that require antibody (blue) capture. **(d)** Both genetic programming and chemical conjugation allow the attachment of different molecules to the surface of the virus. Blue, green and yellow elements are additional affinity tags, functional enzymes, enzyme (protease) inhibitors, etc.

Gleba et al., 2007. Current Opinion in Biotechnology 18:134-147

植物病毒作為基因靜默載體



The Plant Journal (2001) **25**(2), 237–245

TECHNICAL ADVANCE

Tobacco rattle virus as a vector for analysis of gene function by silencing

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Received 11 August 2000; revised 18 October 2000; accepted 23 October 2000.

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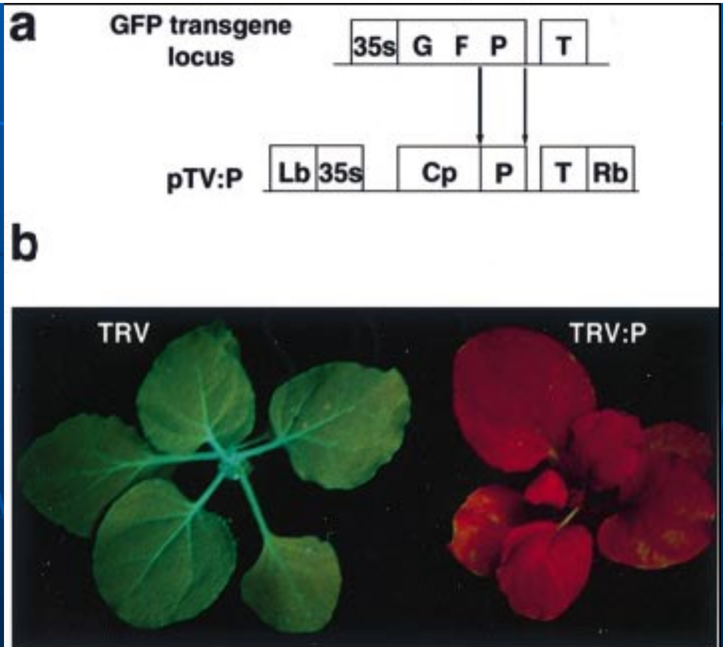
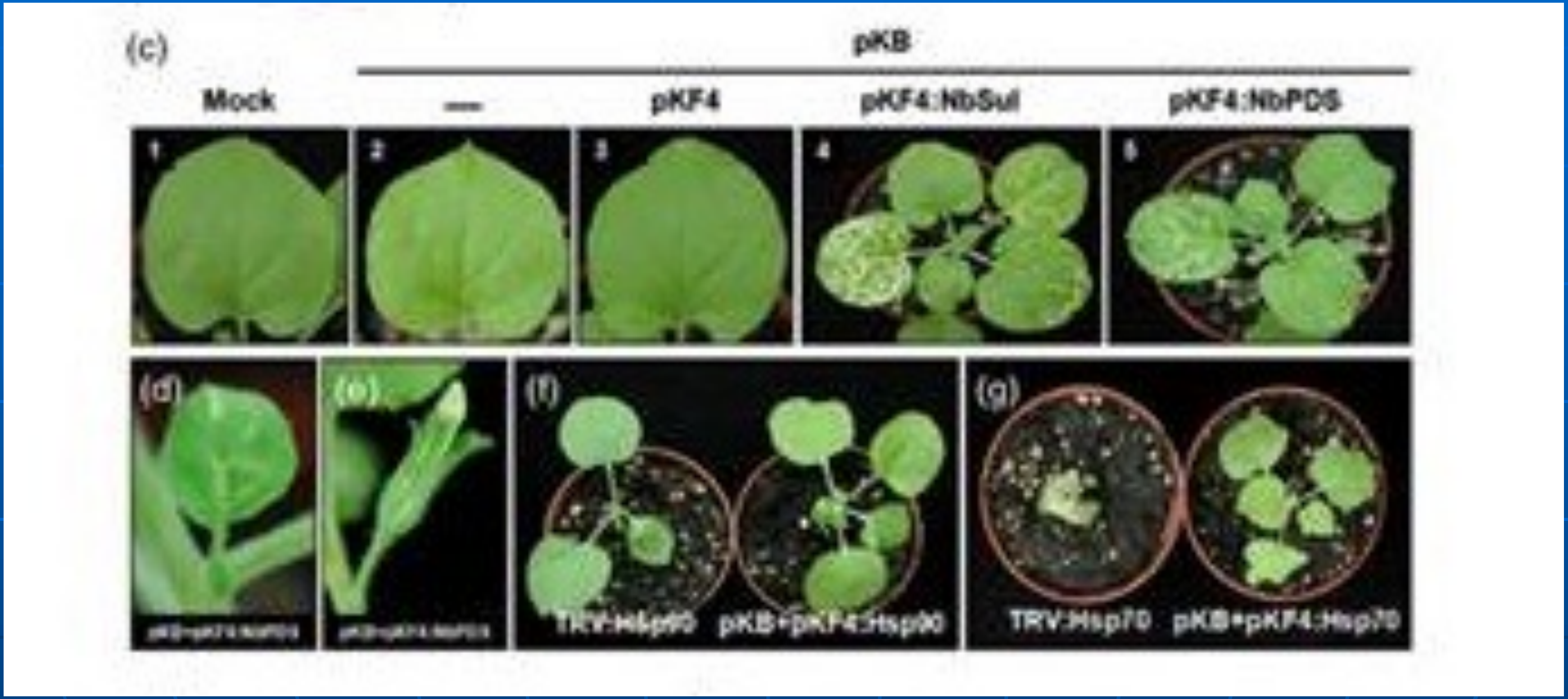
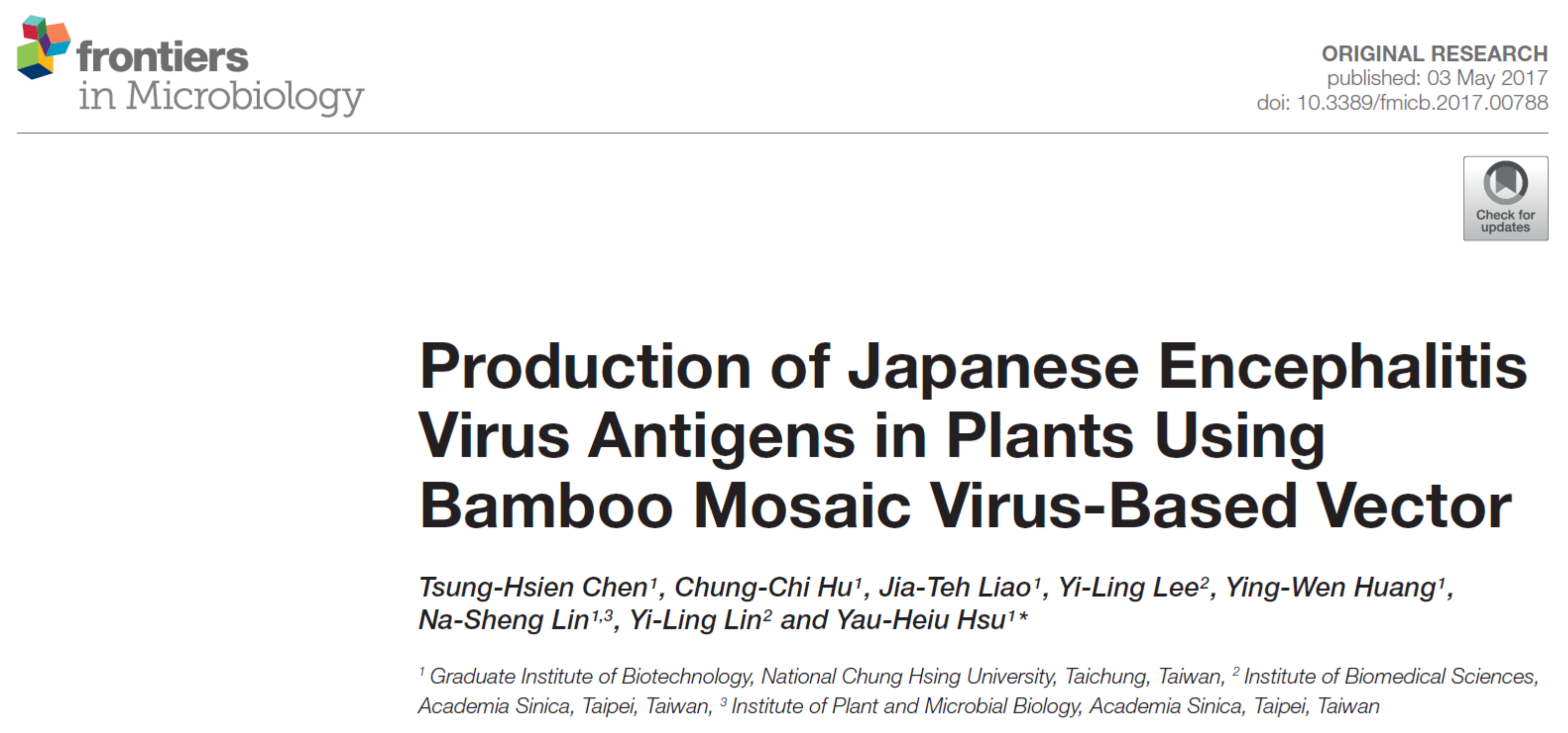


以植物病毒及衛星核酸作為基因靜默載體

A dual gene-silencing vector system for monocot and dicot plants



Liou et al., Plant Biotechnology Journal
Volume 12, Issue 3, pages 330-343, 28 NOV 2013 DOI: 10.1111/pbi.12140
http://onlinelibrary.wiley.com/doi/10.1111/pbi.12140/full#pbi12140-fig-0002



frontiers in Microbiology

ORIGINAL RESEARCH
published: 03 May 2017
doi: 10.3389/fmicb.2017.00788

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Production of Japanese Encephalitis Virus Antigens in Plants Using Bamboo Mosaic Virus-Based Vector

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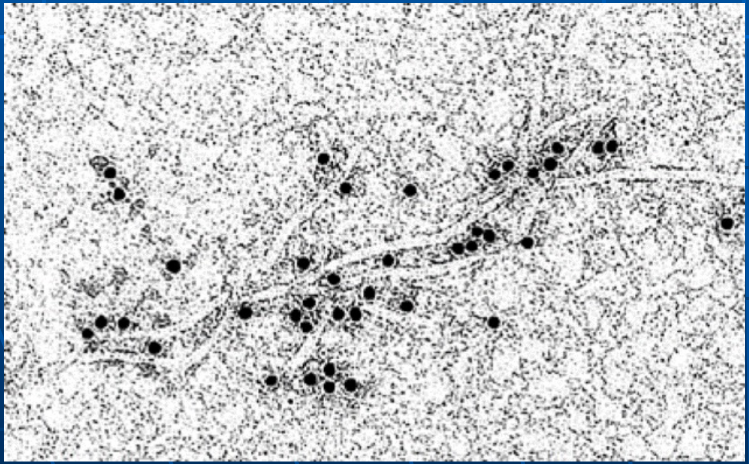
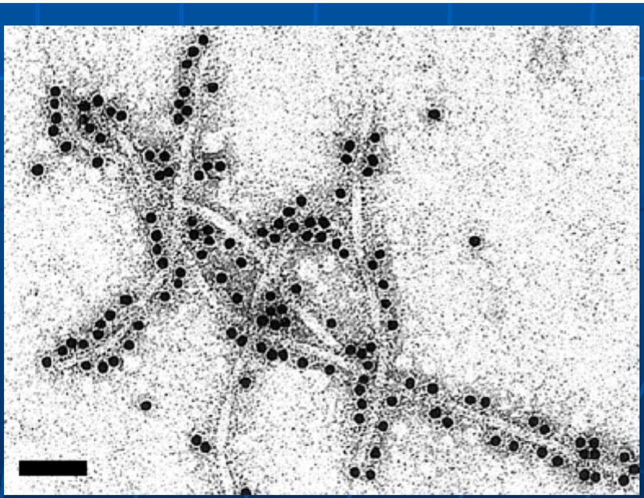


Anti-JEV EDIII



Anti-BaMV CP

利用病毒調控寄主植物的基因表現

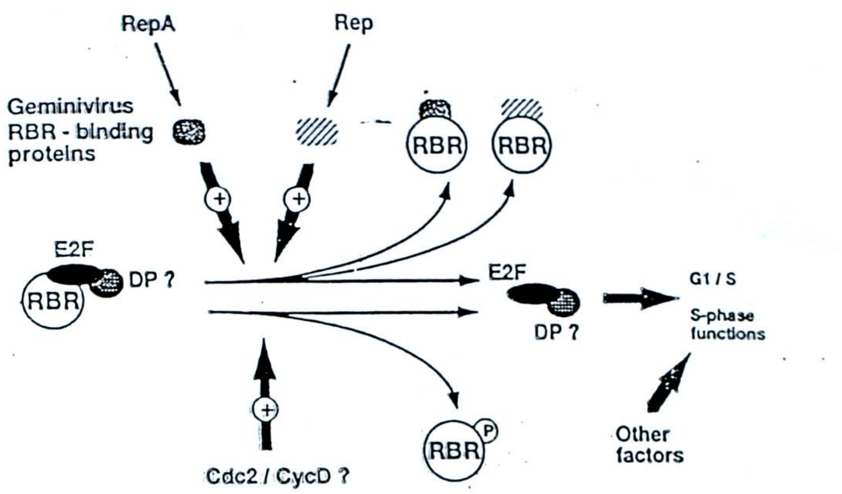


Fig. 3. Model proposed for the interference of geminivirus proteins with the retinoblastoma-related (RBR) pathway. The G_1–S transition in plants is controlled, most probably, by a pathway dependent on the retinoblastoma-related (RBR) protein which associates with the E2F–DP heterodimeric transcription factor(s). In cycling cells, phosphorylation of RBR by a CDK–cyclin complex (Nakagami *et al.*, 1999) is thought to release active E2F–DP complexes required to activate G_1–S transition and S-phase progression. S-phase-specific gene expression also occurs through other mechanisms. Geminivirus RBR-binding proteins (RepA and Rep) would bypass this control by sequestering RBR from the ternary RBR–E2F–DP complex.

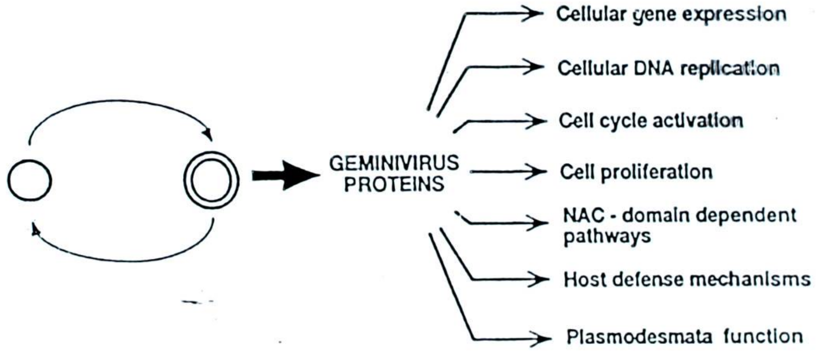


Fig. 4. Impact of geminivirus proteins on different host cell pathways. Viral proteins produced from the transcriptionally active dsDNA template have effects on a number of cellular pathways, some of which are still a matter of speculation. See text for details.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC305619/

重點回顧

- 病毒基本定義: 以”組裝”方式增殖
- 常見病徵與經濟重要性:嵌紋、變形，減產
- 植物病毒的種類: RNA(多)， DNA(少)
- 植物病毒的基本結構: 螺旋對稱、球型對稱
- 移動與傳播: 細胞間、系統性、植株間(蟲媒)
- 植物病毒的基本防治策略: 拒病、抗病
- 植物病毒在生物科技上的應用: 載體 (用途無限)

謝謝

敬請指教