# 医学分子病毒学概论
## 过去，现在和将来

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# 病毒的定义与特点

- 病毒的定义：是一类比较原始的、有生命特征的、能够自我复制和严格细胞内寄生的非细胞生物。
- 病毒的特点：
  ① 形体微小，具有比较原始的生命形态和生命特征，缺乏细胞结构；
  ② 只含一种核酸，DNA或RNA；
  ③ 依靠自身的核酸进行复制，DNA或RNA含有复制、装配子代病毒所必须的遗传信息；
  ④ 缺乏完整的酶和能量系统；

# 病毒学和医学分子病毒学

- **病毒学**是一门以病毒为研究对象的学科，是微生物学的一个分支。其研究领域包括，病毒的结构、分类、进化、感染细胞的机制、复制以及所引发的疾病等；也包括病毒纯化和培养技术的开发和病毒在研究和治疗中的应用。
- **医学分子病毒学**是病毒学的一个重要分支，主要是从分子水平上研究与病毒及其致病等相关的蛋白质与蛋白质，蛋白质与核酸以及蛋白质与脂类之间相互作用的学科。
- **医学分子病毒学**研究范围包括病毒本质，传播模式及致病机制，以及应用层面的药物及疫苗研究。它和分子生物学及药物学等关系密切。

Viruses and viral diseases have been at the centers of science, agriculture, and medicine for millennia, and some of our greatest challenges and triumphs have involved virology. Smallpox is a prime example.

By L.W. Enquist, 2009
( Princeton University)

# 分子病毒学的发展史凝练了分子生物学的精华
# 而医学分子病毒学是用分子病毒学解决人类疾苦的最有效的理论与实践学科

The history and progress of molecular virology contains the essence of molecular biology, while molecular medical virology plays the most effective roles in solving the health problems of mankind , via its theoretical research and practical application.

# 学习医学分子病毒学的意义

## 医学

**1.病毒性疾病占传染病的75%，除少数病毒，目前尚无治疗病毒病的有效措施。**

**2.病毒引起的持续性感染 ( persistent infections)是未解决的大问题。**

**3. 病毒与肿瘤 ( tumor) 自身免疫病 ( autoimmune diseases)等相关。**

**4.病毒结构与功能的解析 ( strucutral and functional analysis) ，与发现新的疾病作出贡献，整合多种医学学科 ( mltidiscipline) ，寻找药靶及疫苗的源泉。**

## 生物学

**5.病毒作为最小的生物体,基因组的结构与功能研究进展快。研究基因调控的模型。**

**6.病毒与细胞相互作用是研究细胞生物学的重要工具。**

**7.生命的起源 ( origin of life)**

**8.发展结构生物学**

# 纲 要

- 病毒学发展史
- 医学分子病毒学研究重大事件和主要进展
- 医学分子病毒学研究热点和或重点
- 医学分子病毒学总结和展望

# 病毒学发展史

- 病毒的发现
- 病毒学研究方法的发展
- 医学分子病毒学的发展

# 病毒病的由来

- 许多记述表明至少在公元前二至三个世纪印度和中国就存在天花，中国从公元十世纪宋真宗时代就有接种人痘预防天花的记载了。

- 考古学的发现说明早就存在某些人类病毒病。在古埃及石刻浮雕中一个主要人像就带有患过引起跛足的脊髓灰质炎的标记。
- 阿里斯多德（Aristotle）在公元前四世纪就记述了病犬的疯狂和暴怒，通过咬啮还能将病魔传给其他的动物，此病也能传染给人（人畜共患疾病），在人体上这种病常被称作恐水病。

# 病毒的发现
# 烟草花叶病毒（TMV）

- 一百多年以来，烟草花叶病毒在病毒学发展史乃至遗传学、生物化学以及当代基因工程中起到了里程碑的作用。在病毒学研究的许多阶段，它都扮演着重要角色，它使人们了解到什么是病毒、病毒的结构、病毒的侵染、复制以及抗病毒基因工程等等。时至今日，它仍然是病毒学工作者的宠儿。

# 病毒的发现

- 1859年斯威腾（Van Swieten）是最初描述烟草花叶病症状的人。
- 1886年在荷兰工作的德国人麦尔（Adolf Mayer）把烟草花叶病株的汁液注射到健康烟草的叶脉中，引起了烟草的花叶病，证明这种病是可以传染的。
- 1892年俄国的伊万诺夫斯基（Ivanowski）不但重复了麦尔的试验，而且发现其病原能通过细菌所不能通过的过滤器，可是他本人抱怨他用的过滤器出了毛病，保持了几个月都未污染细菌的事实也没能改变他的看法。没有足够的勇气冲破思想上的无形禁区。
- 荷兰的一位细菌学家贝叶林克（Beijerinck）敢于正视现实，于1898年重复和肯定了伊万诺夫斯基的结果并且证明显微镜下看不到病原物，试管里用培养细菌的方法也培养不出来，但它能扩散到凝胶中。因此认为病原是一种比细菌还小的“有传染性的活的流质”，给病毒起拉丁名叫“Virus”也是他。

伊万诺夫斯基和贝杰林克通过他们创造性工作发现了烟草花叶病毒，从而开创了病毒学独立发展的历程

Virus一词传到中国，有人把它译成“毒素”。我国微生物学界的老前辈俞大绂先生最初直译为“威罗斯”，后来改为“病毒”即能致病的毒物。同时我国著名的植物病毒学家周家炽先生建议把“疒”和“毒”字加在一起，成为一个双音的单字，没有被大家接受。

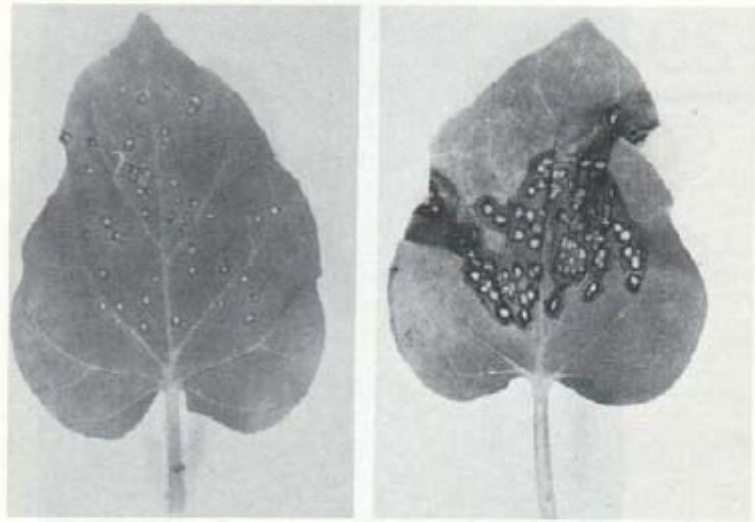
# Milestone I
# 发现病毒

1892 Ivanovski
发现TMV 滤液
引起疾病

1899 Berjernick
系列传代

1935 Stanley 获
得TMV晶体

纯核酸( RNA)可
引起感染



# 人类病毒的发现

- 黄热病自15世纪以来，一直流行并死亡率很高，人们知道科通过蚊子等媒介传播，但找不到病原；
- 1901年WALTER REED 和JAMES CARROLL将患者的血经过过滤后给三个没有免疫力的健康志愿者使用，其中二人引起了黄热病的症状，从而证实了其病原体是可过滤的病毒，1909年，KARL LANDESTEINER等发现了人类脊髓灰质炎的病原体--脊髓灰质炎病毒

# 病毒学研究方法的进展

- 病毒学的发展很大程度上依靠实验技术和实验体系的发展，病毒学发展史实际上就是研究病毒的实验工具和体系发展的历史。
- 病毒学实验技术的发展，开创了生物学（病毒学，细胞生物学及分子生物学）研究的全新领域。

# 活的宿主体系

- 最初的病毒学研究建立的活的宿主体系上；
- 最初的动物病毒研究采用活体动物进行，甚至采用志愿者；
- 二十世纪初：鸡胚，第一个繁殖培养和高效分离病毒的体系，也是第一个定量分析动物病毒的体系，其应用使病毒学研究出现了较大进展，分离鉴定了多种病毒
- 动物宿主体系迄今还在进行应用；
- 转基因动物和植物得到广泛应用。

# 组织培养体系

- 组织培养技术的建立和发展为病毒学研究奠定了良好的基础，使病毒学进入了细胞水平研究阶段。

- 我国学者黄祯祥早在1943年就利用鸡胚组织块在试管内进行病毒传代、定量滴定及中和试验。
- 1949年J.J.Enders利用单层细胞培养繁殖脊髓灰质炎病毒取得成功，并且由于他对脊髓灰质炎病毒的开创性研究，而于1954年获得诺贝尔奖。
- 1952年Dulbecco利用细胞单层培养进行了蚀斑试验，1953年Salk用细胞培养的脊髓灰质炎病毒制备出灭活疫苗，1957年Stewart用细胞培养技术还分离出多瘤病毒。我国已故微生物学和病毒学的奠基人高尚荫院士，1958年在国际病毒学研讨会上宣读了《培养脓细胞的组织培养方法研究》论文，从此揭开了中国昆虫病毒学研究的新篇章。
- 许多学者采用这一新技术，相继分离了上百种过去对动物不敏感的新病毒，如腺病毒、副流感病毒、鼻病毒、呼吸道合胞病毒、Echo病毒和柯萨奇病毒，大大拓宽了病毒学的研究范围。

# 组织培养体系

- 组织培养技术不仅发展了临床病毒学，而且还可用于研究病毒的复制和遗传，使人们对病毒本质有了进一步的认识。

  组织培养技术还推动了病毒学研究方法的发展： 空斑分析技术---分析，定量病毒

- 组织培养技术对动物病毒研究所作的贡献主要包括：病毒转录新途径和翻译新途径的发现；病毒对宿主范围的选择；某些肿瘤病毒引起的细胞转化；某些病毒侵染引起的细胞融合；发现有的病毒核酸由若干片段组成；有的病毒核酸具有极性的不同，如小RNA病毒为正链RNA病毒，正粘病毒为负链RNA病毒。

- 目前组织培养技术已广泛应用于未知传染因子的分离，病毒病诊断，疫苗生产，以及病毒感染和复制的基础研究。

# Milestone IV 病毒的细胞培养

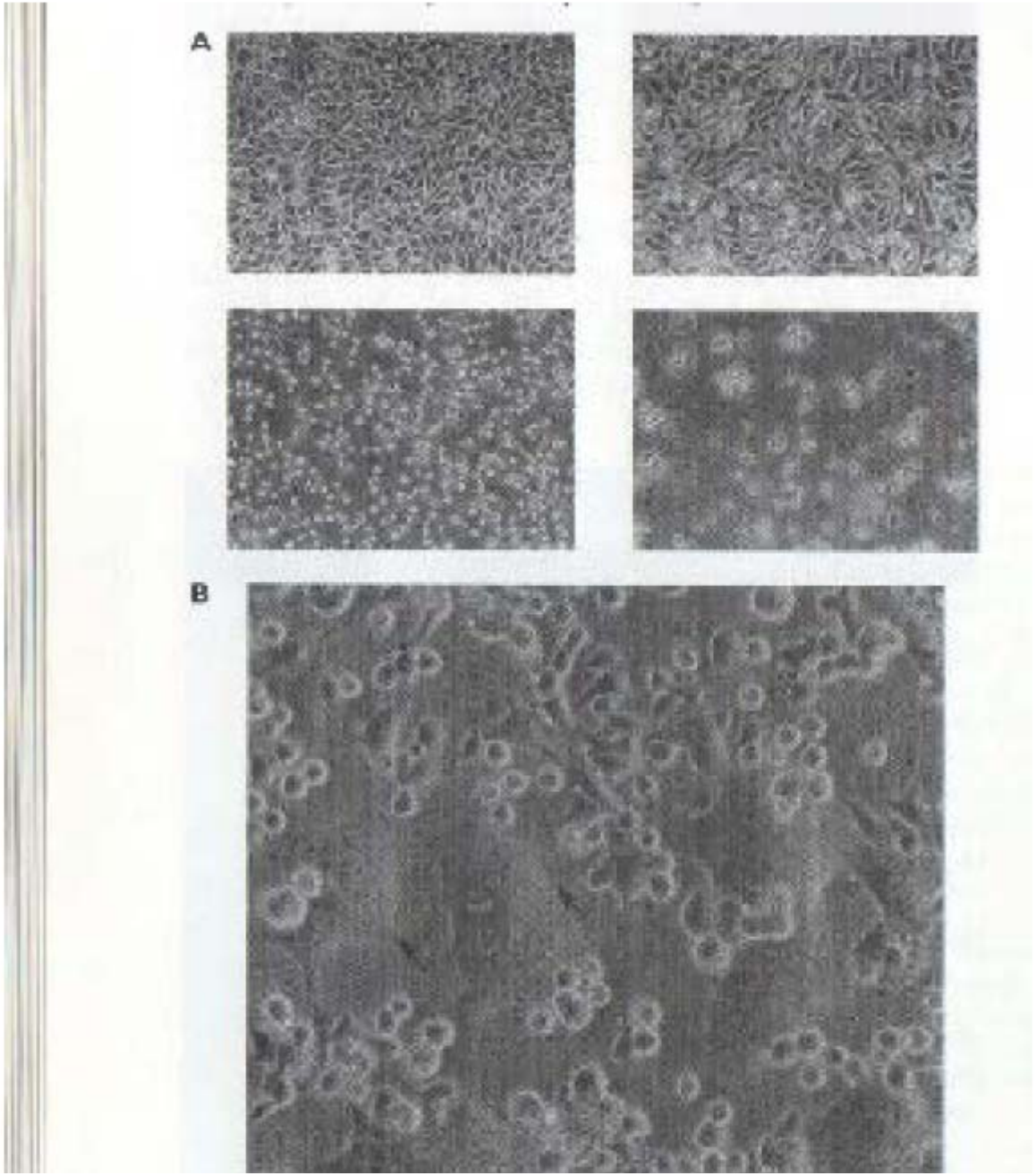
CPE

纯化病毒

疫苗生产

病毒抗原分析

病毒与各种细胞间的作用



# 组织培养与病毒研究的细胞水平时期

**利用大肠杆菌研究噬菌体的感染过程取得了迅速发展。**

以M.Delbruck和A.D.Hershey等领导的“噬菌体小组”围绕噬菌体与感染细菌细胞的相互关系进行了大量而深入的研究。这一时期的突出贡献在于：

1940年M.Delbruck阐明了噬菌体的复制周期；

1950年A.Lwoff揭示了溶原性噬菌体诱导的原理；

1952年A.D.Hershey证明了噬菌体DNA的感染性；

1952年N.D.Zinder发现了噬菌体的转导现象；

1952年E.Wollman发现了溶原性噬菌体。

对病毒学的发展产生了深远的影响，也为分子生物学和分子病毒学的发展奠定了基础

# 血清学和免疫学方法

免疫学为病毒及其致病的研究提供了技术方法和与机体相互作用的认识

目前，免疫荧光技术是研究病毒侵染过程、定位及其病毒基因功能的重要手段。

# 病毒超微结构的研究

- 物理学方法：
- 化学分析方法
- 电子显微镜

# 物理学方法

- 物理学方法测定病毒粒子
- 超速离心机：
- X射线晶体衍射：TMV的结晶及其化学本质的发现是对医学和生物科学的巨大贡献，它不仅引导人们从分子水平去认识生命的本质，而且为分子病毒学和分子生物学的诞生奠定了基础。鉴于Stanley在TMV研究中的突出贡献，1946年他被授予诺贝尔奖，这是病毒学领域第一个获此殊荣的科学家。

# 化学分析方法

- 测定病毒蛋白质和核酸的特性，组成，装配及作用方式；

# Milestone II

用同位素S 及P分别标记蛋白及核酸证实核酸决定感染性，病毒核酸指令宿主改变其生物合成

病毒与宿主细胞的关系

## 病毒的生物合成



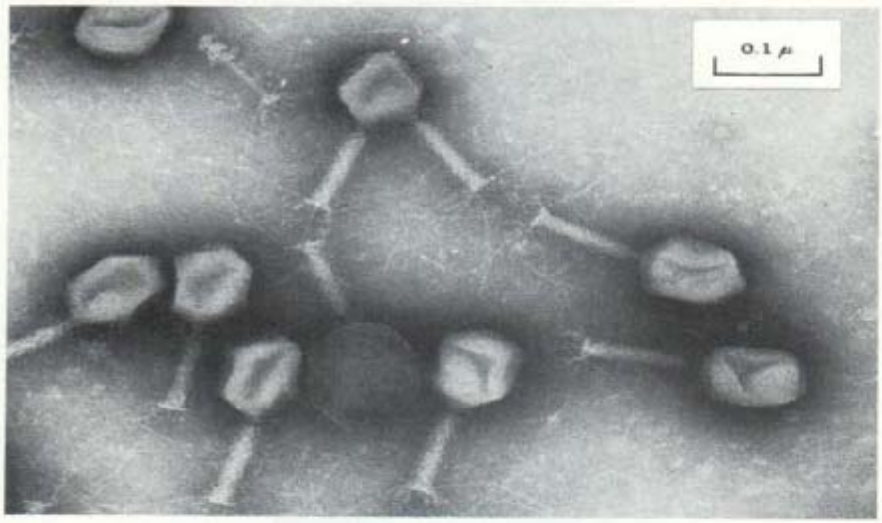
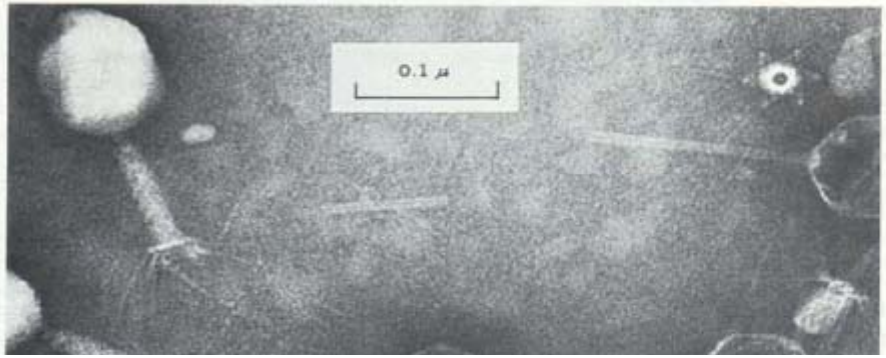



*FIGURE 3-16. Virions of bacteriophage T4; one with two tails, one with exploded head. Courtesy Dr. E. Boy de la Tour.*

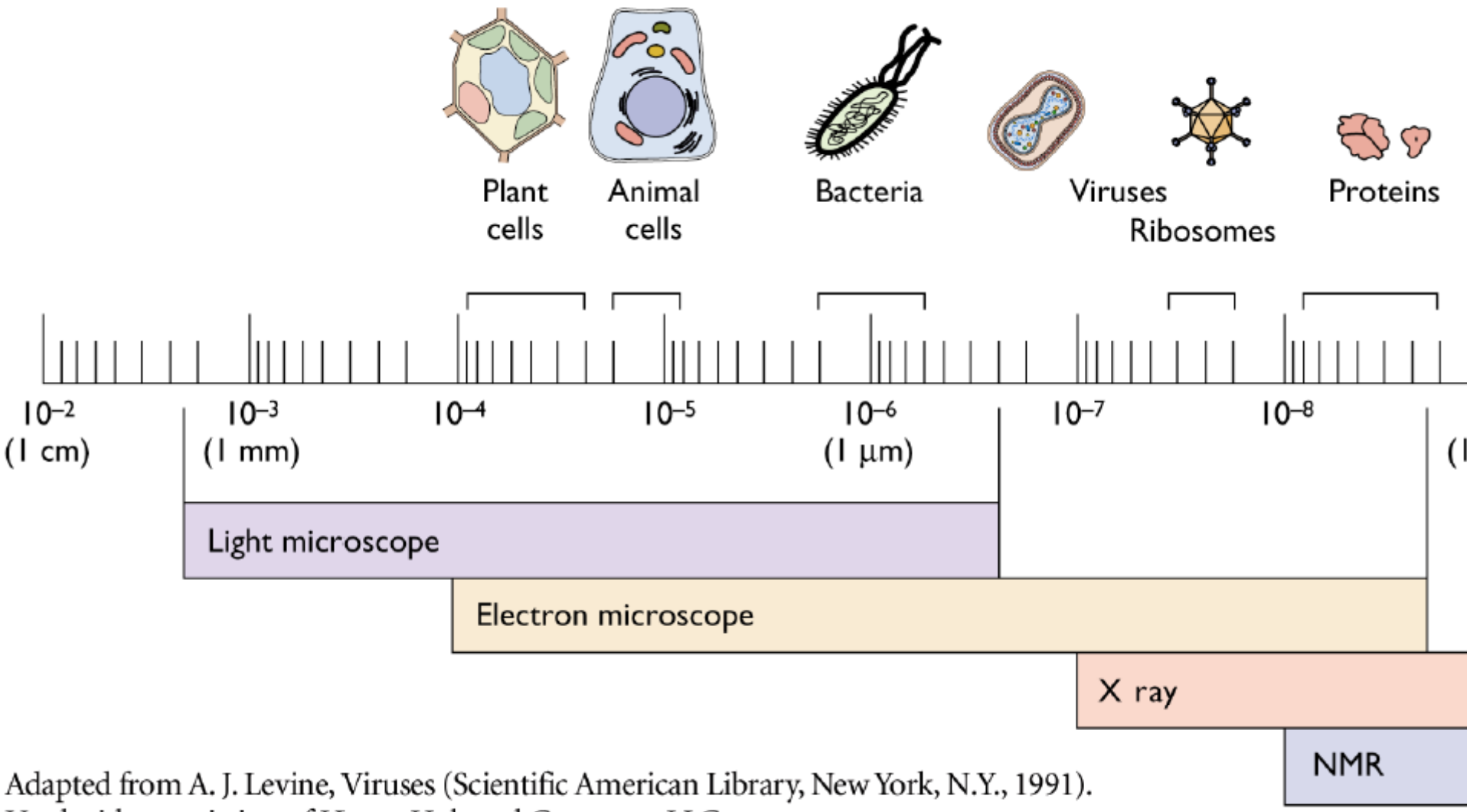
*FIGURE 3-17. Details of the structure of bacteriophage T2L in negative staining. Note the absence of a collar, such as is present in phage T4. Upper right corner: a tail plate, showing hexagonal symmetry with spoke-like appendages. One empty virion with normal tail, one broken tail core, one core attached to an empty capsid. Another empty virion has a contracted sheath. Courtesy Dr. E. Boy de la Tour.*



# 电子显微镜技术

- 1939年，G.A.Kansche在电镜下直接观察到了TMV，指出TMV是一种直径为1.5nm，长为300nm的长杆状的颗粒，而番茄黄化花叶病毒（Tomato yellow mosaic virus, TYMV）颗粒为球形，直径为25nm。
- 早期电镜学家获得的最令人振奋的发现之一是细菌病毒----噬菌体，d’Herelle的噬菌体最初的电镜照片曾引起很大的轰动。噬菌体虽然非常微小，仅为10nm，但它们具有高度整齐而复杂的结构，它们有圆的头和起初被认为是尾巴的附属物，像个小蝌蚪。

# Milestone III-形态学（电子显微镜负染）-病毒的大小




Adapted from A. J. Levine, Viruses (Scientific American Library, New York, N.Y., 1991).
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# 分子病毒学的研究时期

- 自从1953年DNA双螺旋结构理论建立以来，由于分子生物学的迅速发展，新技术和新方法的应用，使得病毒学的研究步入了分子病毒学的发展时期。
- 而分子病毒学的发展对分子生物学的发展也起到巨大的推动作用，特别是噬菌体和植物病毒为此做出了巨大的贡献。
- 分子病毒学的发展是各相关学科如分子生物学、细胞生物学、遗传学、免疫学与病毒学理论和技术相互渗透的结果。

# 分子病毒学的研究时期

- 1953年，Watson和Crick建立了DNA双螺旋结构理论，它使人们开始从分子水平上去认识遗传物质--DNA的结构基础和复制特性，理解基因表达与性状的关系，从而为分子生物学和分子病毒学的创立奠定了基础。

  1962年，D.L.D.Casfar阐明了许多病毒的二十面体结构，明确了病毒核衣壳二十面体的构成规律，这是对病毒超微结构认识的重大突破。

- 1962年，D.Nathans成功地进行了噬菌体RNA的体外翻译；1965年，S.Spiegelman成功地在体外复制出Qβ噬菌体RNA；1967年M.Goulian成功地体外复制ΦX174噬菌体。这些工作对以后阐明DNA病毒和RNA病毒 的繁殖机制起了重要作用。

- 1967年，T.O.Diener发现了类病毒，他在试图分离马铃薯纺锤形块茎病的病毒时，发现其病原不是病毒，而是一种不含有蛋白质，分子量为105左右的裸露RNA。这样小的RNA分子不编码任何蛋白质。根据其特殊的性质，Diener把这类致病因子称为“类病毒（Viroids）”。随后的研究表明，类病毒RNA还有特殊的复制机制。类病毒的发现在分子病毒学史上是一个重要事件，它不仅揭示了自然界存在着比病毒更简单的生物，而且也使人们加深了对生命起源的认识。

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# 分子病毒学的研究时期

- 1968年，P.H.Duesberg发现流感病毒的多节段RNA基因组，随后在其他一些病毒中如呼肠孤病毒、大麦条纹花叶病毒中也发现了病毒基因组分节现象的存在。

- 1970年，P.H.Duelerg发现Rous肉瘤病毒含有癌基因v-src，而且在正常鸡以及其他脊椎动物和无脊椎动物的DNA中，也发现有癌基因v-src的同源序列存在，推测病毒癌基因是来自于细胞正常基因。随着其他肿瘤病毒致癌基因的发现，肿瘤病毒的细胞培养系统建立，以及肿瘤病毒对细胞转化诱导作用的确定，使人们对肿瘤发生的机制有了更深刻的了解。

- 1970年，H.M.Temin和D.Baltimor分别发现了病毒的逆转录酶。逆转录酶基因组RNA在逆转录酶的作用下，首先合成原病毒DNA，然后原病毒可整合到宿主染色体DNA上。除了病毒癌基因外，原病毒在宿主DNA上的插入、整合，也可以引起细胞癌基因的激活和细胞转化，逆转录酶和逆转录过程的发现，是对Crick 1958年提出的遗传学中心法则的重要补充和发展，说明遗传信息不仅可以从DNA RNA，也可由RNA DNA。

# 分子病毒学的研究时期

- 1971年，限制性内切酶技术的发现为DNA序列分析和病毒基因的定位创造了条件，利用这一技术曾经成功地为乳头瘤病毒、多瘤病毒、腺病毒、疱疹病毒构建了酶切图谱。另一些新技术如基因转移方法、Southen blot的相继诞生，也加快了病毒特异性基因，尤其是转化基因的定位和病毒核酸序列分析的进程。

- 1977年，英国剑桥大学的Sanger完成了ΦX174-DNA全部序列的测定,为此Sanger第二次获得诺贝尔奖。根据ΦX174-DNA全部序列的分析结果，Sanger意想不到地发现了基因重叠现象。随后，在DNA噬菌体如R17、MS2、F2、Qβ中也证实了基因重叠现象的存在，这是病毒利用有限的遗传信息执行更多的功能，提高自身在进化过程中适应能力的一种表现。

- 1977年，L. T. Chow阐明了腺病毒转录过程中的mRNA拼接现象，随后在SV40、多瘤病毒中也相继发现了mRNA转录后的拼接过程，从而证实了真核基因的不连续性，明确了内含子（intron）和外显子（exon）的概念。

- 1978年，W. Fiers和V.B.Reddy测定了SV40-DNA的一级结构由5224个碱基对组成。SV40是第一个全部核苷酸序列被搞清楚的真核病毒，它含有结构基因VP1、VP2、VP3以及转化基因T和t，整个基因组有12.5%非编码区或非翻译区，在这些区域中包含启动子、增强子序列和其他调节序列，可对病毒基因组复制、转录、翻译进行调控。由于SV40既是研究真核基因结构和表达的良好模型，又是研究癌变机制的理想材料，因此，SV40-DNA一级结构的测定具有重要意义。

- 1979年，T. Taniguchi用载体成功地表达了人干扰素基因。这是基因工程的一项大突破。

# 分子病毒学的研究时期

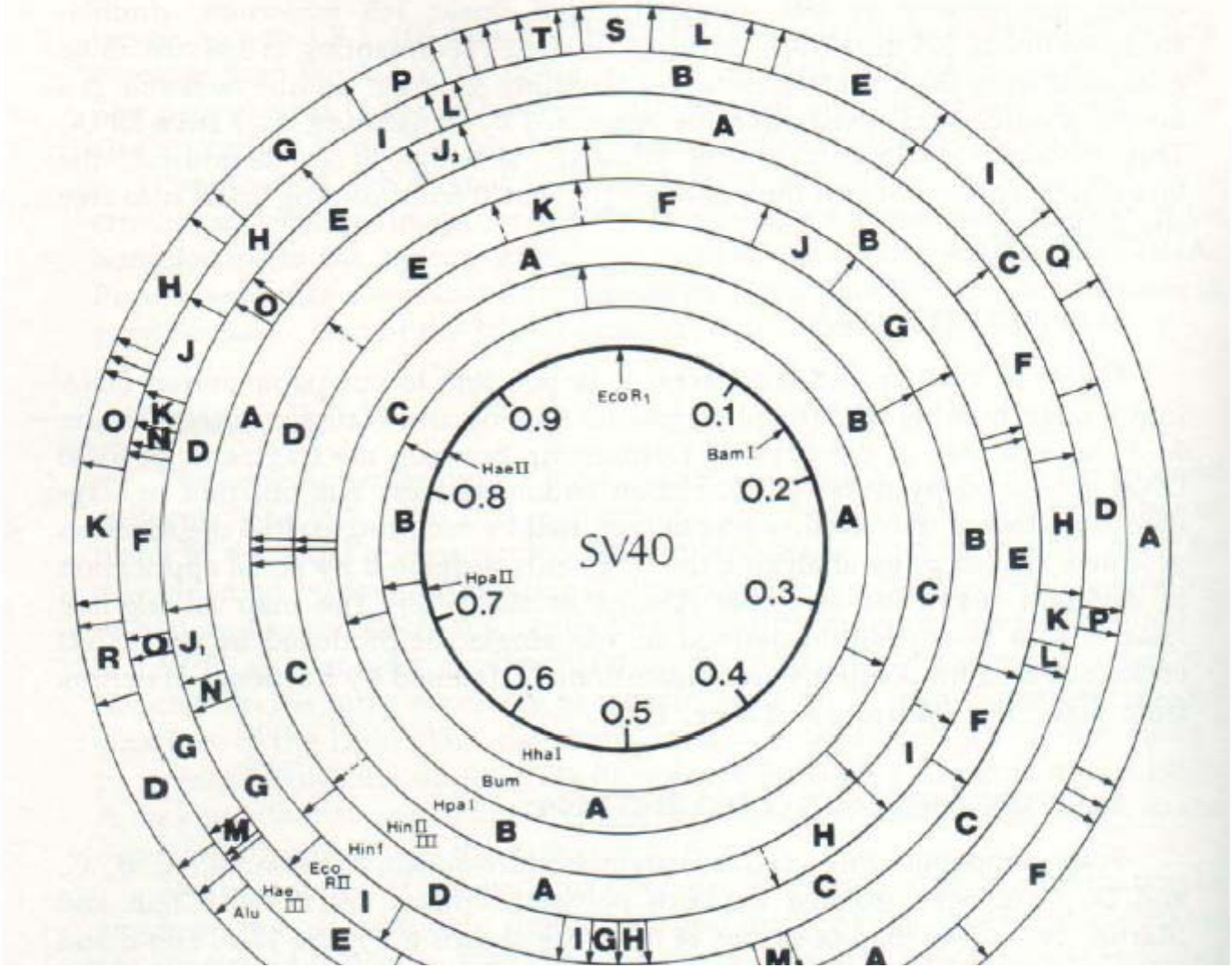
进入八十年代后，分子病毒学研究在深度和广度都有了很大的发展。

- 1981年，D.K.Kleid等利用重组DNA技术制备出口蹄疫病毒疫苗；
- 1982年，J.Summers等发现乙型肝炎病毒DNA复制中有逆转录过程；
- 1982年，B.Moss和E.Paoletti用痘苗病毒作为载体表达外源基因；
- 1983年，Montagnier和R.C.Gallo分别分离到与AIDS相关的人类逆转录病毒（HIV）；
- 1985年，H.Vonder Patten等在3A下阐明了鼻病毒的晶体结构;
- 1988年，Chuo和Yamaya用弱病毒全长cDNA导入产生抗病毒的转化植株；
- 1990年以来，PCR技术在分子病毒学领域得到了广泛应用。
- 1991年，Han等将Moloney鼠白血病毒的反义表达序列导入小鼠受精卵中，从而培育成功对该病毒有抗性的转基因小鼠。
- 1992年，Desrosiers等利用SIV mac239/nef缺失突变株制备出减毒活疫苗，取得了抗SIV感染成功，也给HIV疫苗的研究赋予了许多启示。
- 1995年，HIV天冬氨酰蛋白酶三维结构的鉴定，使得一些针对病毒蛋白酶活性位点的抑制剂先后问世。
- 1996年，David Ho利用逆转录酶抑制剂与蛋白酶抑制剂配成的“鸡尾酒”式药，成功地抵抗了HIV感染，因而1996年称为AIDS希望年。
- 1997年，美国加利福尼亚大学的神经病学和病毒学教授S.Prusiner由于发现了羊瘙痒病的致病因子是朊病毒（prion），以及提出了疯牛病、Creutz-feldt-Jakob氏病、Kuru病等脑退化性疾病是由朊病毒引起的理论。

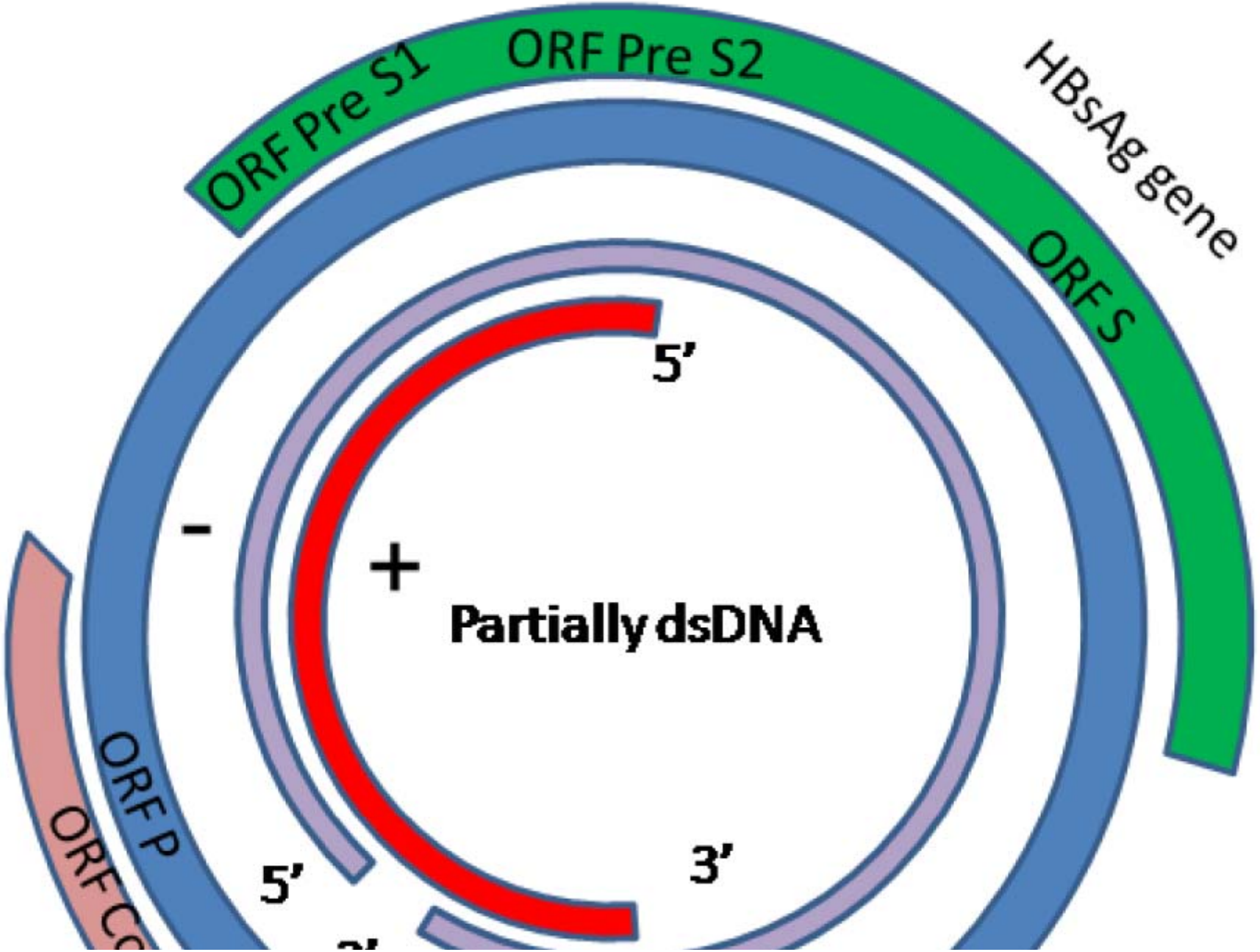
# Milestone V 病毒的基因组克隆与分析

## SV40 Papova virus,（猴肾生产polio 疫苗）





# Hepatitis B virus genome organisation



Milestone VI

Mouse pox

以鼠痘为模型研究

病毒在体内播散，研究凡病机制

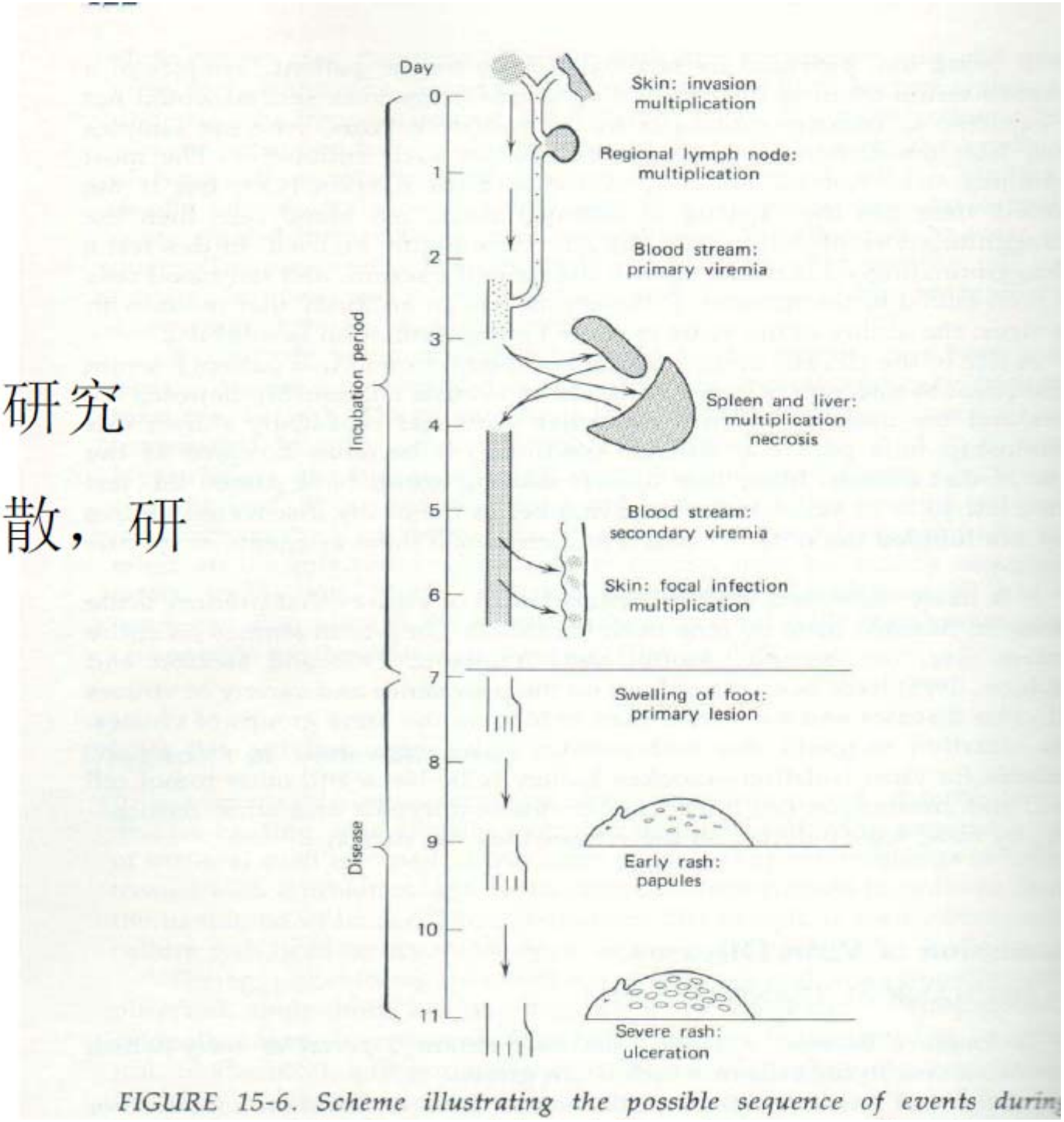
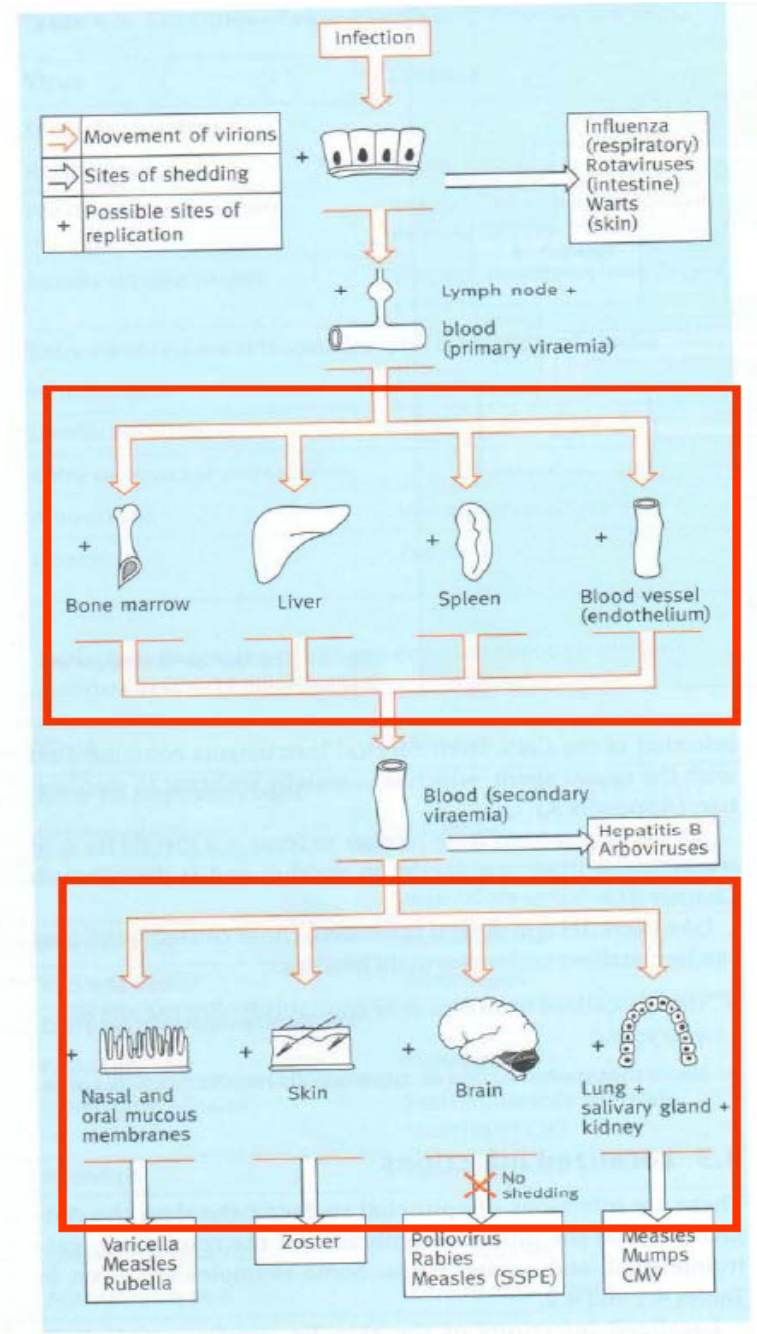



*FIGURE 15-6. Scheme illustrating the possible sequence of events durin*

多种病毒病在体内的播散及病毒排出体内的途径



# Milestone VII 病毒与肿瘤

**Peyton Rous** ( 1878-1970) 1911 Cell-free extract indu
Sarcoma,

不了解机制

认为是细胞DNA发生改变，并可以传代，但病毒的物质发生变化？还是消失了？

**Dulbecco** 1950年发现除鸡肉瘤（RNA,）

鼠中的白血病

通过病毒的诱导可以使正常细胞转化（transform）
瘤细胞与病毒复制产生大量自带病毒不同

**1966 Received Nobel Prize**

# Temin ( 1934-1994) 为什么RSV会致转化？“离谱”有逆转录酶？

1970 David Baltimore ( 遗传学家）

发现Reverse  Transcriptase

1975- shared Nobel prize with Temin

# 病毒学发展总结（Eras in virology）

| Era | Years | Description |
|---|---|---|
| Protovirology | 1796-1885 | Before viruses were recognized |
| Auroravirology (named for the Roman goddess of dawn) | 1892-1933 | Dawn of virology |
| Meridiovirology (from Latin for midday, sequel to dawn) | 1934-1955 | From the demonstration that bacteriophages are composed of protein and nucleic acid and the crystallization of TMV to the in vitro assembly of infectious TMV from purified RNA and protein |
| Janovirology (named for the Roman god of endings and beginnings) | 1956-1975 | Spans the interval between classic virology and the beginning of the era dominated by viral sequence information; encompasses the elucidation of essential features of gene structure, expression, and regulation and the development of essential techniques, including cloning and restriction sequence mapping |
| Neovirology | 1976-present | Begins with the first complete sequencing of viral genomes and atomic resolution structures of intact viruses |

# Discoveries recognized by a Nobel prize

- **1911 Discovery of first solid tumor virus (RSV) (Rous)**
- **1935 Crystallization of TMV (Stanley)**
- **1938 Development of yellow fever vaccine (Theiler)**
- **1943 Discovery of genetic origins of mutations (bacteriophage) (Luria, Delbruck)**
- **1946 Replication of poliovirus in nonneuronal cell cultures (Enders, Weller, Robbins**

# Discoveries recognized by a Nobel prize

- **1952 Plaque assay of animal virus (poliovirus) (Dulbecco) ;Discovery that viral genome is nucleic acid (Hershey, Chase); Transduction of genetic information by bacteriophage (Zinder, J. Lederberg)**
- **1958 Discovery of bacteriophage λ regulation paradigm (Pardee, Jacob, Monod, Lwoff)**
- **1962 Studies of virus structure (Klug, Caspar)**
- **1966 Experimental transmission of spongiform encephalopathy to primates (kuru) (Gajdusek, Gibbs, Hadlow)**

# Discoveries recognized by a Nobel prize

- **1967 Discovery of hepatitis B virus (Blumberg)**
- **1970 Discovery of retroviral reverse transcriptase (Temin, Baltimore)**
- **1972 Development of first recombinant DNA molecules (phage λ, SV40) (Berg)**
- **1973 Development of first restriction map (SV40) (Nathans) ;Discovery of major histocompatibility locus restriction of viral antigen recognition (Doherty, Zinkernagel)**
- **1976 Demonstration that retroviral oncogenes are derived from cells (J. M. Bishop, Varmus)**
- **1977 Discovery of RNA splicing (adenovirus) (Roberts, Sharp)**

# Discoveries recognized by a Nobel prize

- **1982 Development of antiviral and other drugs (Elion, Hitchings) ;Definition of prions (Prusiner)**

- 1989 J. Michael Bishop and Harold E. Varmus *"for their discovery of the cellular origin of retroviral oncogenes"*

- 1997 was awarded to Stanley B. Prusiner *"for his discovery of Prions - a new biological principle of infection"*.

- **1998 Discovery of gene silencing by double-stranded RNA, an antiviral response (Fire, Mello)**

- 2008 one half awarded to Harald zur Hausen *"for his discovery of human papilloma viruses causing cervical cancer"*, the other half jointly to Françoise Barré-Sinoussi and Luc Montagnier *"for their discovery of human immunodeficiency virus"*.

# 中国学者对病毒学的发展作出过贡献

汤飞凡1925年在美国对疱疹病毒进行过研究，他和他的导师Zinsser用物理学方法研究病毒的本质，证明病毒是存在于宿主细胞内能自我复制的颗粒，为病毒学的创立做出过重要贡献。

# 中国学者对病毒学的发展作出过贡献

1943年，黄祯祥在美国发表了对病毒学研究有重大影响的论文“西方马脑炎病毒在组织培养上滴定和中和作用的进一步研究”。这一研究成果，使病毒在试管内繁殖成为现实，从此摆脱了人工繁殖病毒靠动物、鸡胚培养的原始落后的方法。

新技术可以概括为：

第一步，用人为的方法将动物组织处理消化成单层细胞，并给以一定的营养成分使其在试管内存活；

第二步，将病毒接种在细胞内，经过一段时间细胞就会出现一系列病理改变。观察者只要用普通显微镜观察细胞有无病理改变，即可间接观测和判断有无病毒繁殖。

这一新技术把病毒培养从实验动物和鸡胚的动物水平．提高到体外组织培养的细胞水平。也正是这项新技术拓宽了国际上病毒学学者的思路。许多病毒学家采用或改良这一技术，成功地发现了许多病毒性疾病的病原，分离出许多新病毒，解决了当时还鲜为人知的一些疾病的病毒病因问题。有些学者还采用组织培养技术制备了疫苗，实现了人群的主动免疫，为控制和消灭病毒病做出了贡献。

50年代美国著名病毒学家恩德斯(Enders)获得诺贝尔奖，就是在采用黄祯祥这一技术的基础上取得的成果。美国1982-1985年出版的《世界名人录》称这一技术为现代病毒学奠定了基础。

# 中国学者对病毒学的发展作出过贡献

朱既明1948年在英国发现同一病毒可因变异而呈不同形态，并首次将流感病毒裂解为有生物活性的亚单位，并在此基础上提出了病毒的结构模型；

这一假说为后来阐明各种病毒结构开创了先河，并为研究亚单位疫苗准备了理论和方法的基础。

朱既明还发现了正常血清中抑制流感病毒血凝作用的β-抑制素。

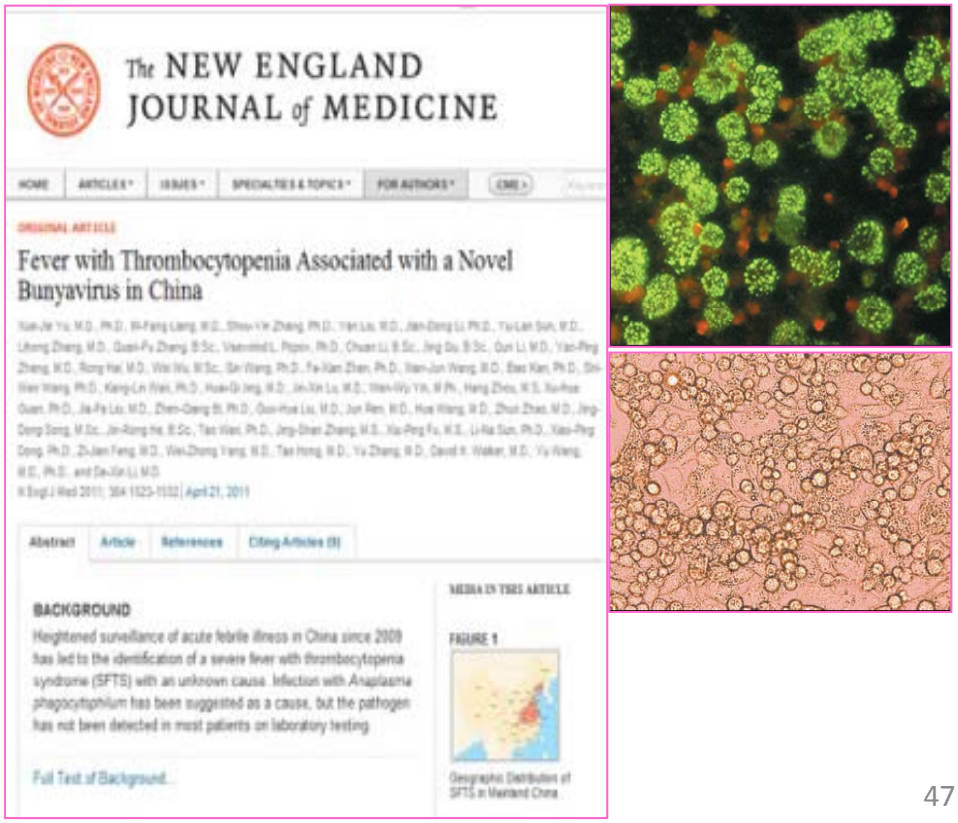
高尚荫1943年在美国进行烟草花叶病毒研究时曾观察到病毒感染不同宿主后仍保持稳定理化和血清学特征，在病毒学发展史中有一定地位。

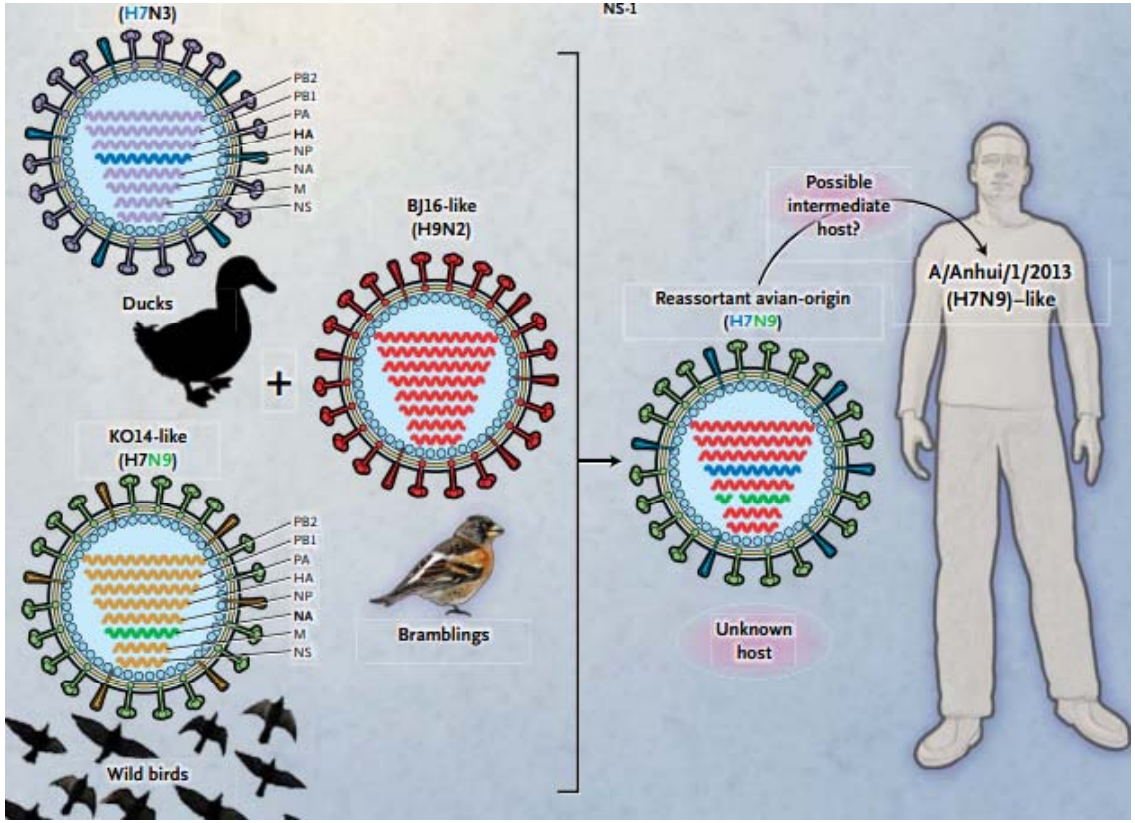
# 新病原的发现与研究

## 在全球首次发现新型布尼亚病毒

《新英格兰医学杂志》，2011

- 是继ＳＡＲＳ冠状病毒发现以来全球在病原学研究领域中的又一个重大突破，得到了国际科学界的重视和认同，表明我国病原学及新发传染病的研究达到一个更高水平！






2013年3月，中国上海和安徽发现3例感染H7N9禽流感病毒的病例。通过病毒培养和序列分析显示，这种甲型H7N9流感病毒是新型重配病毒。

（Gao R, Cao B, Hu Y, et al. Human infection with a novel avian-origin influenza A (H7N9) virus. N Engl J Med, 2013.DOI: 10.1056

# 纲 要

- 病毒学发展史
- 医学分子病毒学研究重大事件和主要进展
- 医学分子病毒学研究热点和或重点
- 医学分子病毒学总结和展望

# 21世纪病毒研究重大事件

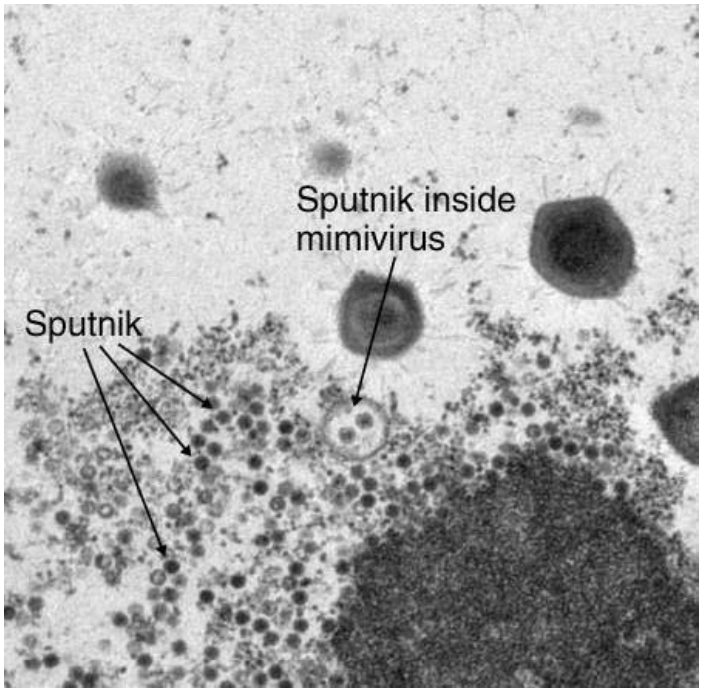
# 新型禽流感病毒H7N9在中国的爆发

H7N9型禽流感是一种新型禽流感，于2013年3月底在上海和安徽两地率先发现,是全球首次发现的新亚型流感病毒。目前全国已报告104例H7N9禽流感确诊病例，其中21人死亡，13人康复。

工作人员正在家禽市场取样，检测H7N9

从H1N1、H2N2、H3N2 、H5N1 至H7N9，甲型流感病毒每隔十几年就发生一次大变异

# Nature：能感染病毒的病毒




法国马赛地中海大学的细菌学家Bernard La Scola和微生物学家Didier Raoult在2008年8月7号的《Nature》杂志上发表的一项研究指出，在阿米巴里发现一种巨型的病毒，他们发现大病毒体内还感染有另一种小病毒。他们戏称称这种病毒为卫星病毒，取名为“噬病毒体”。

# 天然DNA-RNA嵌合病毒的发现

| BLASTp RESULTS FOR REP PROTEINS | | | | | | |
|---|---|---|---|---|---|---|
| Accession | Description | Max score | Total score | Query coverage | E value | Max ident |
| | Global Ocean Survey | | | | | |
| 142008899 | GOS_10666 | 156 | 169 | 67% | 7.0E-49 | 50% |
| 134313056 | GOS_6801 | 132 | 147 | 67% | 4.0E-40 | 43% |
| | Circovirus | | | | | |
| 189916555 | PCV2 | 149 | 149 | 79% | 2.0E-45 | 33% |
| 96980765 | StCV | 140 | 140 | 79% | 2.0E-42 | 33% |
| | Cyclovirus | | | | | |
| 324309805 | DfCyV | 114 | 147 | 61% | 3.0E-33 | 38% |
| | Integrated Protozoan | | | | | |
| 253743285 | Giardia intestinalis ATCC 50581 | 115 | 149 | 76% | 6.0E-33 | 35% |
| 183234913 | Entamoeba histolytica HM-1-IMSS | 56.2 | 70.8 | 40% | 4.0E-14 | 32% |
| | Phytoplasmal plasmids | | | | | |
| 13434985 | Onion yellows phytoplasma | 46.2 | 46.2 | 29% | 7.0E-10 | 29% |
| 327202124 | Periwinkle leaf yellowing phytoplasma | 43.1 | 59.3 | 33% | 6.0E-09 | 44% |
| | Nanovirus | | | | | |
| 71532925 | BBTV | 40 | 72.4 | 37% | 4.0E-08 | 57% |
| 20530225 | SCSV | 40 | 73.9 | 34% | 4.0E-08 | 36% |
| | Geminivirus | | | | | |
| 20564197 | TPCTV | 21.9 | 84.3 | 37% | 2.4E-02 | 57% |
| 10257478 | HrCTV | 13.9 | 13.9 | 5% | 7.8E+00 | 33% |
| | Microvirus | | | | | |
| 9791179 | CPAR39 | 17.7 | 17.7 | 2% | 5.2E-01 | 55% |
| 12085145 | phiMH2K | 14.2 | 14.2 | 1% | 6.8E+00 | 63% |

在2013年4月19日的《Biology Direct》杂志上发表了一篇来自美国波特兰州立大学的研究人员的论文，研究人员从冒泡的火山温泉有害环境中，发现了一个新病毒基因组，看起来它是一种DNA病毒和RNA病毒的重组产物——一种前所未见的天然嵌合体。

# 高通量测序技术在发现新的病毒中的应用

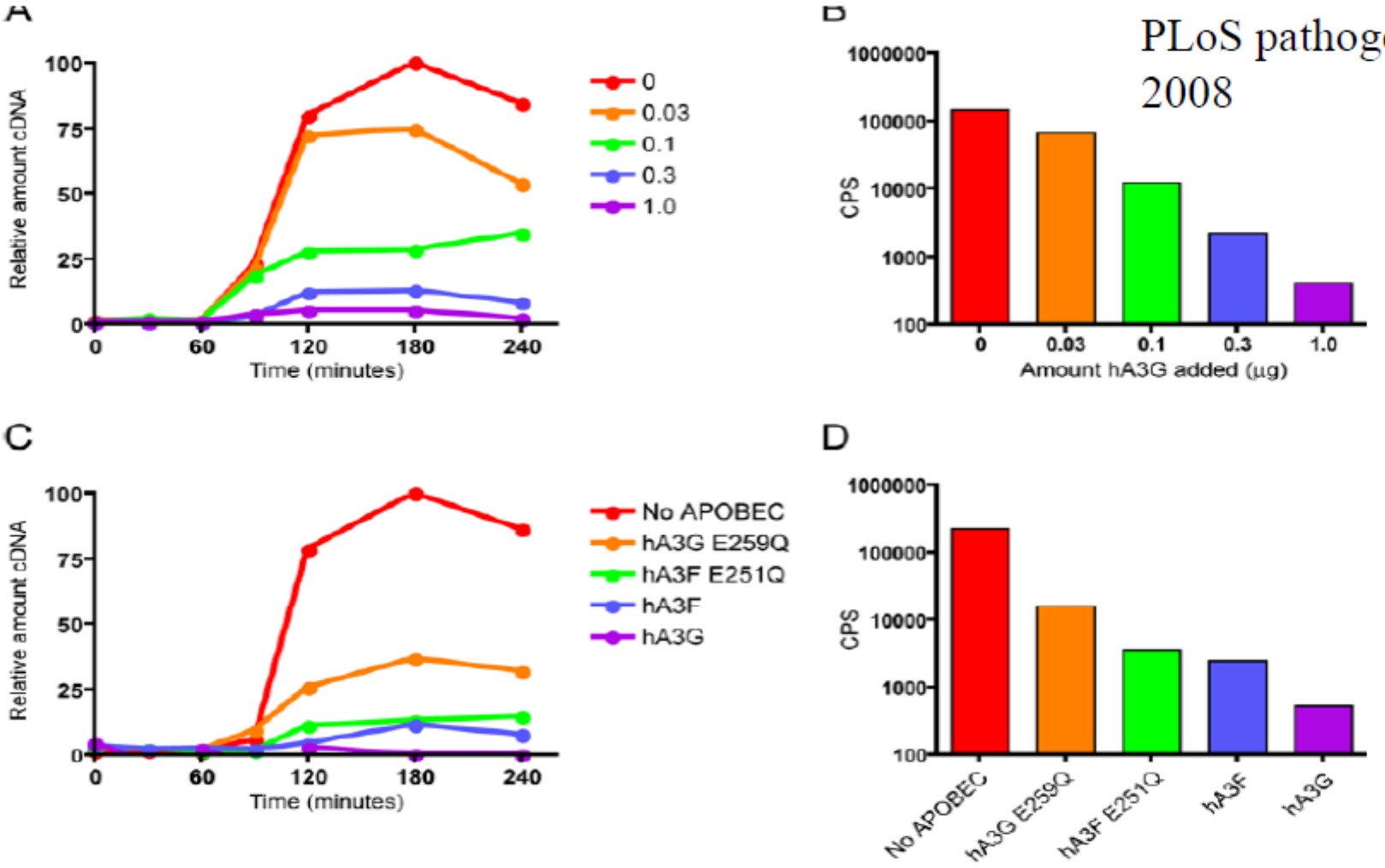
21世纪，测序技术迅猛发展，高通量测序技术能一次并行对几十万到几百万条DNA分子进行序列测定。

通过对呼吸道分泌物和胃肠道内容物测序，发现了新的致病和非致病的新病毒。结合基因组、宏基因组分析，新的多瘤病毒、海洋病毒及噬菌体也相继被发现。

# New progress

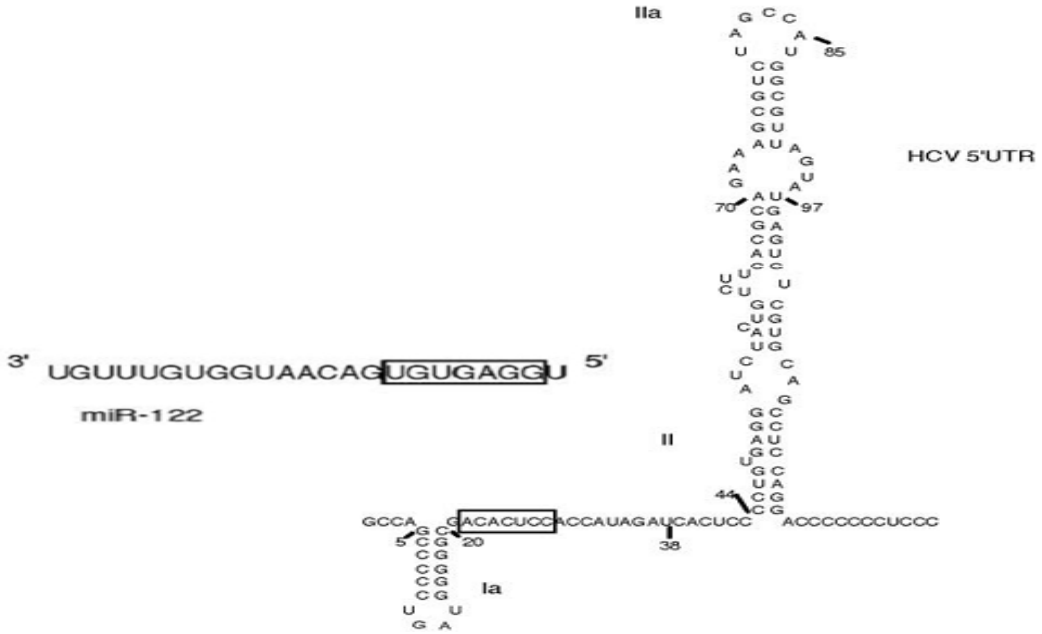
# Virus-host interactions

Kate N. Bishop
APOBEC3G Inhit
Elongation of HIV
Reverse Transcrip
PLoS pathogen, D
2008



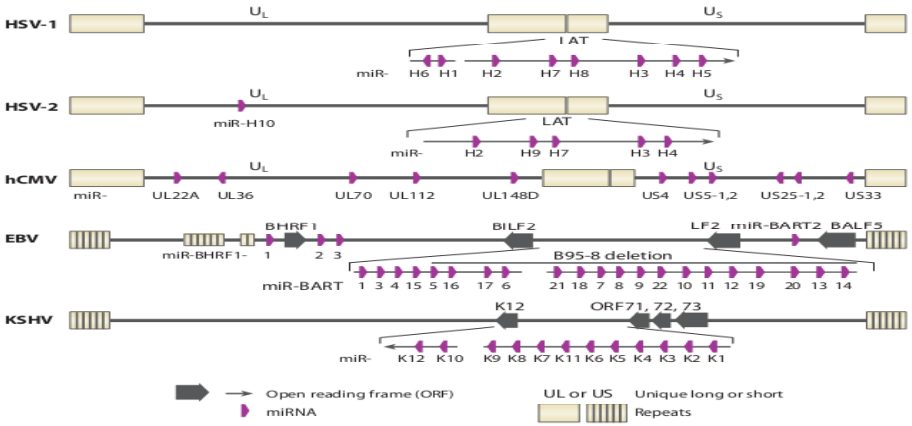
# Science：颠覆miRNA在病毒中的作用

通过高通量测序，人们发现人类70%的基因被转录成RNA，而事实上只有不到2%的基因编码蛋白，越来越多的非编码RNA被发现在人类的生命活动过程中扮演着重要的角色。




斯坦福大学医学院的研究人员发现了在肝脏肝炎C型（丙型）病毒积累过程中miRNA的新作用——肝脏特有的microRNA-122(miR-122)与肝炎病毒mRNA5’非编码区相互作用引起病毒增殖，这一结果发表在Science上。

# 病毒世界的非编码RNA




非编码RNA不单单存在于人类这种灵长类大生物，病毒同样具有非编码RNA。2004年，Pfeffer等首次在EB病毒中发现了5个miRNA，到目前为止，已经发现了超过200种的病毒miRNAs，主要是在疱疹病毒、多瘤病毒和腺病毒。

| Length | Name | Virus | Characteristics |
|---|---|---|---|
| <200 nt | *EBERs* (*EBER1*, *EBER2*) | Epstein–Barr virus | ~170 nt, play roles in oncogenesis and modulate innate immune signaling |
| | *HSURs* (*HUSR1*, *HUSR2*) | Herpesvirus saimiri | *HSUR1* (143 nt), *HSUR2* (115 nt); *HSUR1* directs degradation of *miR-27* to manipulate host T-cell gene expression |
| | *VA I* and *II* | Human adenovirus | ~160 nt, block PKR activity, avoiding phosphorylation of eIF-2α and inhibition of viral mRNA translation; can be processed by Dicer into small RNAs that are incorporated into RISC |
| >200 nt | *β2.7* | Human cytomegalovirus | 2.7 kb, binds to the mitochondrial enzyme complex I, protecting virus-infected cells from apoptosis, resulting in continued ATP production |
| | *sfRNA* | Flaviviruses | 0.3–0.5 kb, produced from the incomplete degradation of the viral genome by the host exonuclease XRN1 and required for virus-induced cytopathicity and pathogenicity |

随后，病毒编码的长非编码RNA（Long Nocoding RNA,lncRNA）也相继被发现,它们具有多种功能，如加强病毒的复制、调节宿主的免疫功能及促进肿瘤的形成等。

# HIV相关研究的最新进展

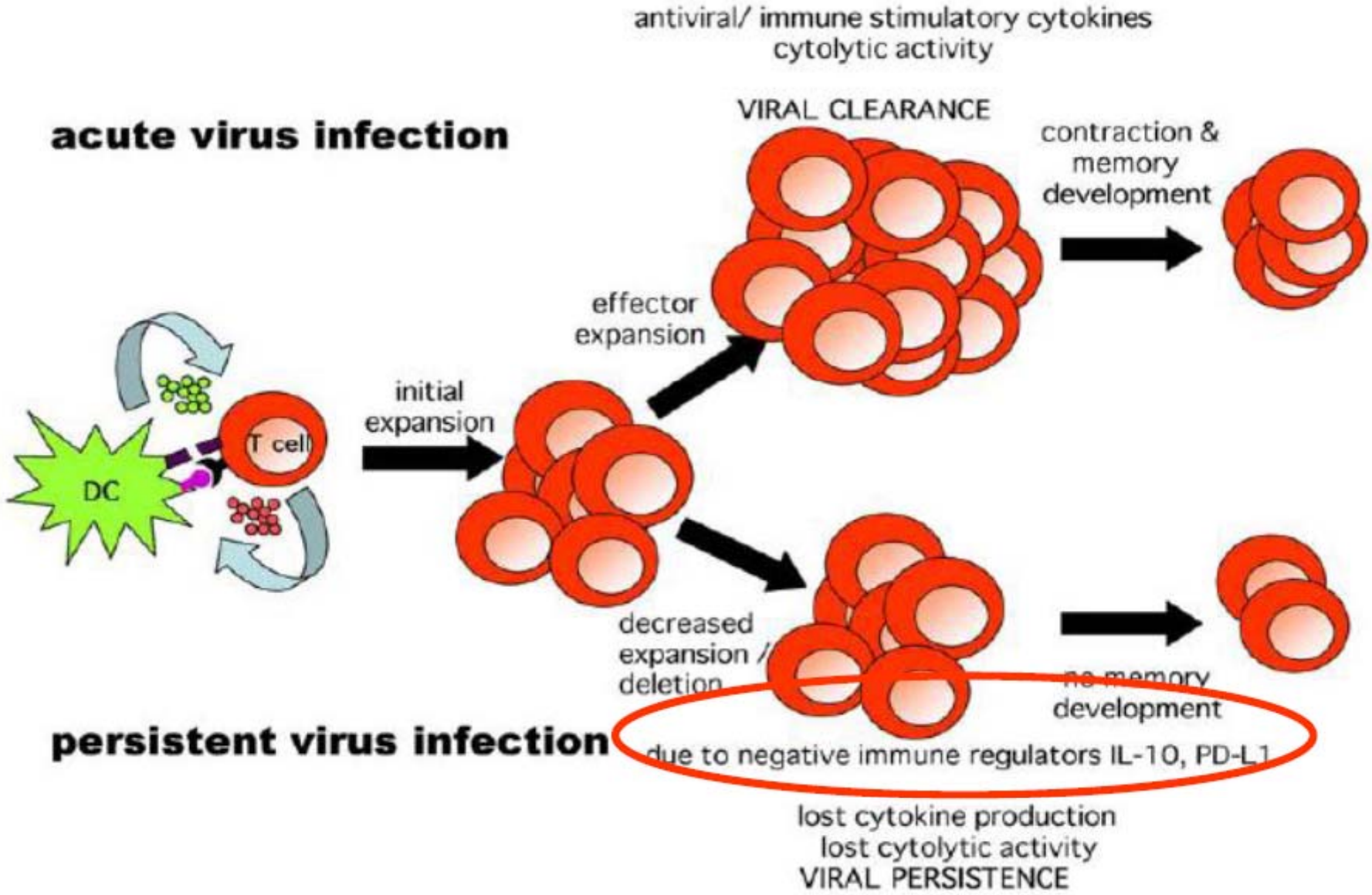
Tetherin：一种可阻止艾滋病病毒扩散的蛋白质分子的发现

HIV依赖因子：利用RNAi技术筛选出273个与HIV复制、增　　殖有关的宿主基因

CBFβ调节HIV-vif介导的免疫逃逸：在没有CBFβ存在的情况下，病毒无法抵抗宿主体内的天然抗病毒因子，从而无法完成复制

IFN-I启动HIV持续性感染：在小鼠体内证实，IFN-I启动了持续性感染，限制了产生有效的抗病毒反应

# New Progress :Persistent infections



# New progress: Vaccines –

- Therapeutic human papillomavirus DNA vaccination strategies to control cervical cancer

T.-C. Wu

Departments of Pathology, Oncology, Obstetrics and Gynecology ar
MolecularMicrobiology and Immunology, The Johns Hopkins Medical Institutions, Baltimore,
MD, USA

- A Randomized Controlled Phase IIb Trial of Antigen-Antibody Immunogenic Complex Therapeutic Vaccine ir Chronic Hepatitis B Patients

Dao-Zhen Xu1, Kai Zhao2, Li-Min Guo1, Xin-Yue Chen3, Hui-Fen Wang4, Ji-Ming Zhang5, Qin Xie6, Hong Ren7, Wen-Xiang Wang8, Lan-Juan Li9, Min Xu10, Pei Liu11, Jun-Qi Niu12, Xue-Fan Bai13, Xin-Liang Shen2, Zheng-Hong Yuan14, Xuan-Yi Wang14,15, Yu-Mei Wen14*

# HPV疫苗的成功应用

2007年，宫颈癌疫苗问世，宣告人类以对抗病毒感染来防止肿瘤发生的时代来临。抗病毒治疗逐渐登上了抗癌的舞台，成为举世关注的焦点。

# New progress- JV Feb

## Papillomavirus Prophylactic Vaccines: Established Successes, New Approaches[▽]

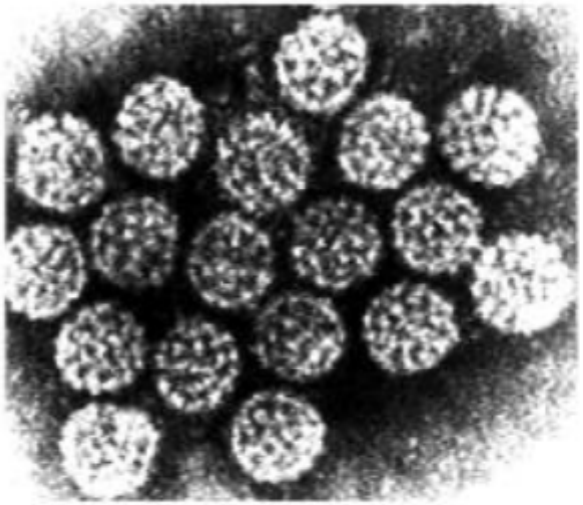
M. Saveria Campo[1]* and Richard B. S. Roden[2]*

*Division of Pathological Sciences, Institute of Comparative Medicine, Garscube Campus, University of G Glasgow G61 1QH, United Kingdom,[1] and Department of Pathology, The Johns Hopkins Universit Baltimore, Maryland 21231[2]*

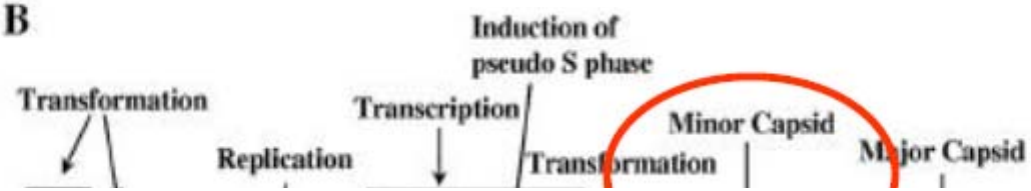
**Vaccines against the human papillomaviruses (HPVs) most frequently associated with cancer of th are now available. These prophylactic vaccines, based on virus-like particles (VLPs), are extremely e providing protection from infection in almost 100% of cases. However, the vaccines present some limi they are effective primarily against the HPV type present in the vaccine, are expensive to produce, and cold chain. Vaccines based on the minor capsid protein L2 have been very successful in animal models a been shown to provide a good level of protection against different papillomavirus types. The pote L2-based vaccines to protect against many types of HPVs is discussed.**

---

**A**



**B**



# New progress: Antiviral drug development

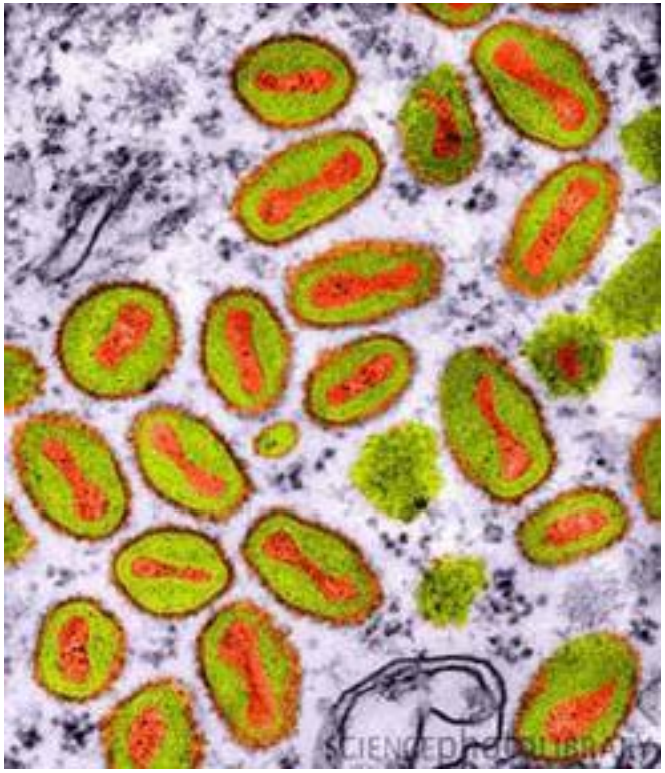
## The Cyclophilin Inhibitor Debio-025 Shows Potent Anti–Hepatitis C Effect in Patients Coinfected with Hepatitis C and Human Immunodeficiency Virus

Robert Flisiak,1 Andrzej Horban,2 Philippe Gallay,3 Michael Bobar

**Debio-025 is an oral cyclophilin (Cyp) inhibitor with potent anti-hepatitis C virus activity *in vitro*. Its effect on viral load as well a influence on intracellular Cyp levels was investigated in a rando double-blind, placebo-controlled study. Mean hepatitis C viral load decreased significantly by 3.6 log10 after a 14-day oral treatment with 1200 mg twice daily ($P < 0.0001$) with an effect ag the 3 genotypes (1, 3, and 4) represented in the study.**

**HEPATOLOGY 2008;47:817-826.**

# 溶瘤病毒：癌症病毒治疗的成功案例



来自加拿大渥太华大学，美国抗癌药研发公司Jennerex等的研究人员于2011年9月1日）在《Nature》杂志上公布了一种溶瘤病毒治疗癌症实验结果，这项研究首次利用一种静脉注射病毒靶向肿瘤，从而不会伤害到正常组织。提示病毒不仅会导致重大疾病，也可以作为治疗疾病的载体对人类的健康带来极大的帮助。

# 高通量测序技术在发现新的病毒中的应用

21世纪，测序技术迅猛发展，高通量测序技术能一次并行对几十万到几百万条DNA分子进行序列测定。

通过对呼吸道分泌物和胃肠道内容物测序，发现了新的致病和非致病的新病毒。结合基因组、宏基因组分析，新的多瘤病毒、海洋病毒及噬菌体也相继被发现。

# TECHNOLOGY DEVELOPMENT

## high-throughput sequencing and gene-mapping techniques

- Research in the 21st century will allow the identification of new families of organisms (including viruses) by high-speed sequencing of RNA and DNA.
- When deep sequencing of nucleic acids in a complex sample is merged with other powerful technologies, including mass spectrometry, proteomics, optical imaging, and high-throughput screening using small molecules and short hairpin RNAs (shRNAs), we can be sure that the discovery pipeline for novel viruses and their antagonists will be full indeed.

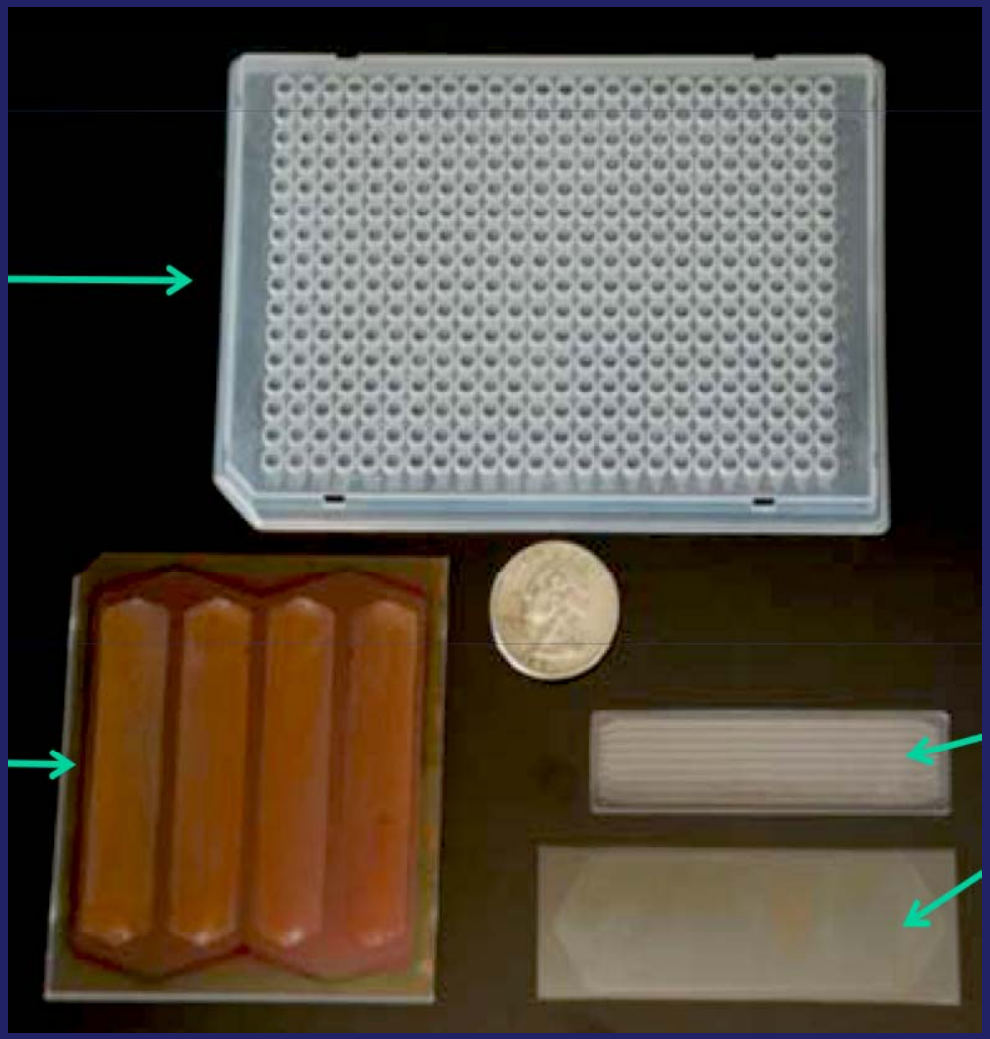
# Deep Sequencing Technologies

## Next-generation protocols

- Pyrosequencing (454 Technology)
  - 300-500 nucleotides long, modest throughput
- Illumina / Solexa technology
  - 150 x 2 nucleotides long, highest throughput
- ABI / SOLiD™ System
  - Sequencing by ligation
- Ion Torrent
  - No dyes, electric readout
- Pacific Biosciences
  - Extremely high read length, but low fidelity

## Comparison of Sequencing ‘Vessel’ Size and Capacity

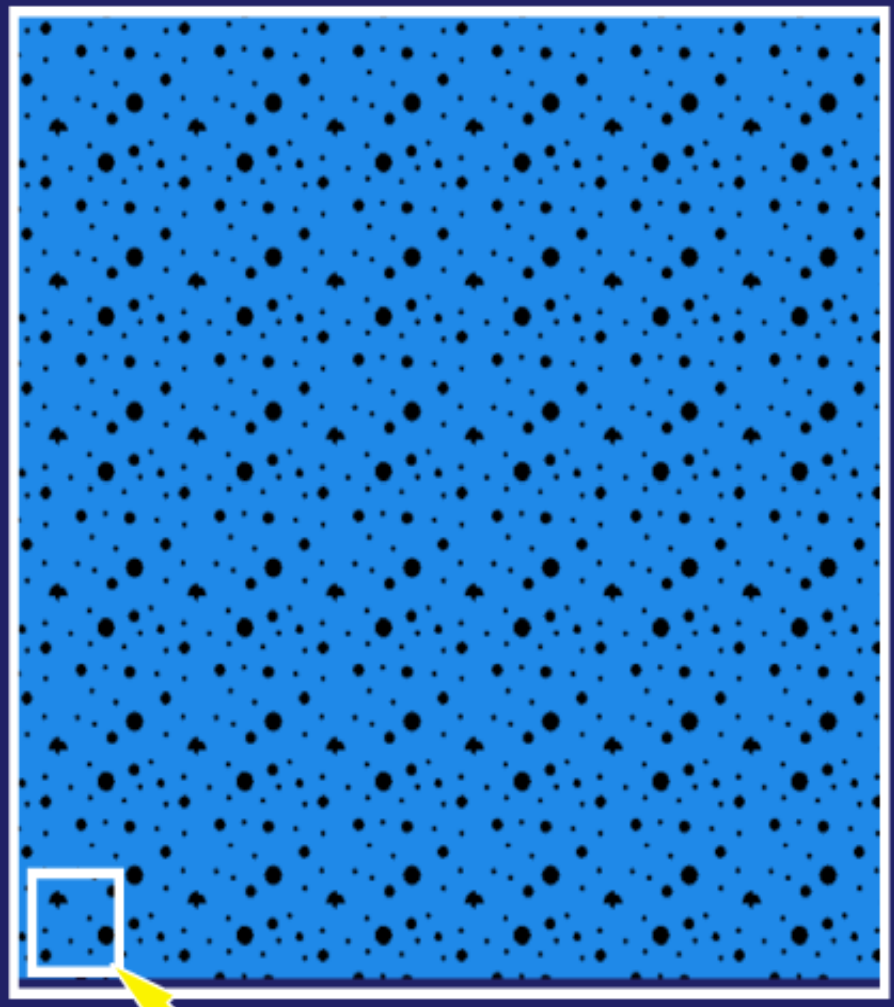
Sanger / 3730
384 rxn
0.0003 Gb



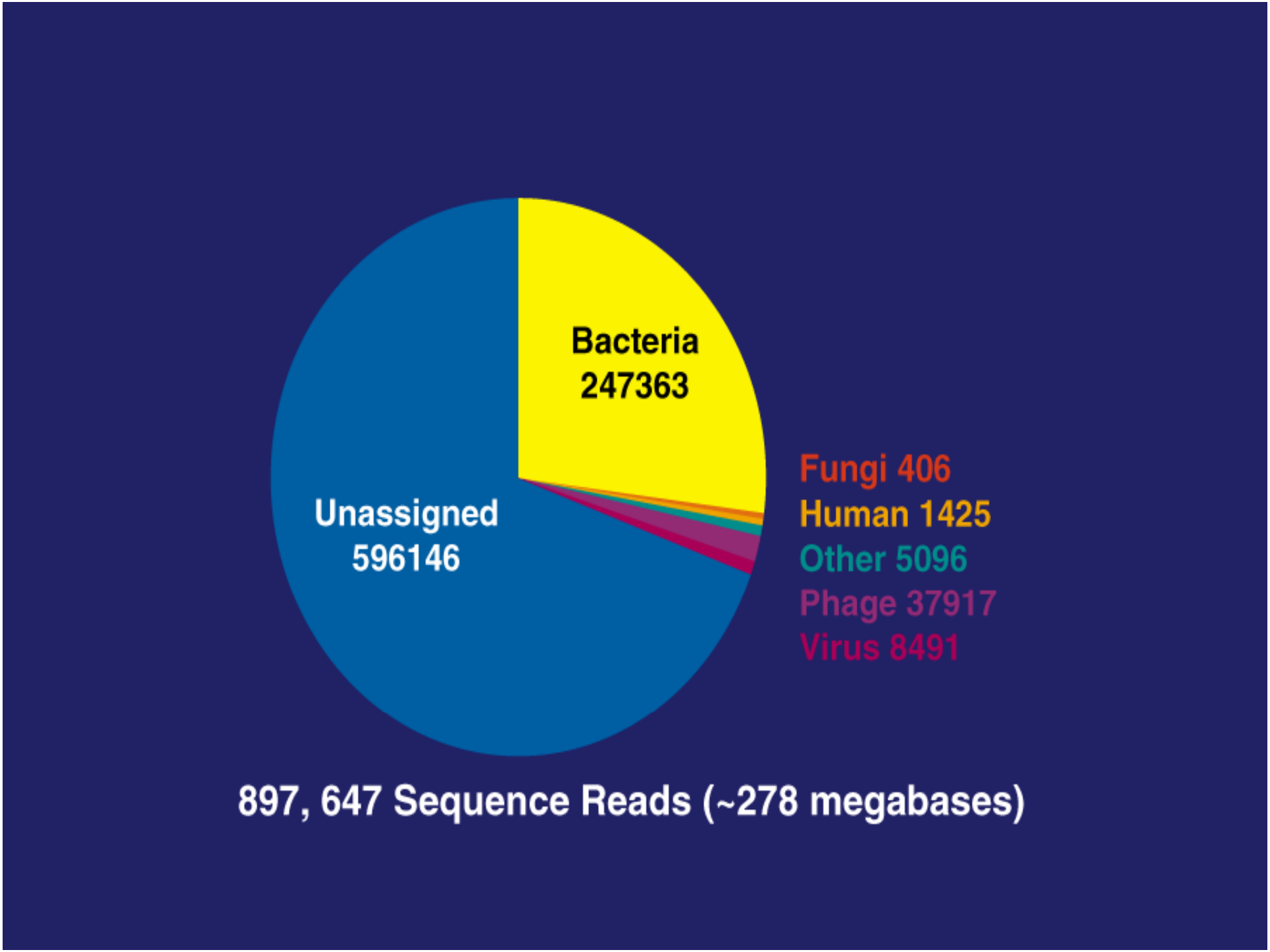
Roche GS
FLX
>1x10$^6$ rxn
0.5 Gb

Illumina GAiiX
or
SOLID 3
~600 x 10$^6$ rxn
60 Gb

# The World of Microorganisms




D. Relman, Sci., 1999


Bacteria
247363
Unassigned
596146
Fungi 406
Human 1425
Other 5096
Phage 37917
Virus 8491
897, 647 Sequence Reads (~278 megabases)

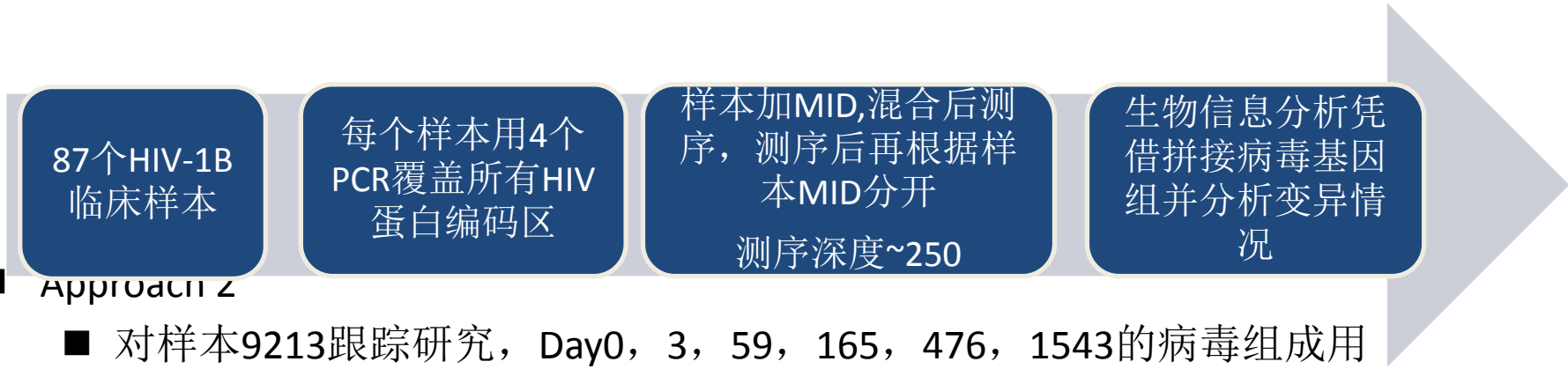
# Human Viruses Present in Raw Sewage

| Family | Species | Genome |
|---|---|---|
| Adenoviridae | Human adenovirus 41 | dsDNA |
| Astroviridae | Astrovirus MLB1 | ssRNA(+) |
| | Human astrovirus 1 | ssRNA(+) |
| Caliciviridae | Norwalk virus | ssRNA(+) |
| | Sapporo virus | ssRNA(+) |
| Papillomaviridae | Human papillomavirus 112 | dsDNA |
| Parvoviridae | Adeno-associated virus | ssDNA |
| | Human bocavirus 2 | ssDNA |
| | Human bocavirus 3 | ssDNA |
| Picobirnaviridae | Human picobirnavirus | dsRNA |
| Picornaviridae | Aichi virus | ssRNA(+) |
| | Human klassevirus 1/ Salivirus NG-J1 | ssRNA(+) |
| | Human parechovirus 1 | ssRNA(+) |
| | Human parechovirus 3 | ssRNA(+) |
| | Human parechovirus 4 | ssRNA(+) |
| | Human parechovirus 7 | ssRNA(+) |
| Polyomavividae | Polyomavirus HPyV6 | dsDNA(+) |

# 454高深度测序发现逃脱早期免疫反应的HIV病毒株

Henn MR, Boutwell CL, Charlebois P, Lennon NJ, Power KA, et al. (2012) Whole Genome Deep Sequencing of HIV-1 Reveals the Impact of Early Minor Variants Upon Immune Recognition During Acute Infection. PLoS Pathog 8(3): e1002529. doi:10.1371/journal.ppat.1002529

- 罗氏454测序系统对可以快速高效且经济地对病毒基因组样本进行测序。
- Approach 1
  - 对87个样本进行高深度的测序，结果进行病毒基因组拼接，检测突变，并与传统方进行对比。




- Approach 2
  - 对样本9213跟踪研究，Day0，3，59，165，476，1543的病毒组成用454FLX测序。可提供关于病毒在病程中快速进化演变、基因组高度变异区域、变异事件发生位点特征信息。

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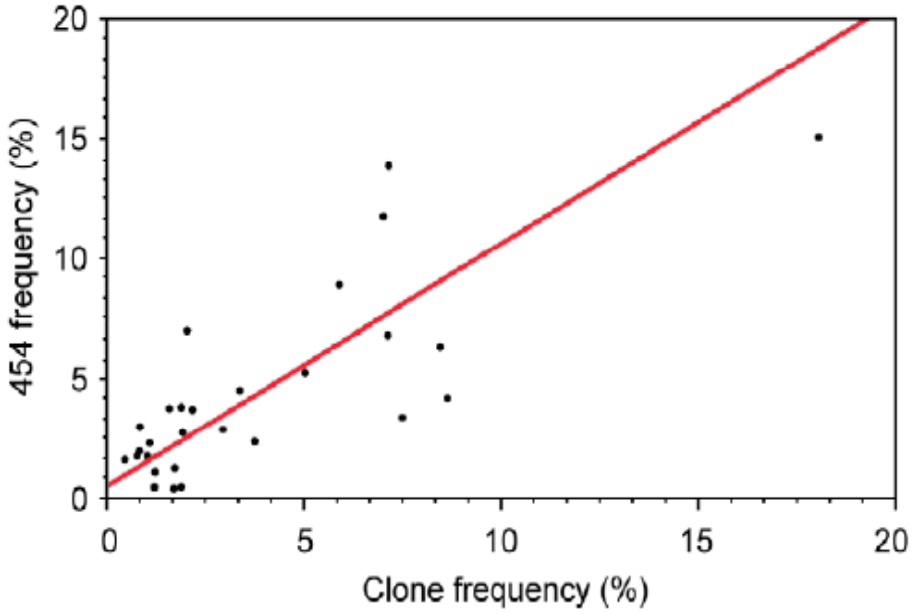


- 454测序以及克隆测序的一致性分析

- 98%以上样本拼接成一个contig。
- 新算法可以从〉x200深度的测序结果中发现低至1.0%的突变。同已知组成的样本比较，该方法的灵敏度可达到100%，准确度高达97%。
- 该方法与传统Sanger的大规模克隆测序以及 SGA方法检测结果比较，一致性达95.6%。证明该实验方法的可行性及正确性。

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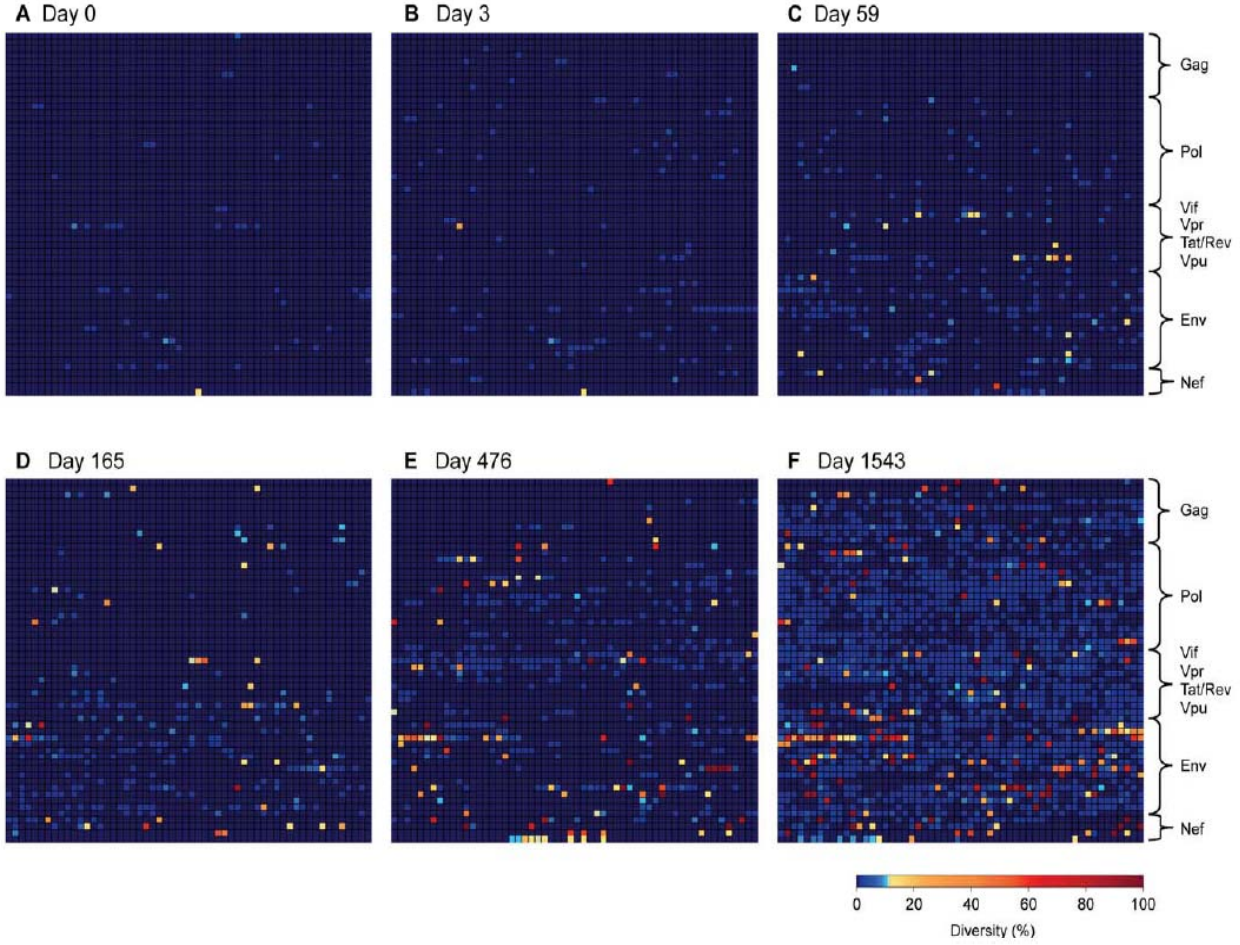
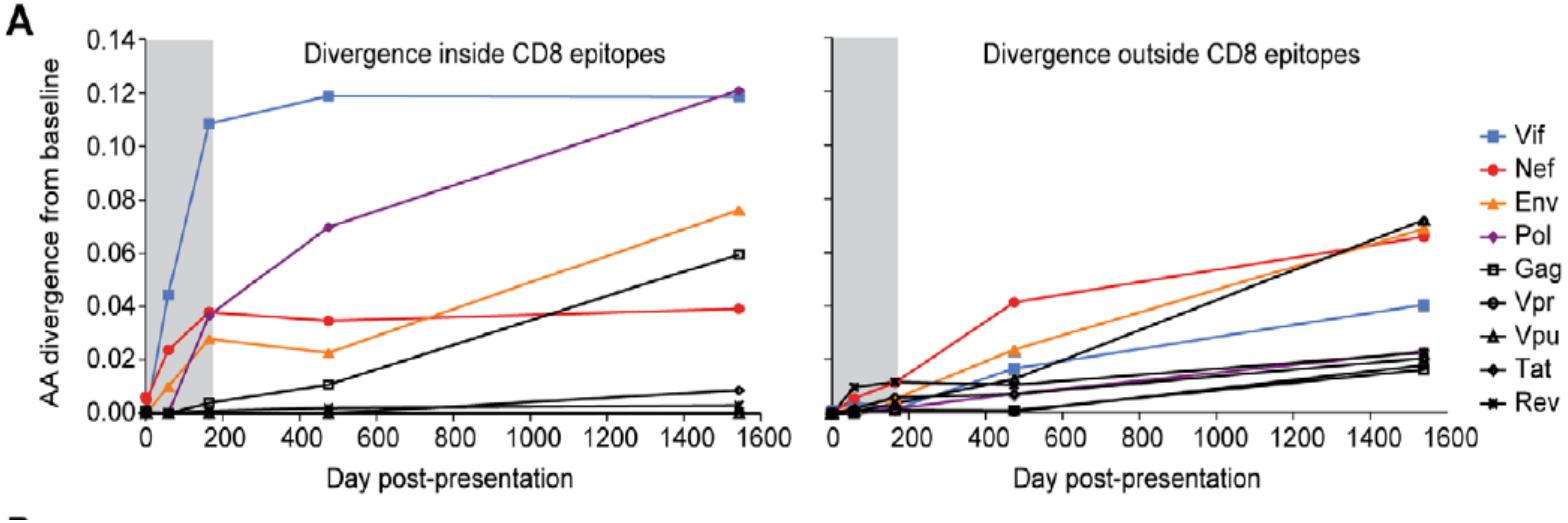



- 跟踪研究中，病毒每个蛋白对应序列在Day0，3，59，165，476，1543的变异情况，蓝色标识保守，红色表示高度变异

# 454高深度测序发现逃脱早期免疫反应的HIV病毒株

Henn MR, Boutwell CL, Charlebois P, Lennon NJ, Power KA, et al. (2012) Whole Genome Deep Sequencing of HIV-1 Reveals the Impact of Early Minor Variants Upon Immune Recognition During Acute Infection. PLoS Pathog 8(3): e1002529. doi:10.1371/journal.ppat.1002529

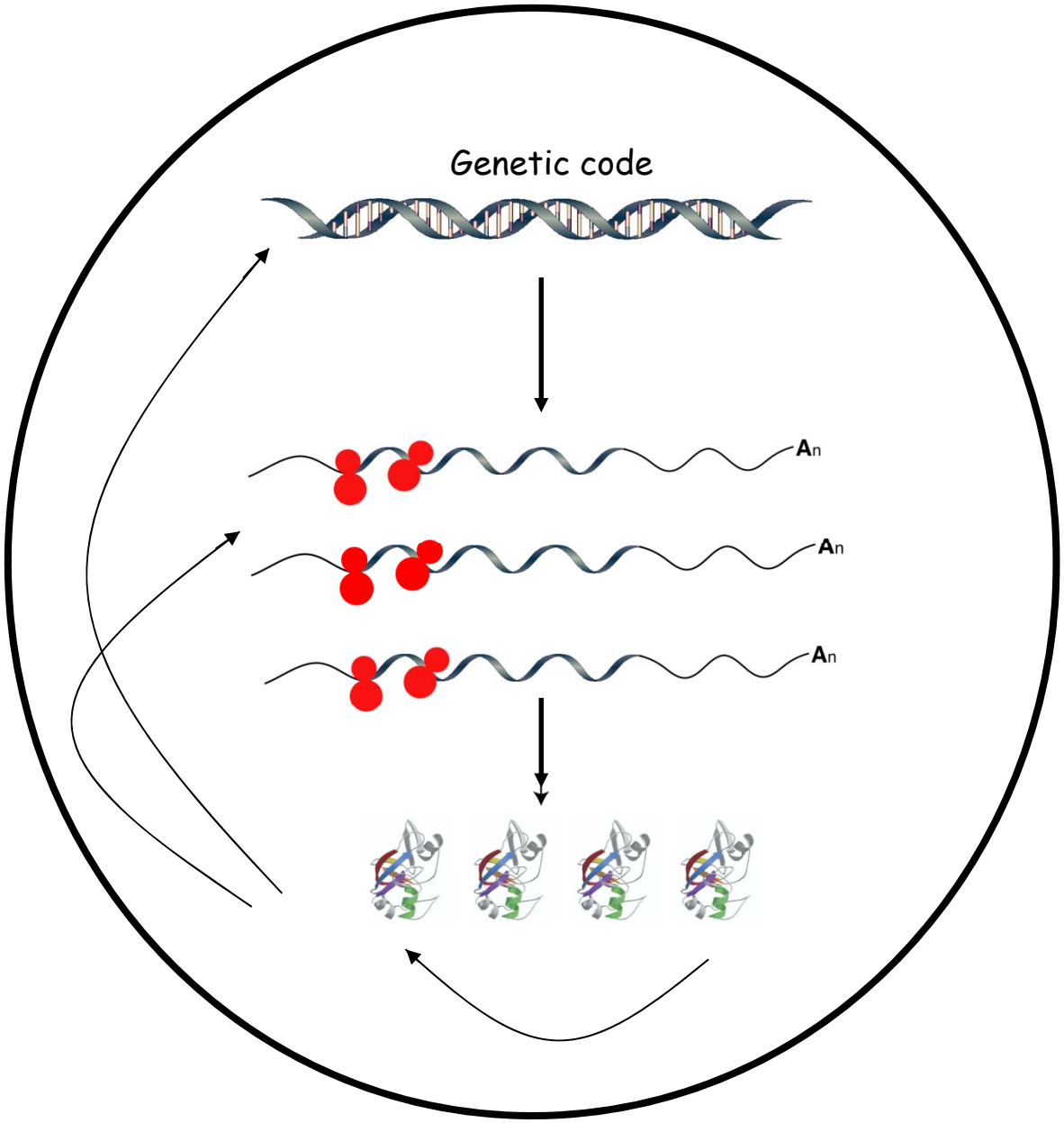
- 跟踪研究表明，在感染初期病人体内的HIV基因组多态性较低，可能只是受到单病毒株的感染，两个月后，病毒多态性迅速增加
- CD8+ T细胞识别的区域变异频率高，推测CD8+ T细胞对变异起到了筛选作用
- 少数含有CD8+ T细胞识别位点的突变病毒株能逃脱急性免疫应答，在感染初期消除这些个体成为艾滋病治疗的重要目标
- 了解病毒基因组进化变异情况，可发现保守区域，并针对性的设计疫苗，为艾滋病新疫苗的研发提供新的依据。




上图为CD8 eptiopes区域内与区域外变异发生情况的比较。横轴为感染的天数。

# Regulation using micro RNAs (miRNA's)

# Basic Unit of Genetic Circuitry



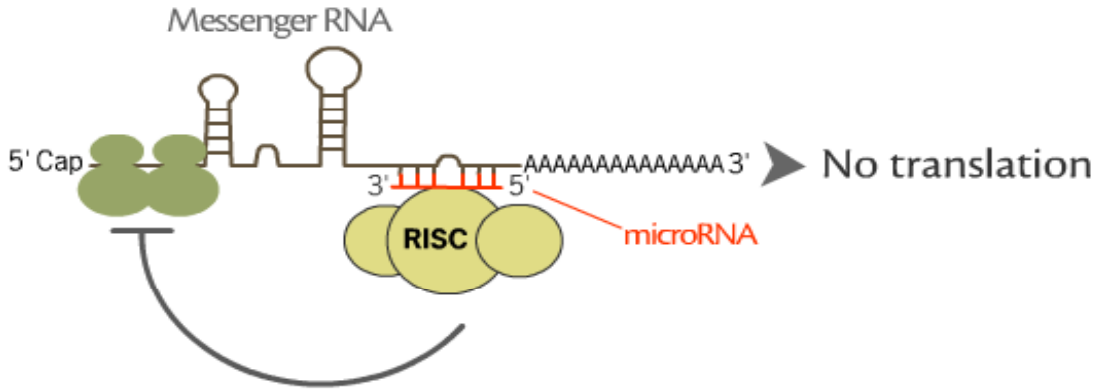

Protein-coding Genes

Biological Function (Proteins)

http://microrna.sanger.ac.uk/sequences/

# What are microRNAs?

Mammalian genomes are predicted to encode 200 to 500 unique miRNAs that together regulate expression of at least one-third of all genes.




**MicroRNA (miRNA): A type of non-coding small RNA (~21–23 nucleotides) produced by Dicer from a stem-loop structured RNA precursor (~70-90 nts ong). miRNAs are widely expressed in animal and plant cells as RNA–protein complexes, termed miRISCs, and have been implicated in the control of development because they lead to the destruction or translational suppression of target mRNAs with homology to the miRNA.**

NATURE | VOL 391 | 19 FEBRUARY 1998

# The Nobel Prize in Physiology or Medicine 2006

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

Photo: L. Cicero/Stanford

Photo: R. Carlin/UMMAS

**Andrew Z. Fire**

1/2 of the prize

USA

Stanford University School of Medicine
Stanford, CA, USA

**Craig C. Mello**

1/2 of the prize

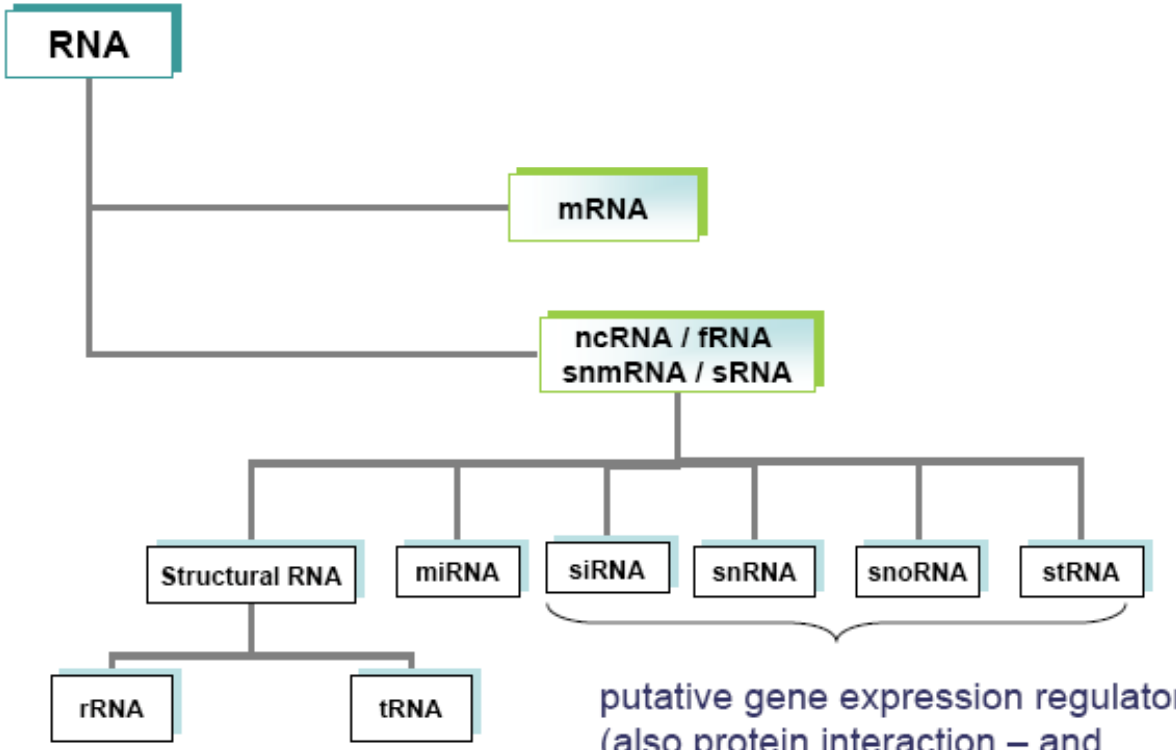
USA

University of Massachusetts Medical School
Worcester, MA, USA

# Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*

Andrew Fire*, SiQun Xu*, Mary K. Montgomery*, Steven A. Kostas*†, Samuel E. Driver‡ & Craig C. Mello‡

# RNome or transcriptome



# RNome or transcriptome

**types of RNA:**

| | |
|---|---|
| **mRNA** | messenger RNA - transcript of a protein coding gene |
| **rRNA** | ribosomal RNA - form large parts of the ribosome, the protein producing machinary |
| **tRNA** | transfer RNA - also involved in protein production, carrying single amino acids to the growing amino acid chain of a protein |
| **ncRNA** | non coding RNA - found in intergenic regions, playing miscellaneous roles |

Non-coding RNA (ncRNA) genes produce functional RNA molecules rather than encoding proteins and here are the nominees:

| | | |
|---|---|---|
| **fRNA** | Functional RNA | essentially synonymous with non-coding RNA |
| **miRNA** | MicroRNA | 21-24 nucleotide RNAs probably acting as translational regulators mRNA |
| **siRNA** | Small interfering RNA | active molecules in RNA Interference |
| **snRNA** | Small nuclear RNA | includes spliceosomal RNAs |
| **snmRNA** | Small non-mRNA | essentially synonymous with small ncRNAs |
| **snoRNA** | Small nucleolar RNA | most known snoRNAs are involved in rRNA modification |
| **stRNA** | Small temporal RNA | for example, lin-4 and let-7 in Caenorhabditis elegans |

# miRNAs play important roles in all aspects of life

- Brain development (miR-430)
- Patterning of nervous system (miR-273)
- Pancreatic islet-cell development (miR-375)
- Adipocyte differentiation (miR-143)
- Limb patterning (miR-196)
- Heart development (miR-1)
- Programmed cell death (miR-14)
- Lymphocyte maturation and organogenesis (miR-181)
- Some viruses encode their own miRNA genes (EBV, CMV,…)

| Sanger release | Date | known miRNAs |
|---|---|---|
| 1.0 | 2002 | 218 |
| 2.2 | 2003 | 593 |
| 5.1 | 2004 | 1420 |
| 7.1 | 2005 | 3424 |
| 9.0 | 2006 | 4361 |

*Homo sapiens*: 474 miRNAs

*Mus musculus*: 373 miRNAs

## List of disease related genes Targeted by siRNA is rapidly Increaseing

| ***Cancer*** | ***siRNA target*** |
|---|---|
| CML | BCR/ABL fusion protein |
| Leukemia | c-raf, bcl-2 |
| Cervical carcinoma | E6, E7 (HPV) |
| Pancreatic carcinoma | K-RAS$^{V12}$ |
| Melanoma | ATF2 |
| | BRAF$^{V599E}$ |
| Ovarian carcinoma | H-Ras |
| | mVEGF |
| | COX-2 |
| Prostate cancer | P110, p110B of PI 3 kinase |
| Wilms' tumor | Wt1, Pax2, Wnt4 |

| ***Virus*** | ***siRNA target*** |
|---|---|
| HIV-1 | LTR, *vif, nef* |
| | *rev* |
| | *Tat, Rev* |
| | Gag |
| | CCR5 |
| | CD4, *Gag* (p24), *nef* |
| | CCR5, p24 |
| | CCR5 |
| | CCR5 |
| | *gag, pol* |
| | *env* |
| | *tat* |
| Poliovirus | Capsid, viral polymerase |
| Hepatitis B | Core region (3.5 kb RNA) |
| | Pregenomic RNA (seven shRNA) |
| Hepatitis C | EMCV-IRE5, NS3, NS5B, NA |
| | Core, NS4B |
| | 5' UTR |
| | NS3, NS5B |
| | NS5A |
| | NS5B |
| Rous sarcoma virus | Gag |
| Respiratory syncytial virus | Phosphoprotein (P) |
| | Fusion protein (F) |
| Influenza A | NP, PA, PB1, PB2, M, NS |
| Rotavirus | VP4 |
| Adenovirus (group B) | CD46 (cellular coreceptor) |
| Gammaherpesvirus | Rta, ORF45 |

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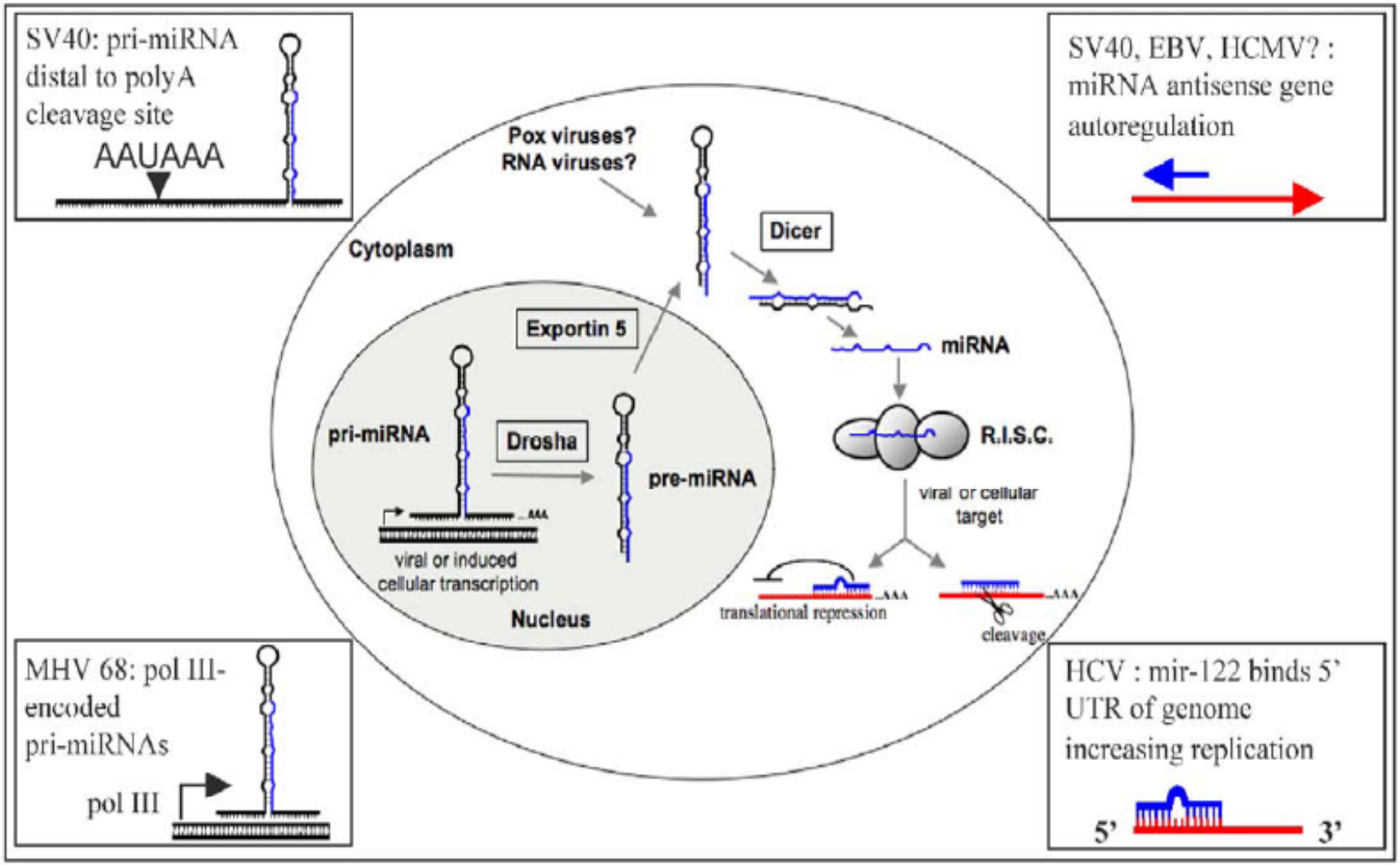
| 9.0 | 2006 | 4361 |

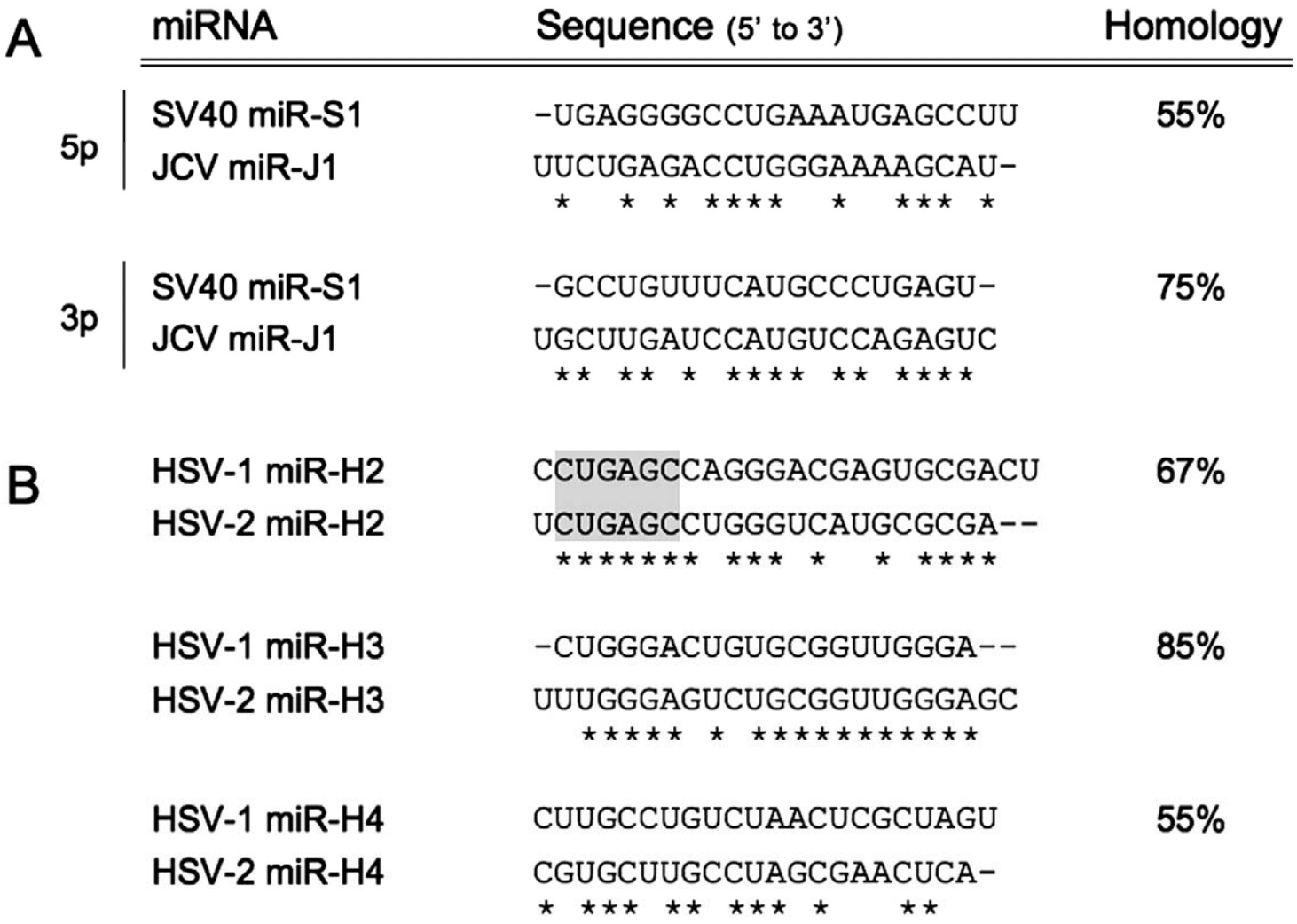
*Homo sapiens*: 474 miRNAs

*Mus musculus*: 373 miRNAs

# Some viruses encode miRNAs



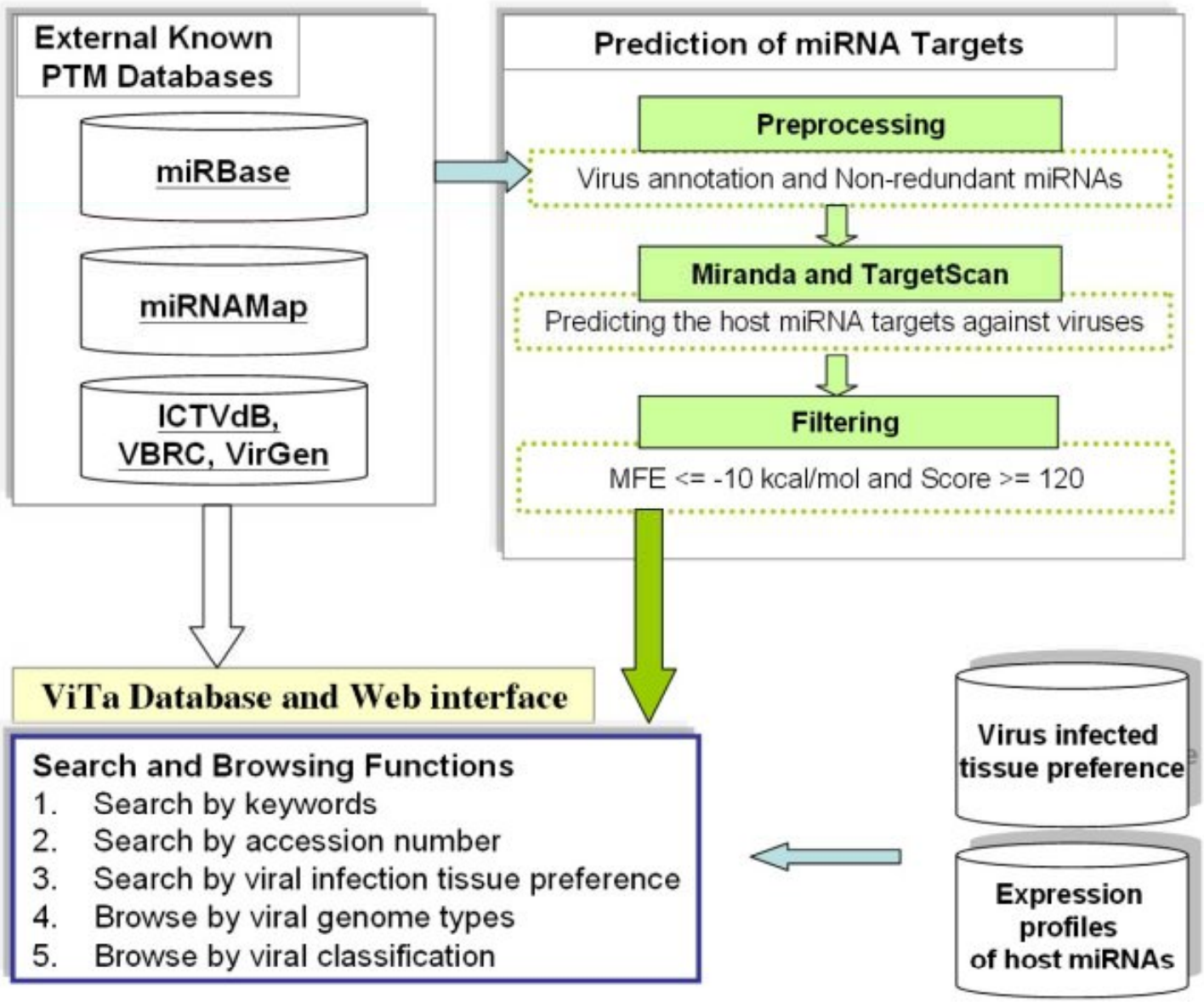
**Several viral miRNAs show sequence similarity but lack a conserved miRNA seed region.**



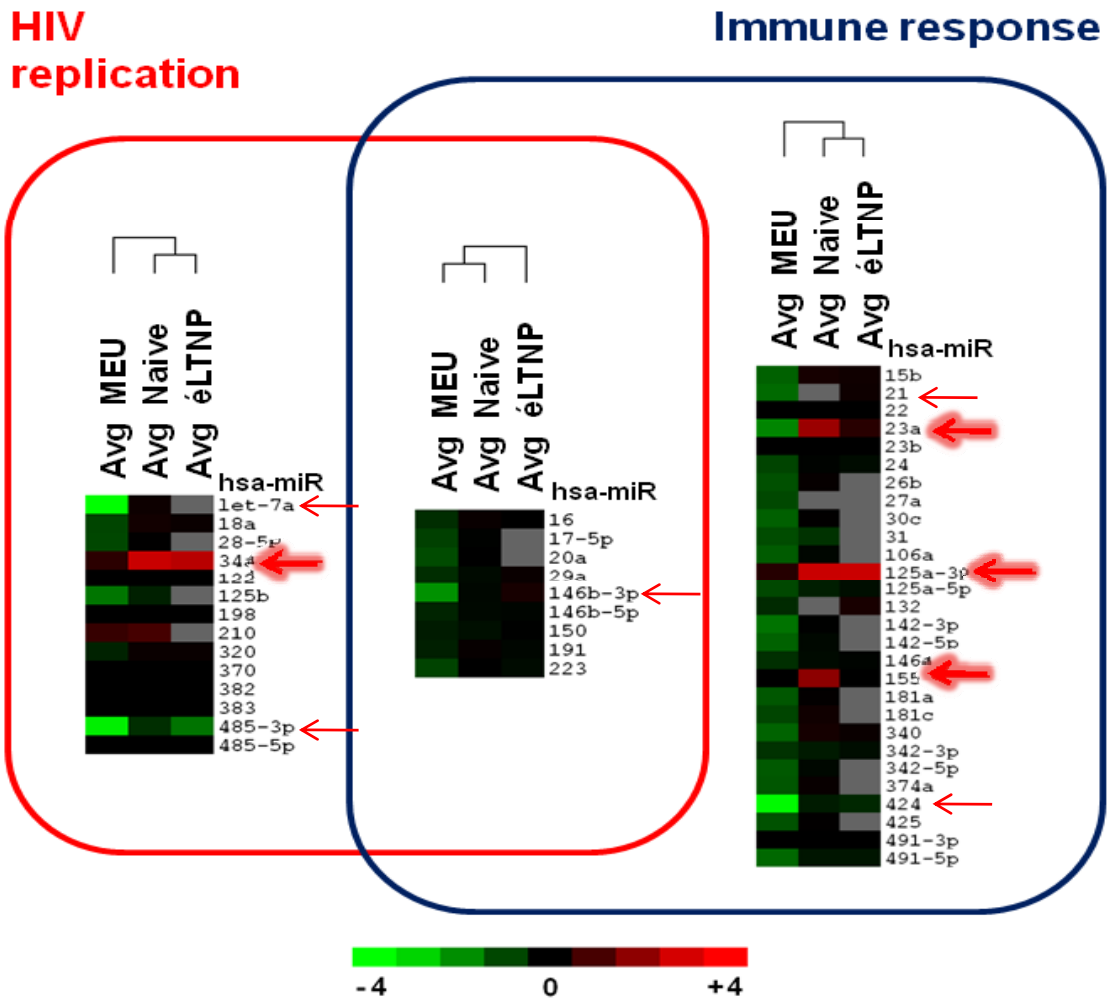

**Umbach J L , Cullen B R Genes Dev. 2009;23:1151-1164**

# Tayloring the RNAi to viral diseases

- Delivery of siRNAs for transient suppression, appropriate for acute viral infections such as influenza or SARS
- Delivery of shRNA expression constructs can provide more sustained RNAi that is suitable for chronic infections such as hepatitis or HIV-1
- Understanding the interplay between virus and RNAi for viral treatment (viral RNAi defence)


External Known PTM Databases
miRBase
miRNAMap
ICTVdB, VBRC, VirGen
Prediction of miRNA Targets
Preprocessing
Virus annotation and Non-redundant miRNAs
Miranda and TargetScan
Predicting the host miRNA targets against viruses
Filtering
MFE <= -10 kcal/mol and Score >= 120
ViTa Database and Web interface
Search and Browsing Functions
1. Search by keywords
2. Search by accession number
3. Search by viral infection tissue preference
4. Browse by viral genome types
5. Browse by viral classification
Virus infected tissue preference
Expression profiles of host miRNAs

# Which are the miRNA involved in HIV-1 replication or immune response?



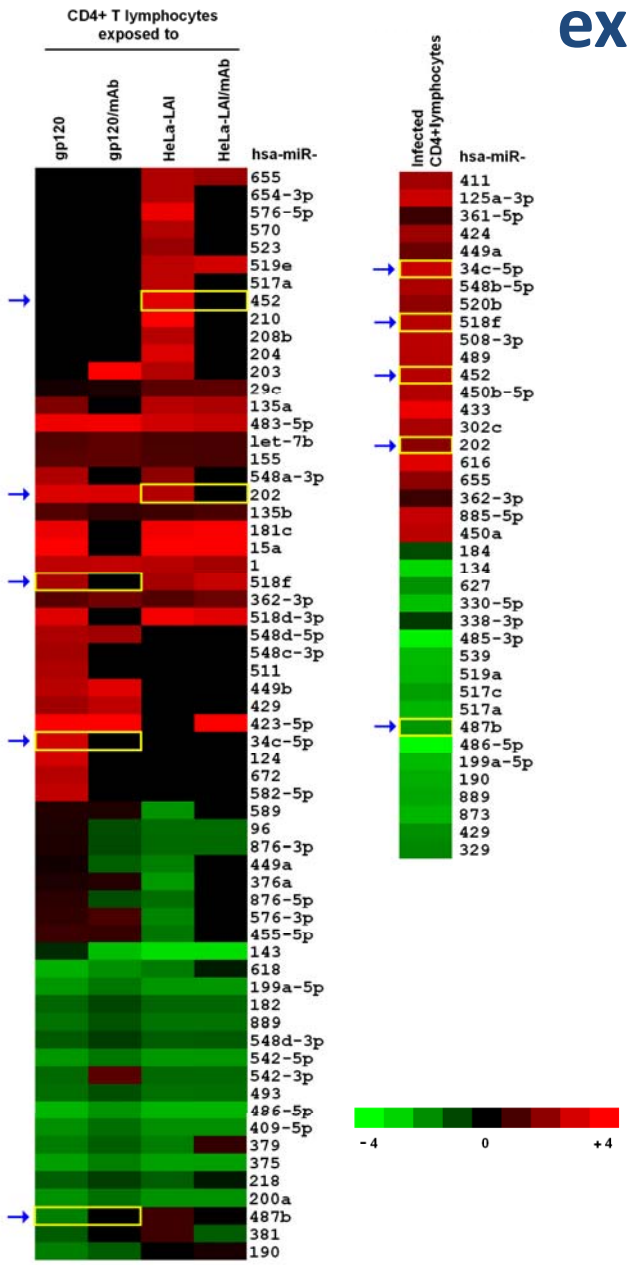

**Out of 377 miRNAs tested, only a few are implicated in HIV replication and immune response.**

**Among these, some (like 34a and 125a-3p) distinguish infected from uninfected patients in HIV replication and immune response, respectively.**

**In addition, 23a and 155 miRNAs characterize Naive from the other two groups.**

**In the MEU group, most of the miRNAs involved in the control of the immune response are down-regulated.**

# Which miRNAs are similarly altered after gp120 exposure and in vitro infection?




**5 out of 23 miRNAs, which expression may be related to CD4/gp120 binding site, were also found altered after in vitro infection with X4 strain.**

**Of note these miRNAs (34c-5p, 518f, 452, 202, 487b) showed the same expression levels in both conditions (gp120 exposure and in vitro infection).**

**Can these miRNAs modulate HIV entry?**

# Conclusions

- **miRNAs discriminate infected from uninfected subjects.**

- **A signature of HIV exposure may be defined.**

- **There is no difference between miRNA profiles of Naive and éLTNP groups.**

**-Only exposure to HIV is enough to induce a marked alteration of miRNA patterns and consequently may influence immune function of CD4+ T cells.**

**-Thus a miRNA pattern may reflect a change in the immune system.**

# Therapy development

| mRNA target | Disease | Method |
|---|---|---|
| HIV-1 | AIDS | AS/siRNA |
| H-Ras R12 | Cancer | siRNA |
| NTFα | Rheu. Arthritis | siRNA |
| BCL-ABL | Leukemia (CLL) | siRNA |
| Influenza virus | Influenza infection | siRNA (T. Smith) |
| RSV virus | RSV infection | 27 mer siRNA (M. Behlke P. McCray) |
| MCAD | MCAD deficiency | STAR |

# 纲 要

- 病毒学发展史
- 医学分子病毒学研究重大事件和主要进展
- 医学分子病毒学研究热点和或重点
- 医学分子病毒学总结和展望

# 分子病毒学研究的热点和主要进展

病毒与宿主细胞的相互作用

病毒与免疫细胞的相互作用

机体对病毒的识别和清除及病毒逃逸或调控细胞的防御功能

# 病毒与宿主细胞的相互作用

- 细胞受体、病毒粘附、细胞表面改变
- 病毒复制及与细胞蛋白等的相互作用
- 影响蛋白代谢
- 影响信号传递
- 影响细胞骨架
- 对胞膜及胞内膜的靶向

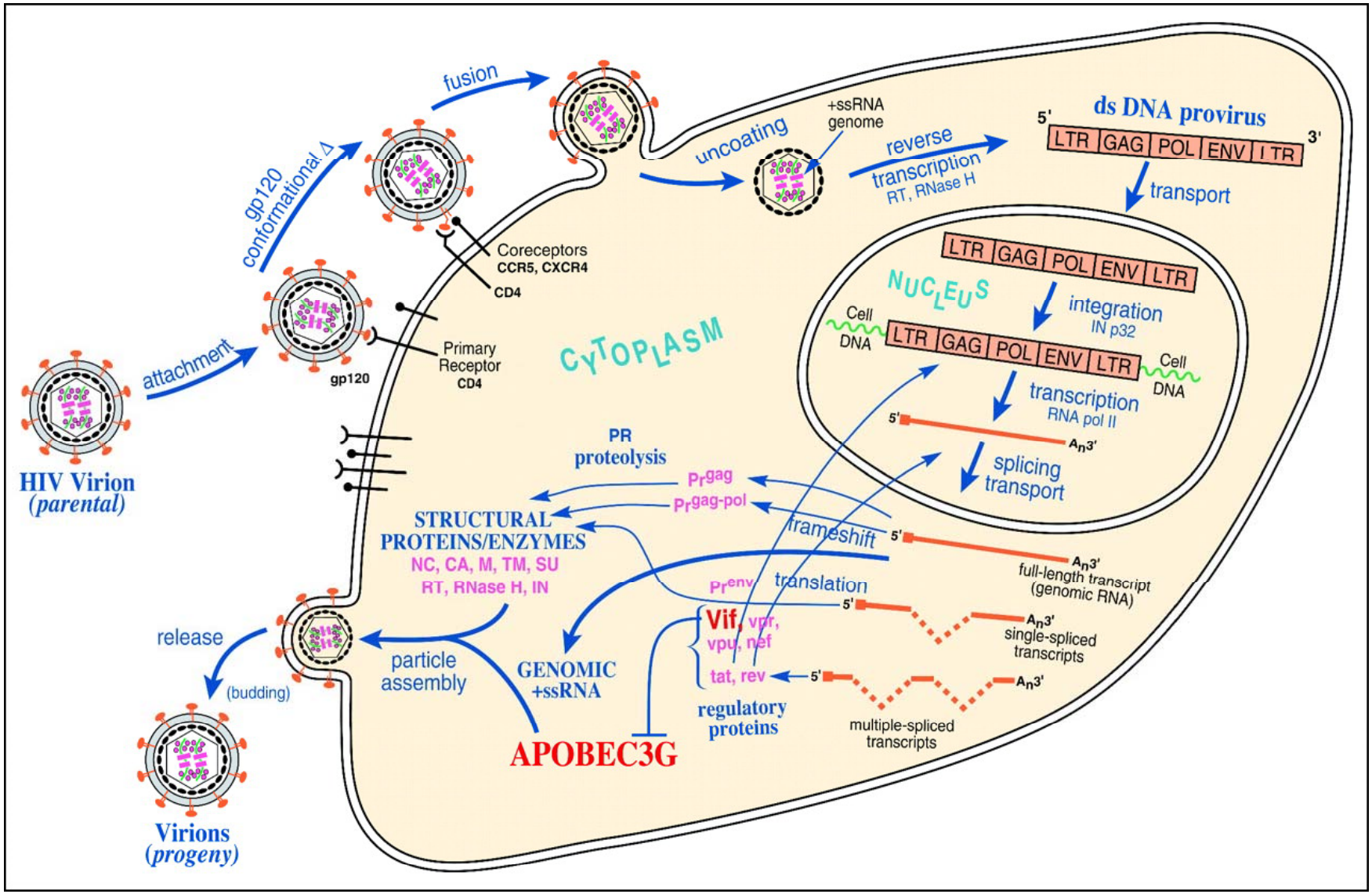
# Steps in the Replicative Cycle of Viruses

**1. Attachment** - through a receptor.
**Specific:** CD4 on T-cells for HIV
ICAM on upper respiratory epithelial cells - Rhinoviruses (common cold
Immunoglobulin-like receptors - polio virus
**2. Entry** - receptor-mediated endocytosis, e.g. Influenza and adenoviru
- membrane fusion, e.g. herpesviruses and paramyxoviruses
**3. Uncoating** - triggered by pH changes in endosomes, e.g. Influenza A virus
**4. Replication & viral protein production**
- early proteins: control the next phase of replicative cycle, e.g. genom replication and late protein production.
- late proteins: usually structural proteins
**5. Assembly and release**

# Quantitation of Virus Particles and Component

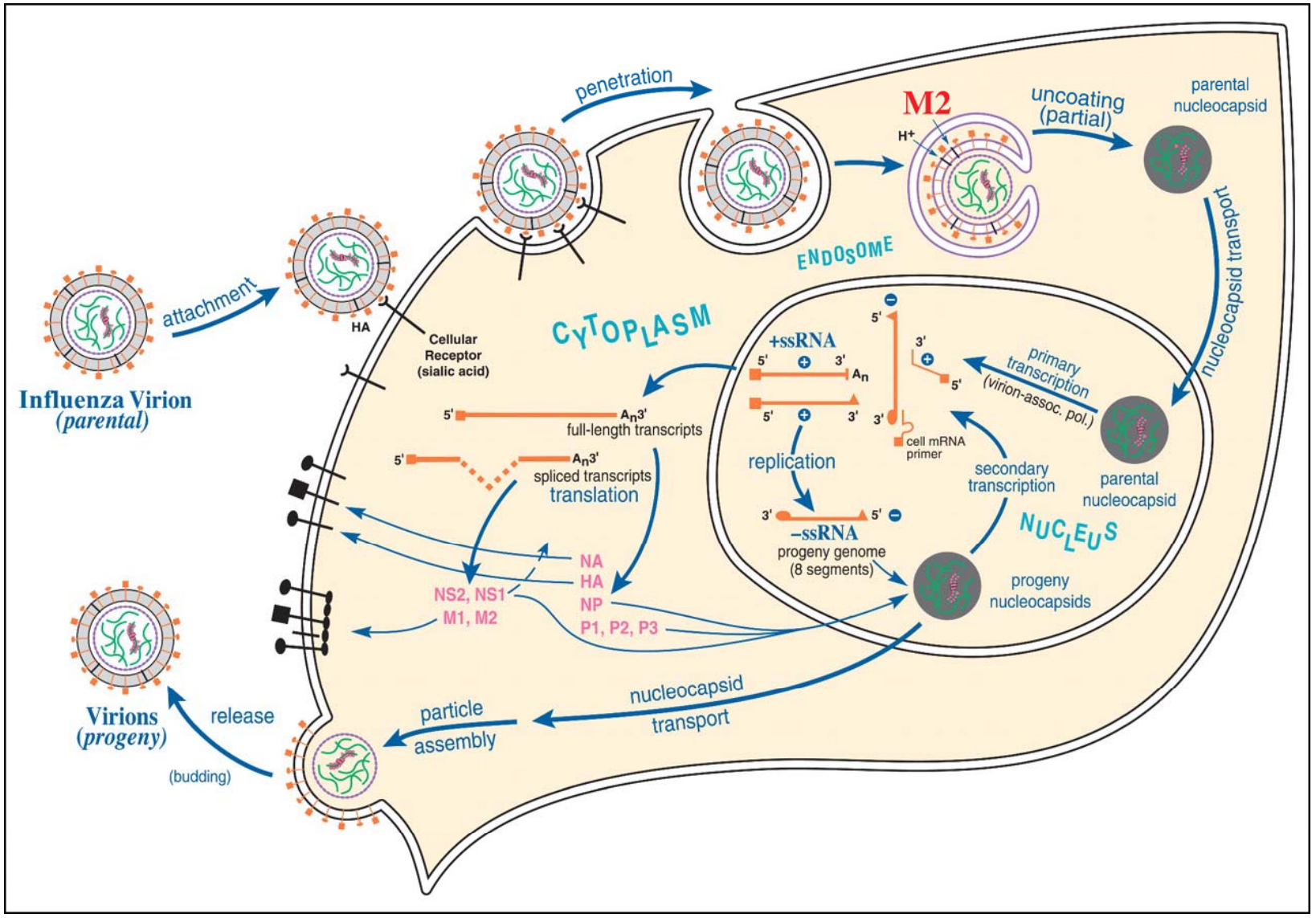
- **EM analysis**
- **hemagglutination assay**
- **Viral enzyme assays: virally-encoded enzymes such as reverse transcriptase (retroviruses, hepatitis B virus) or viral proteases (encoded by many viruses, and used in protein maturation**
- **Serologic Methods**
- **Nucleic acid detection.**

**Schematic diagram of the human immunodeficiency virus multiplication cycle.**



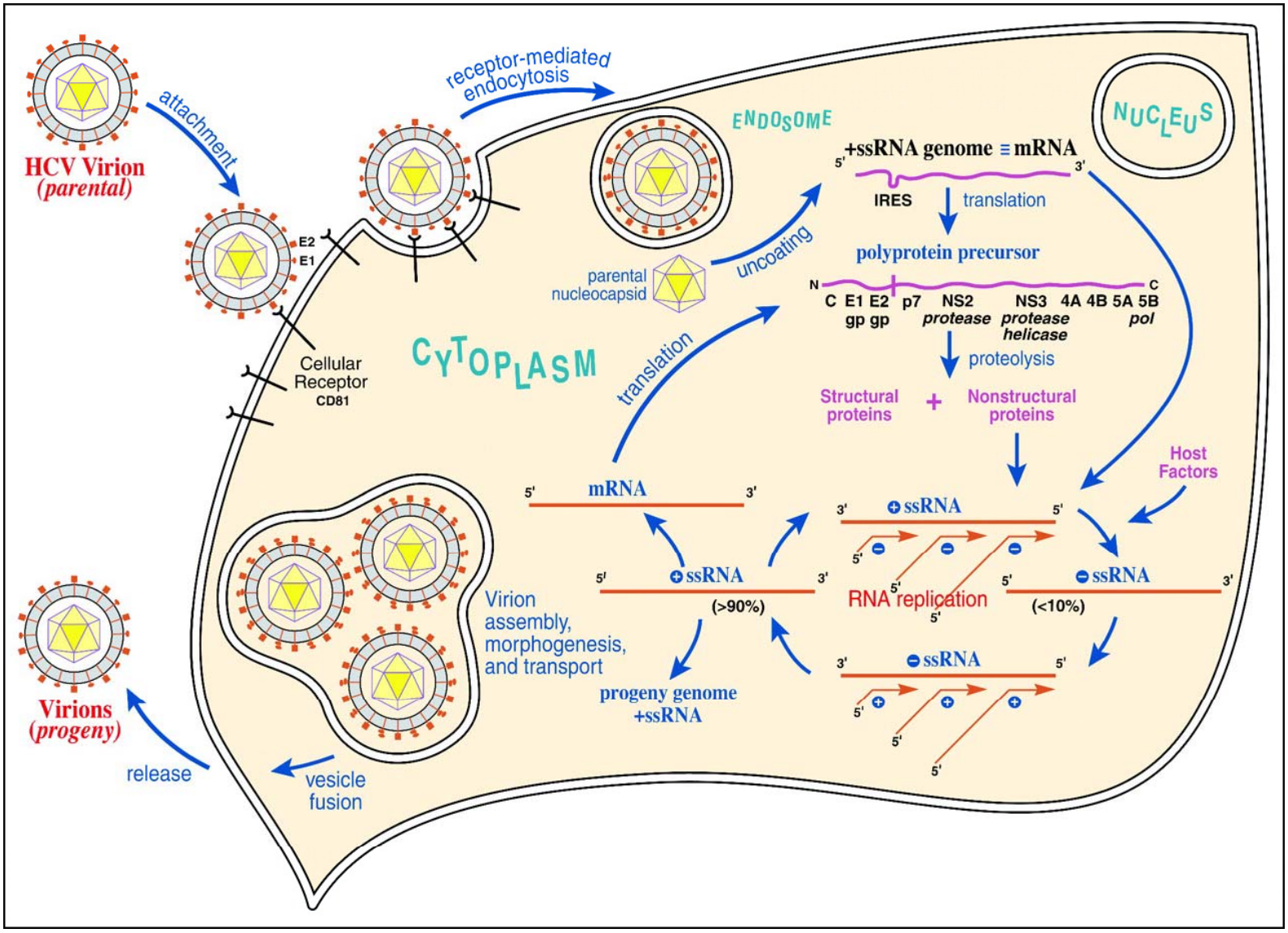

Samuel C E J. Biol. Chem. 2006;281:8305-8307

**Schematic diagram of the influenza virus multiplication cycle.**

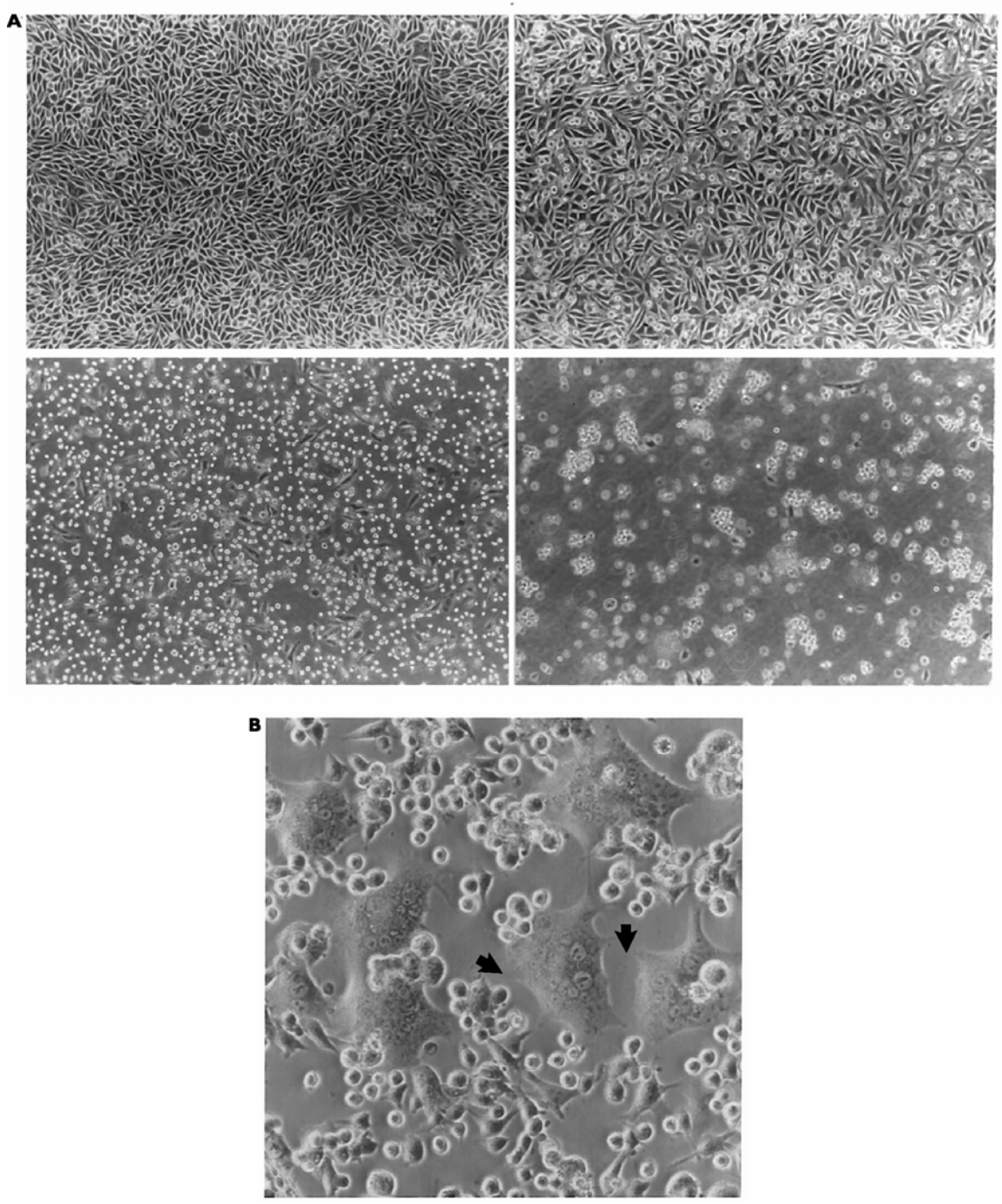



Samuel C E J. Biol. Chem. 2006;281:8305-8307

# Schematic diagram of the hepatitis C virus multiplication cycle.




Samuel C E J. Biol. Chem. 2006;281:8305-8307




# Cytopathic effects (cpe)

- Some viruses kill the cells in which they replicate, often easily visible as cpe, other viruses produce little or no cpe
- Typically rounding or detaching of cells and cell fusion (syncytia)

病毒与细胞相互作用

# 病毒与细胞相互作用

- 病毒在细胞内复制对宿主细胞的影响；
- 机体对病毒的识别及对其在细胞内复制和细胞间播散的干预

# 病毒在细胞内复制对宿主细胞影响

- Effects of viruses on the host cell may be mediated by addition or substitution of a virus-specific macromolecule into a cellular complex or structure;
- Virus may mediate a covalent or noncovalent modification of a host-cell molecule.
- Virus infection may cause a disassembly or rearrangement of a host-cell complex or structure, or virus infection may lead to the assembly of a new infected cell–specific complex or structure in the infected cell.

# Mechanisms of effects of virus on cells

- TAKING OVER THE CELLULAR TRANSCRIPTION MACHINERY;
- REGULATING CELLULAR SIGNAL TRANSDUCTION PATHWAYS ;
- TAKING OVER THE CELLULAR RNA PROCESSING AND TRANSPORT MACHINERY;
- TAKING OVER THE TRANSLATIONAL MACHINERY: STRATEGIES FOR OPTIMIZATION OF VIRAL PROTEIN SYNTHESIS AND ANTAGONISM OF CELLULAR PROTEIN SYNTHESIS
- TAKING OVER THE CELLULAR DNA REPLICATION MACHINERY

# TAKING OVER THE TRANSLATIONAL MACHINERY

**1:** Modification of eIF-4F initiation factor function involved in recruitment of the 40S subunit to mRNA. Picornaviral 2A protease catalyzes cleavage of the eIF-4G component of the eIF-4F complex and also the poly A binding protein; phosphorylation of the eIF-4E component is decreased in cells infected with adenovirus, influenza virus, and encephalomyocarditis virus.

**2:** Modification of eIF-2 initiation factor function involved in binding of tRNA to the 40S subunit. Phosphorylation of the a subunit of eIF-2 is antagonized by several viral RNAs (including adenovirus VA1 and Epstein-Barr virus EBER) and proteins [including reovirus s3, vaccinia E3L and K3L, hepatitis C virus NS5A and E2, influenza virus NS1, and Herpes simplex virus (HSV) g34.5 and Us11].

**3:** Elongation factor function is altered in cells infected with human immunodeficiency virus, HSV, and vesicular stomatitis virus.

**4:** Host-cell mRNA is degraded in cells infected with HSV, influenza virus, and poxvirus.

**5:** Unconventional strategies used for viral protein synthesis include leaky scanning and ribosome shunting during initiation, frameshifting during elongation, and suppression of termination.

# TAKING OVER THE CELLULAR DNA REPLICATION MACHINERY

- Inhibition of Host-Cell DNA Replication ;
- A Secondary Effect of Inhibiting Cell Protein Synthesis
- Displacement of Cellular DNA from Its Normal Site of Replication
- Recruitment of Cell DNA Replication Proteins to Viral Structures
- Degradation of Cellular DNA

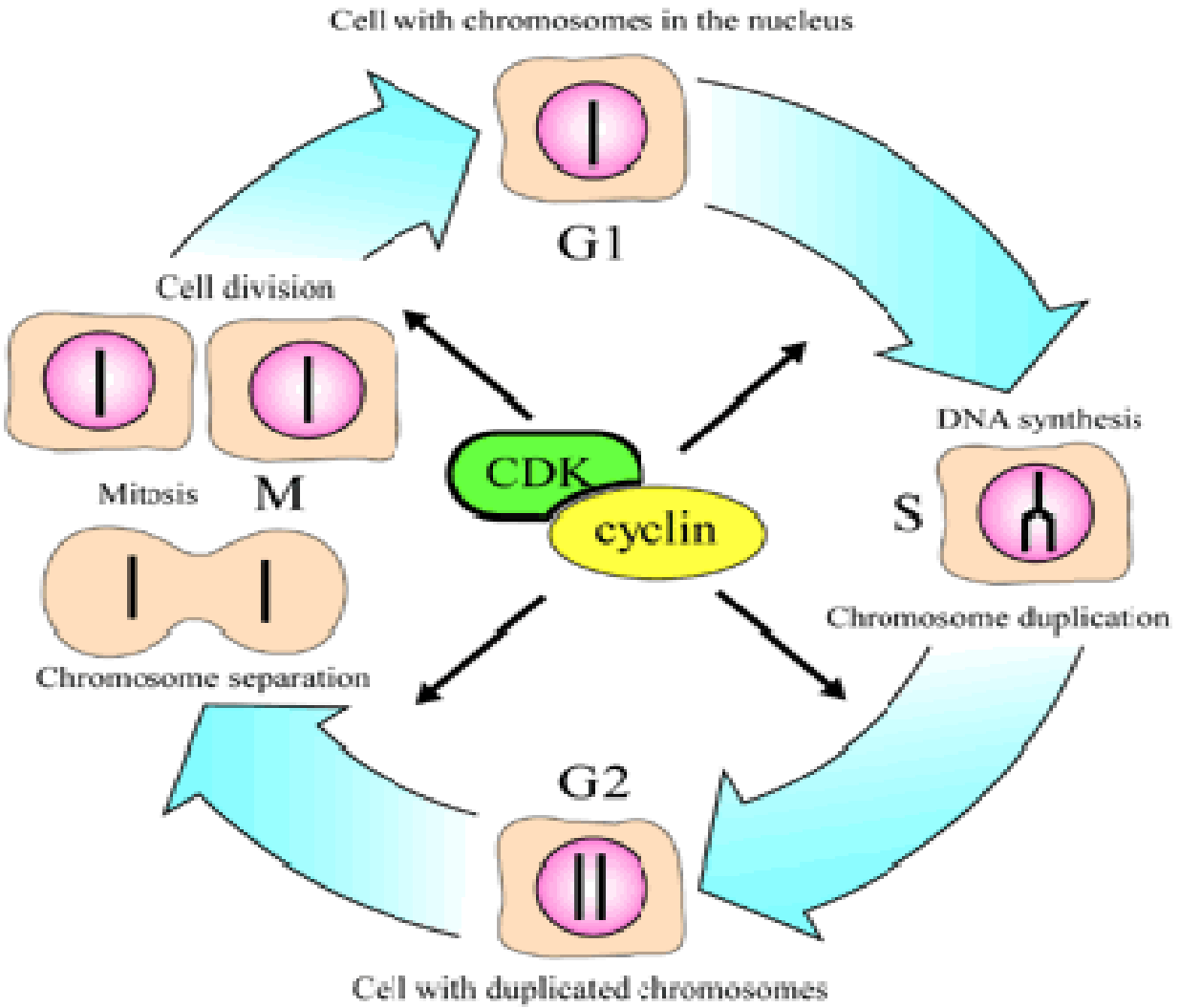
# VIRUSES CAN REGULATE CELLULAR SIGNAL TRANSDUCTION PATHWAYS

by binding of virions to a surface receptor on the cell surface;

by a new protein encoded by modifying the activity of a cellular protein;

by a viral protein mimicking a cellular protein.

# The Cell Cycle



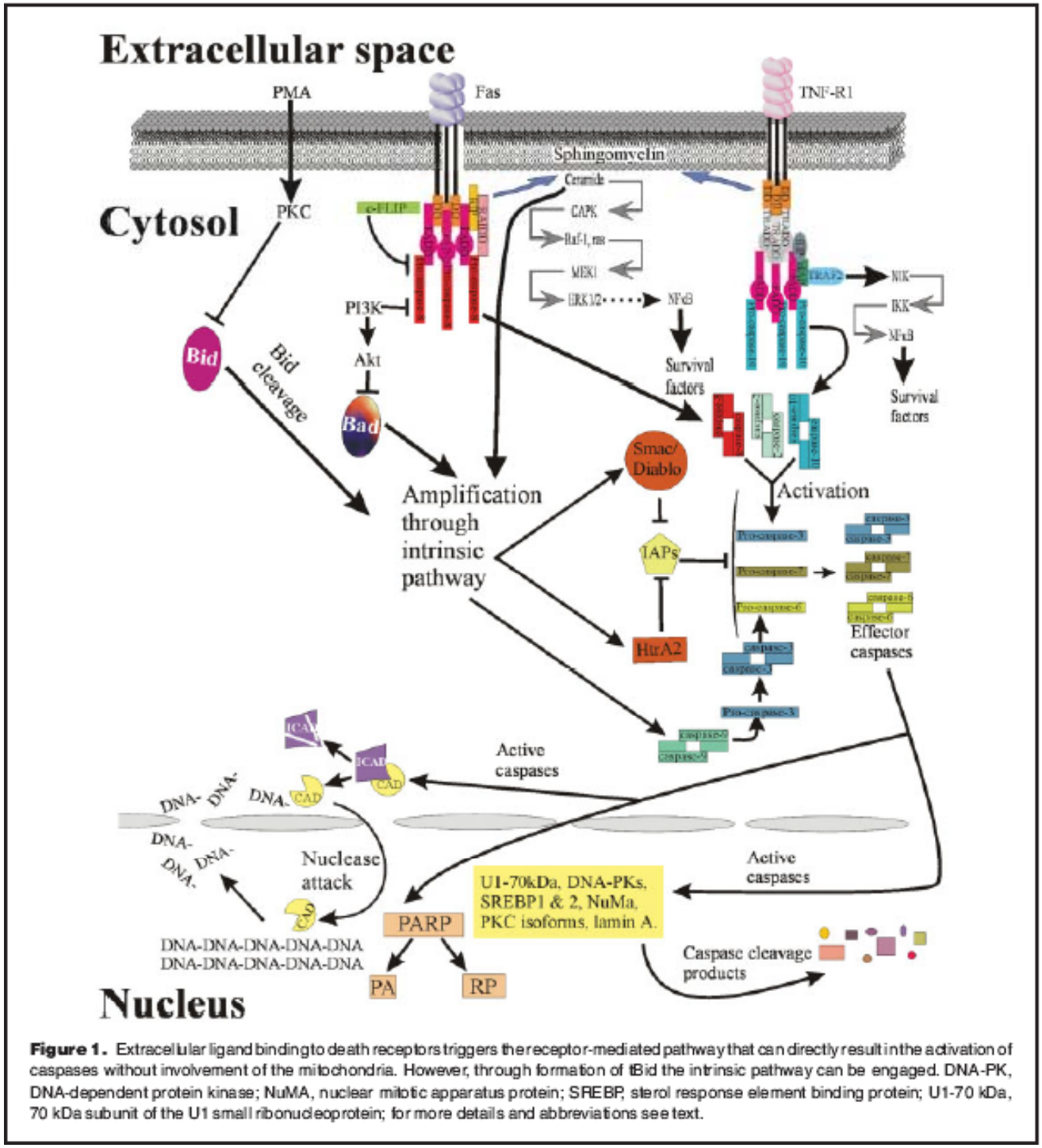



**Figure 1.** Extracellular ligand binding to death receptors triggers the receptor-mediated pathway that can directly result in the activation of caspases without involvement of the mitochondria. However, through formation of tBid the intrinsic pathway can be engaged. DNA-PK, DNA-dependent protein kinase; NuMA, nuclear mitotic apparatus protein; SREBP, sterol response element binding protein; U1-70 kDa, 70 kDa subunit of the U1 small ribonucleoprotein; for more details and abbreviations see text.

**Death receptor-mediated apoptosis**

***Bioessays***. 2003

# Intrinsic pathway

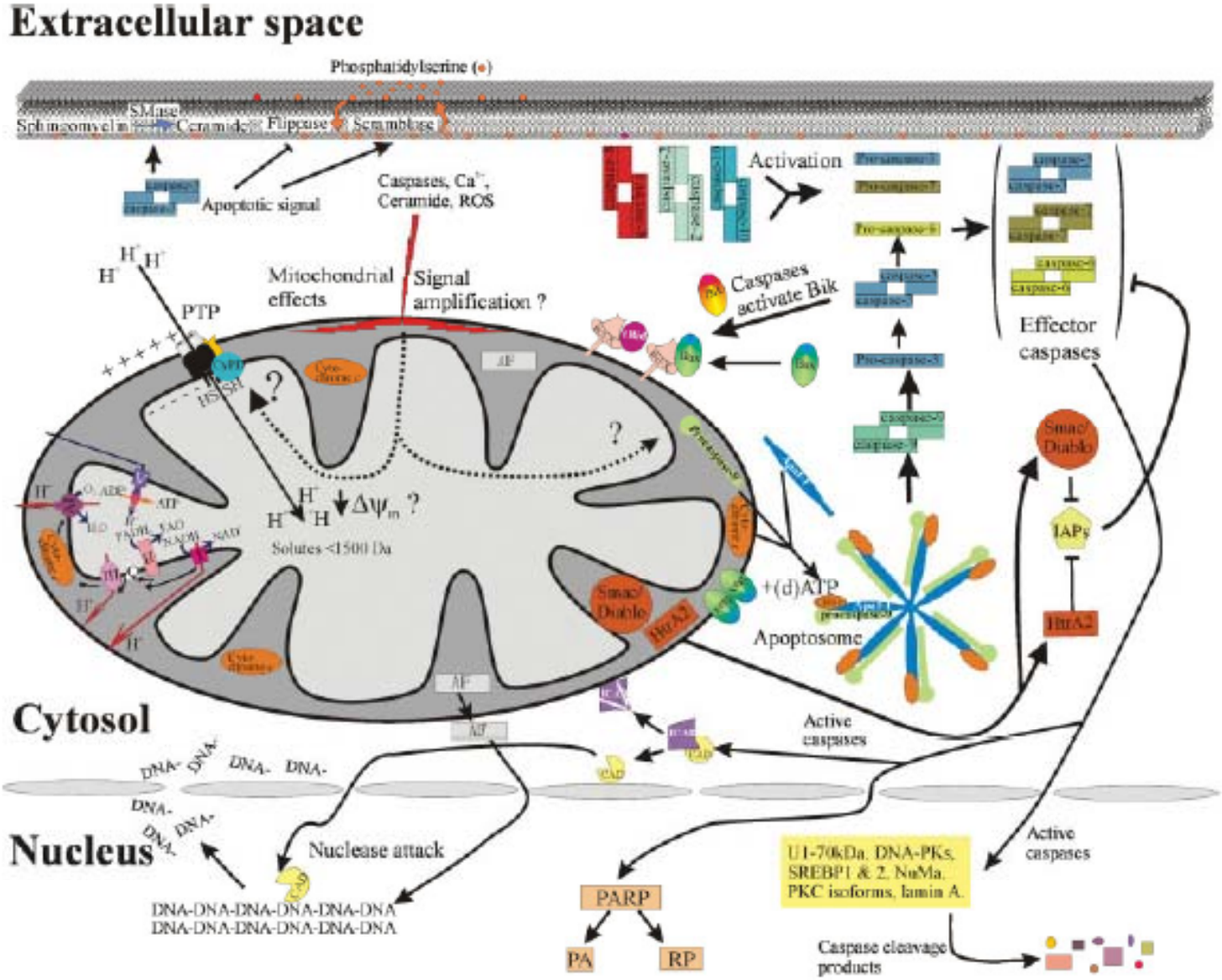
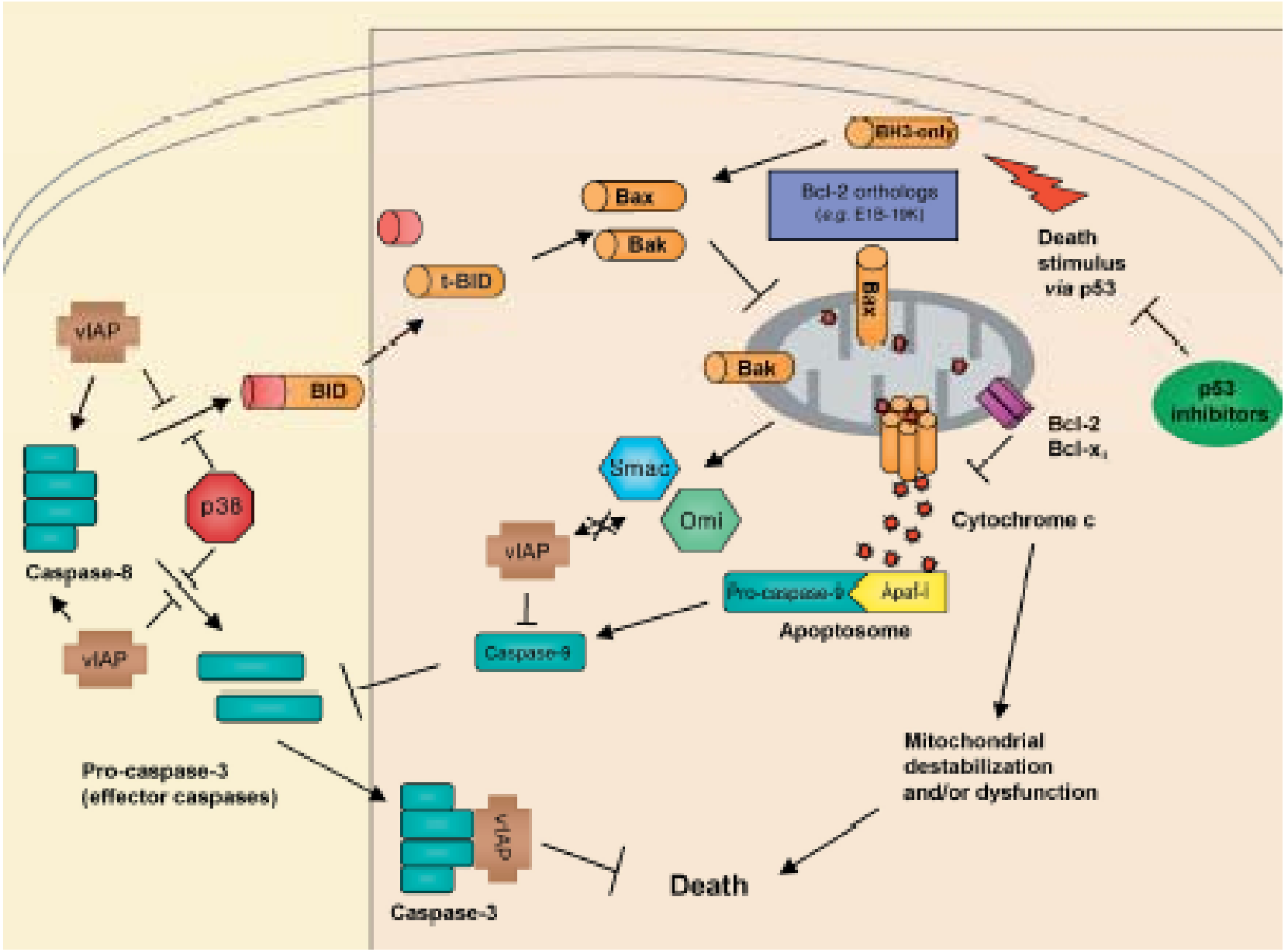



**Figure 2.** Intrinsic pathway to apoptosis. Intracellular stress signals result in the activation of the intrinsic pathway, which leads to cytochrome *c* release from the mitochondria, apoptosome formation and caspase activation. DNA-PK, DNA-dependent protein kinase; NuMA, nuclear mitotic apparatus protein; SREBP, sterol response element binding protein; U1-70 kDa, 70 kDa subunit of the U1 small ribonucleoprotein; for more details and abbreviations see text.

***Bioessays. 2003***

# Viral evasion of apoptosis




*Nature Immu* 2002

# 乙肝病毒感染与细胞周期

通过HBV DNA 整合到宿主染色体、编码具有反式激活作用的X蛋白或截短的PreS2/S基因产物，激活原癌基因(myc)、灭活抑癌基因（P53），以及持续炎症过程中肝细胞反复变性坏死和再生导致细胞染色体和基因的突变，最终通过干扰细胞周期调节导致肝细胞癌的发生。

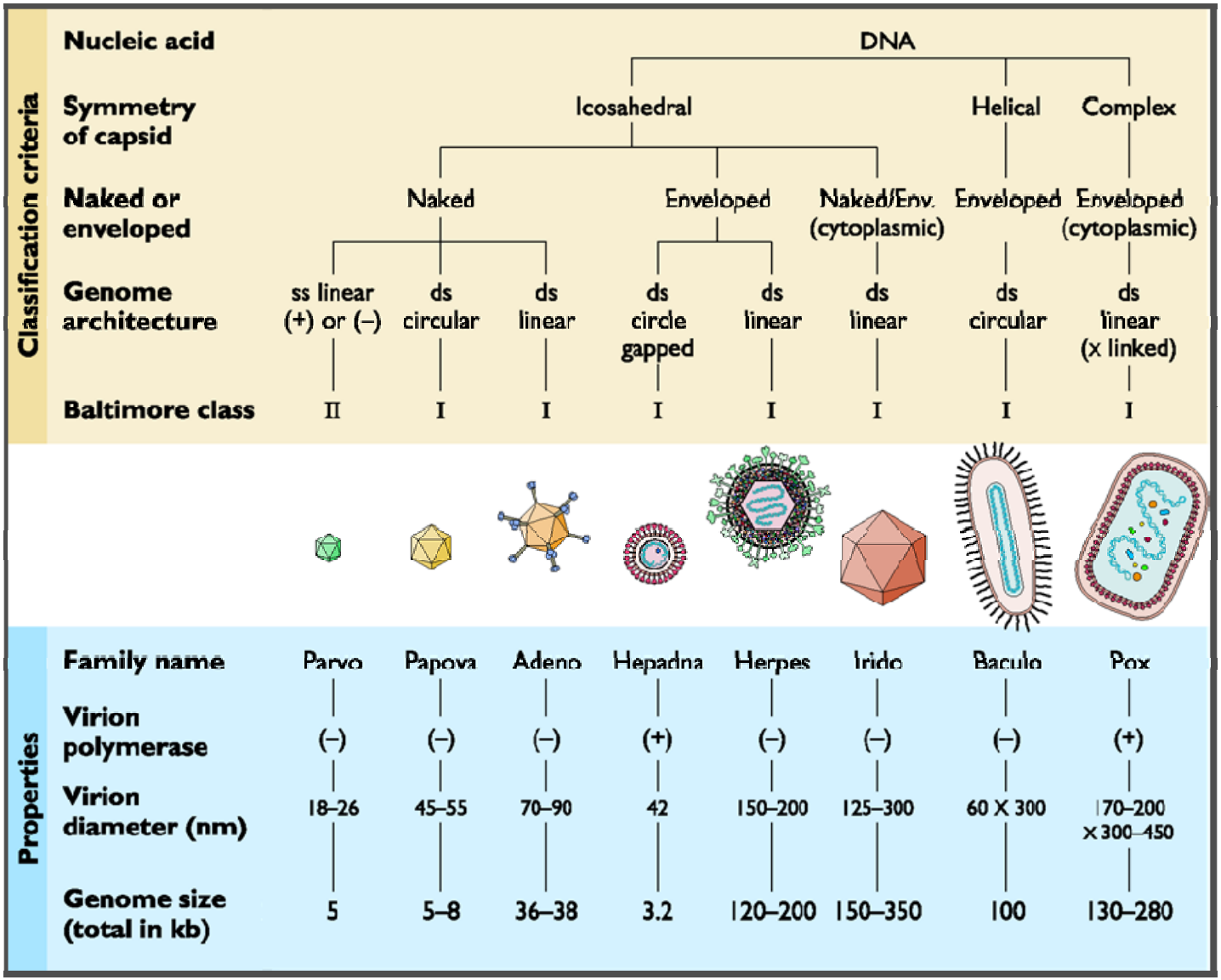
# HCV蛋白抑制凋亡、诱发细胞恶性增殖

- C和NS3对细胞有转化作用；
- C可抑制p53功能、激活NF－κB；
- NS3可与细胞cAMP依赖性蛋白激酶PKA发生作用；
- NS5A可与人囊泡相关蛋白(human vesicle-associated membrane protein-associated protein ,hVAP-33)和细胞转录调节因子SRCAP结合等

# 病毒与细胞相互作用

- 病毒在细胞内复制对宿主细胞的影响；
- 机体对病毒的识别及对其在细胞内复制和细胞间播散的干预

# 我们的机体无时无刻不处在病原体的包围之中

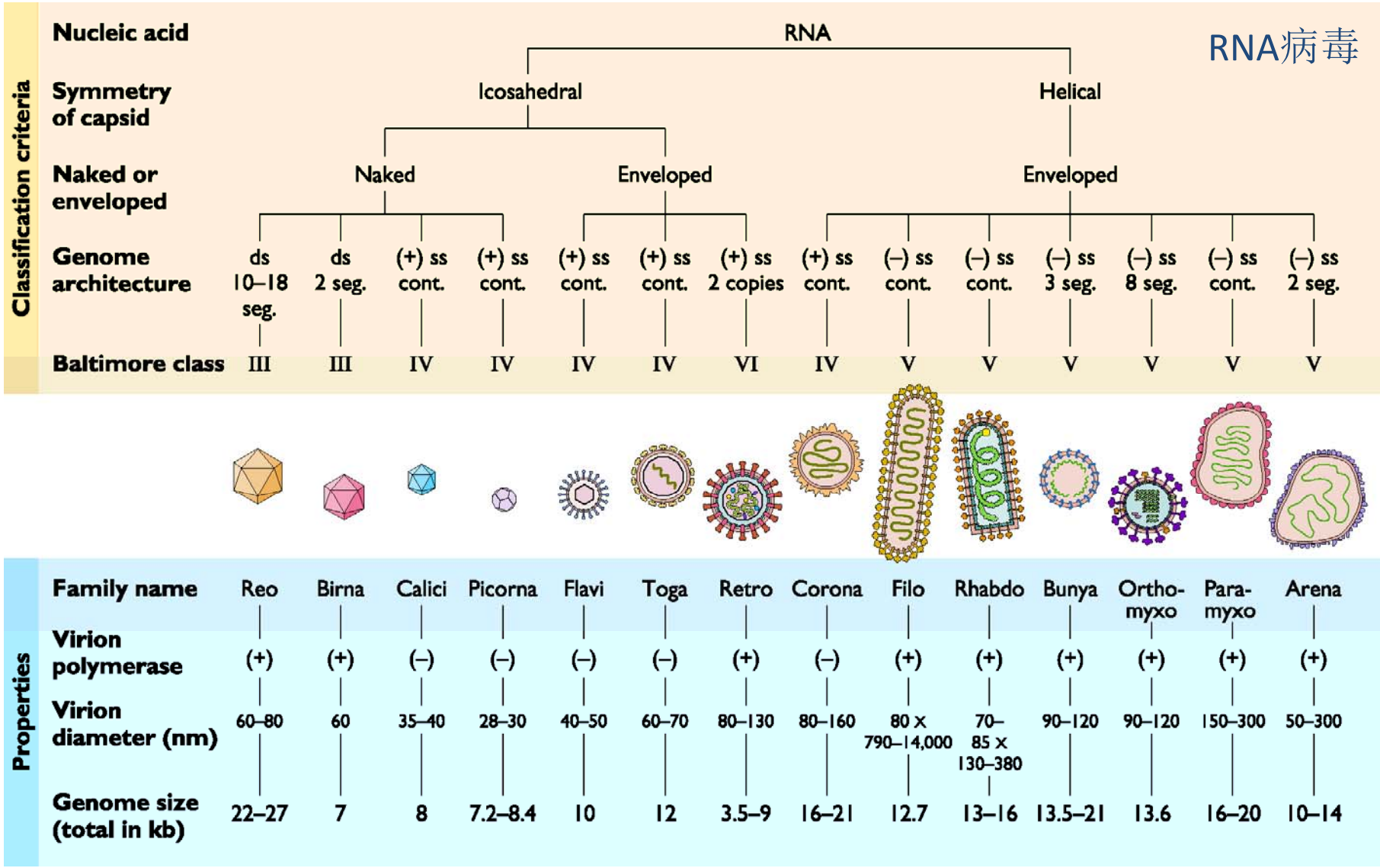



DNA病毒

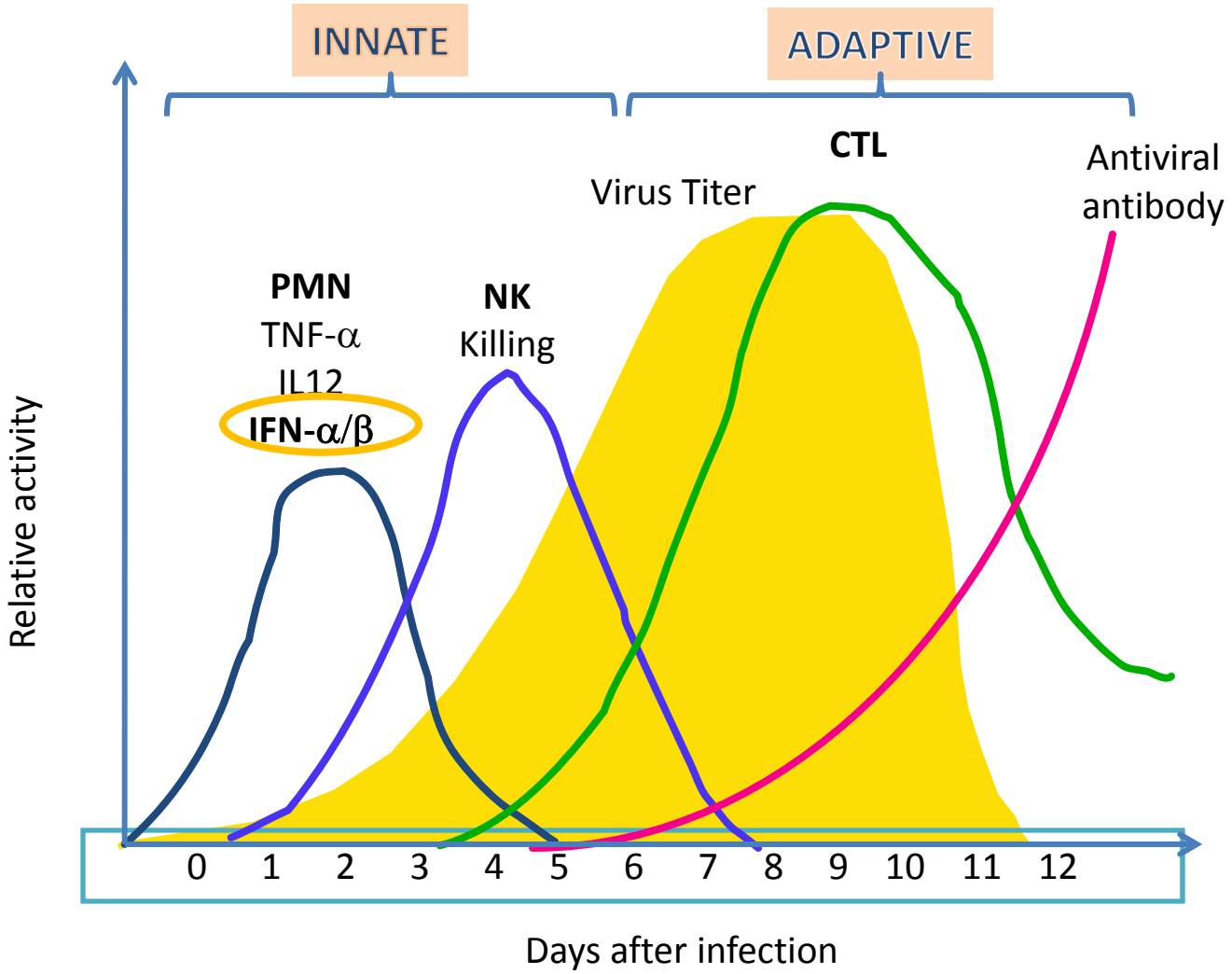
Principle of Virology

# 我们的机体无时无刻不处在病原体的包围之中

RNA病毒



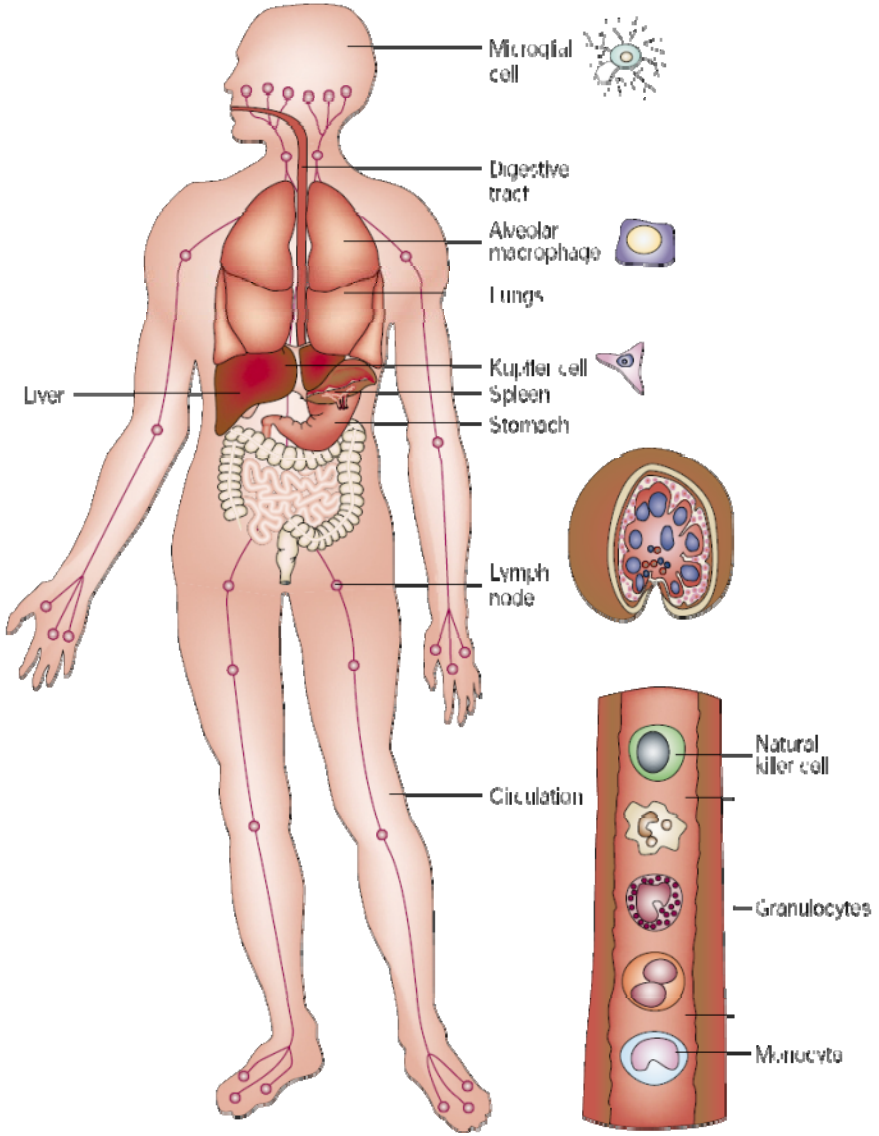
# 病毒感染诱导的宿主免疫应答



# 天然免疫 (Innate Immunity)是机体抵御外来入侵的第一道屏障

## 天然免疫的特点：

- 先天固有；
- **模式识别**；
- 应答迅速，持续时间短；
- 免疫作用广泛，无特异性；
- 是适应性免疫应答的始动者。



Nobelförsamlingen
The Nobel Assembly at Karolinska Institutet

## The 2011 Nobel Prize in Physiology or Medicine

shall be divided, with one half jointly to

**Bruce A. Beutler and Jules A. Hoffmann**

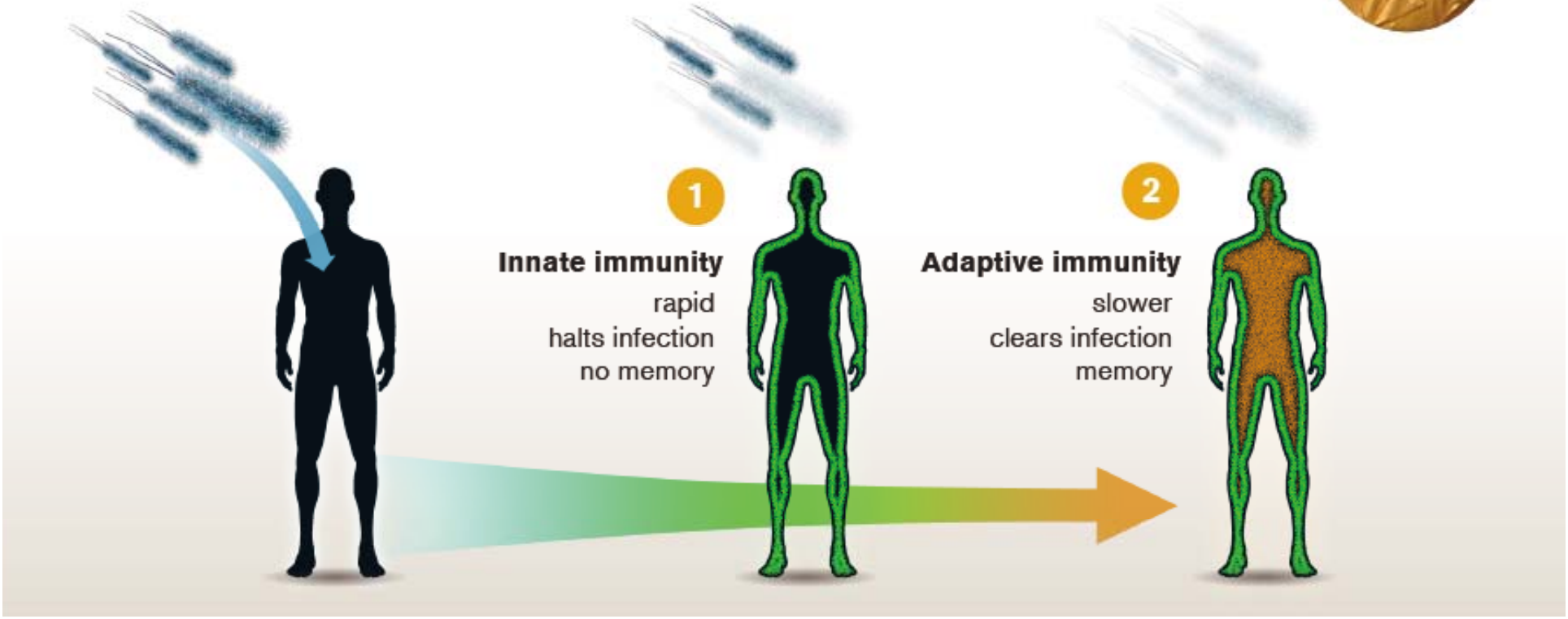
**for their discoveries concerning the activation of innate immunity**

and the other half to

**Ralph M. Steinman**

**for his discovery of the dendritic cell and its role in adaptive immunity**

# **The Nobel Prize** in Physiology or Medicine 2011



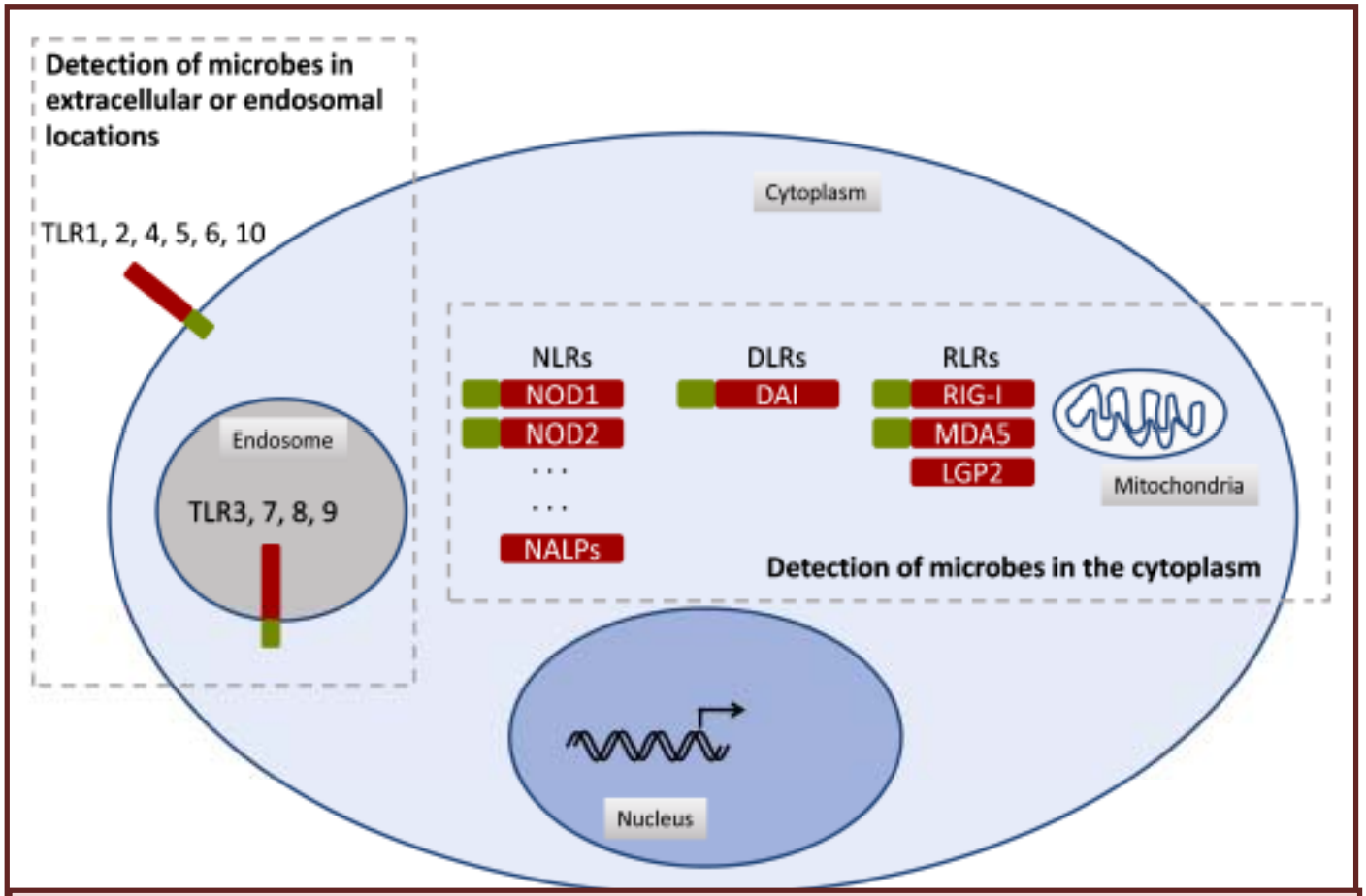

## 1 Innate immunity

Components of microorganisms bind to Toll-like receptors located on many cells in the body. This activates innate immunity, which leads to inflammation and to the destruction of invading microorganisms.

## 2 Adaptive immunity

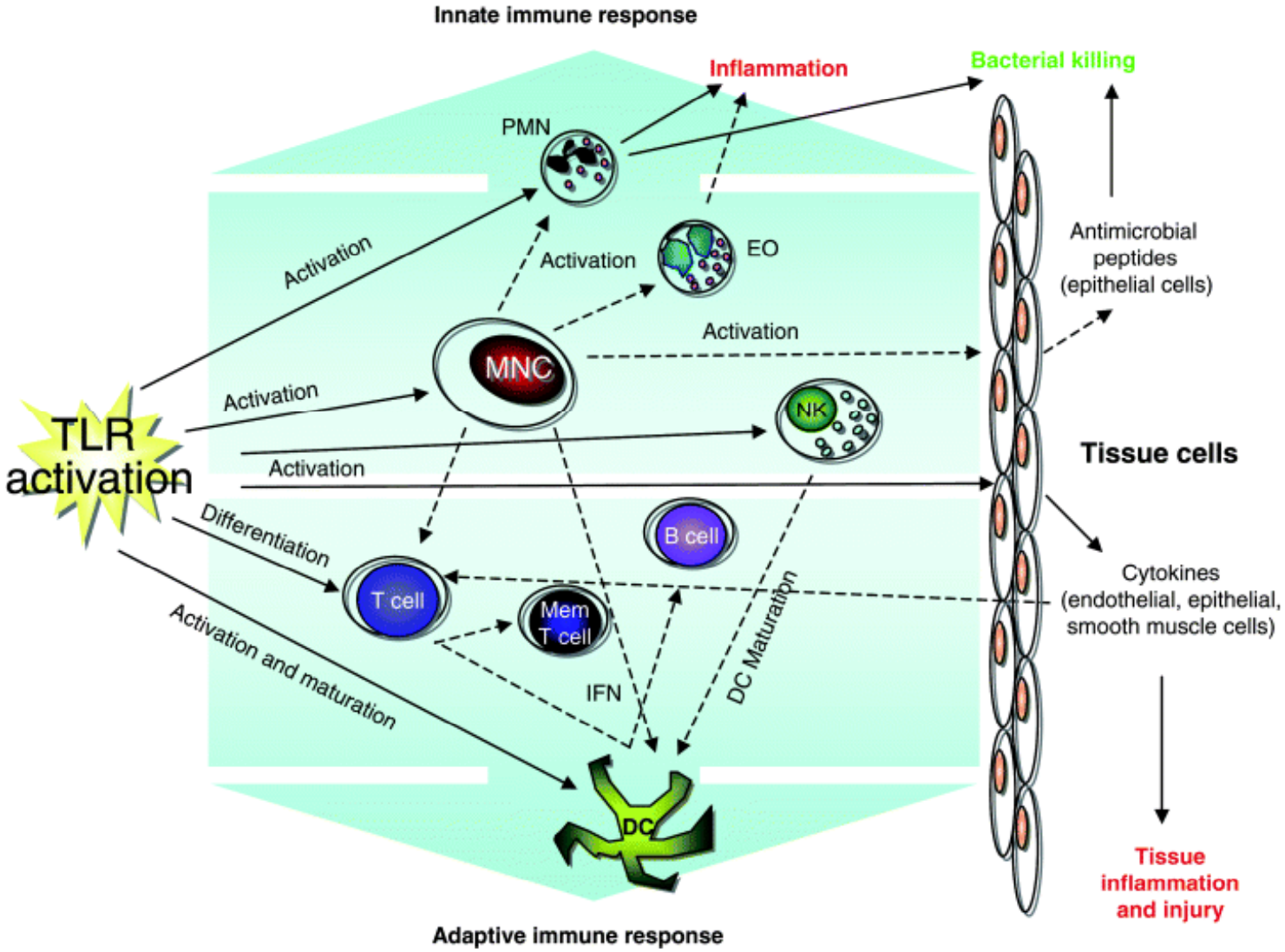
Dendritic cells activate T lymphocytes, which initiates adaptive immunity. A cascade of immune reactions follows, with formation of antibodies and killer cells.

# 模式识别受体(PRRs)在识别病原体中发挥重要作用

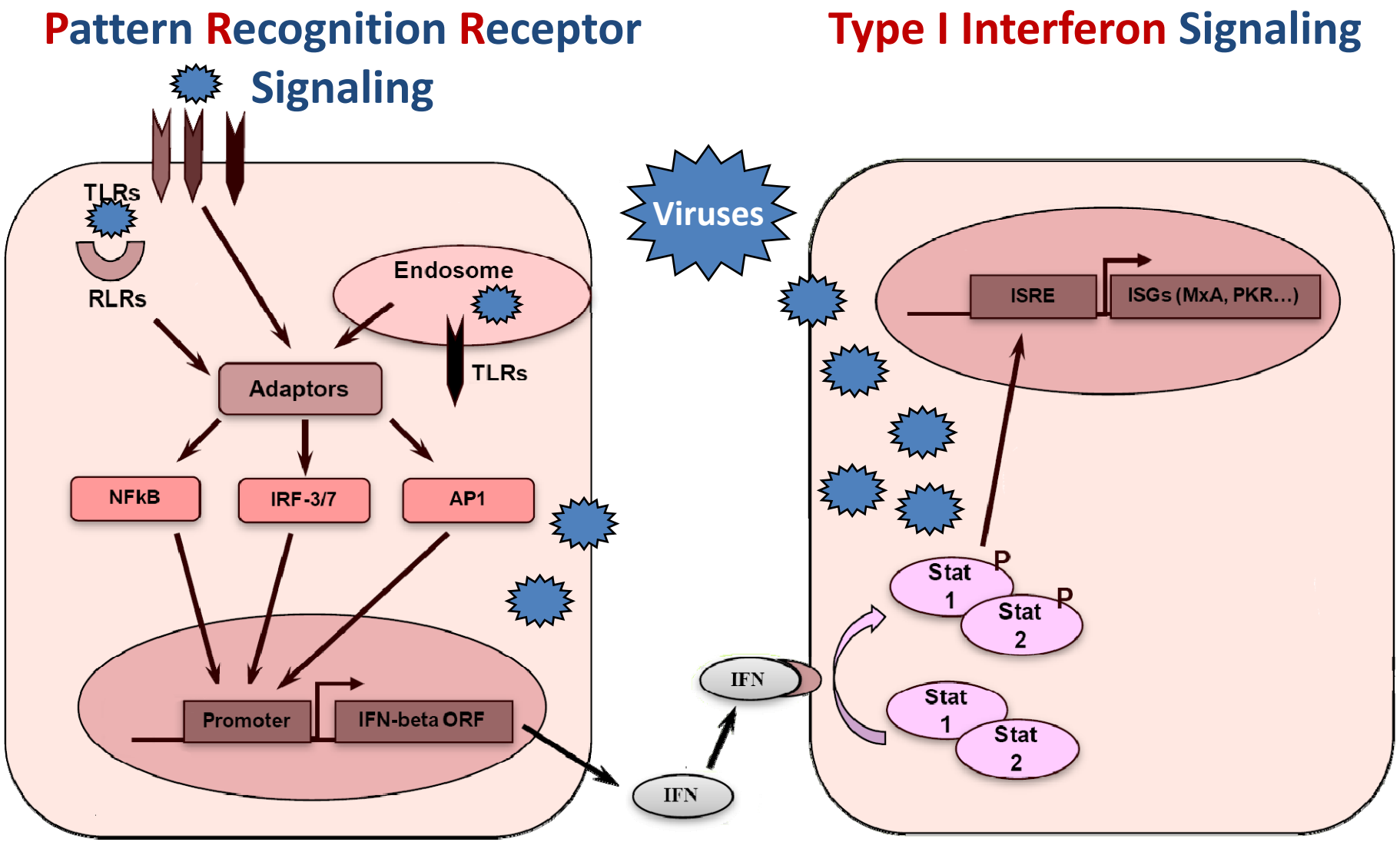



APMIS 117: 323–337.

# PRRs识别病毒，激活天然免疫应答并诱导适应性免疫应答

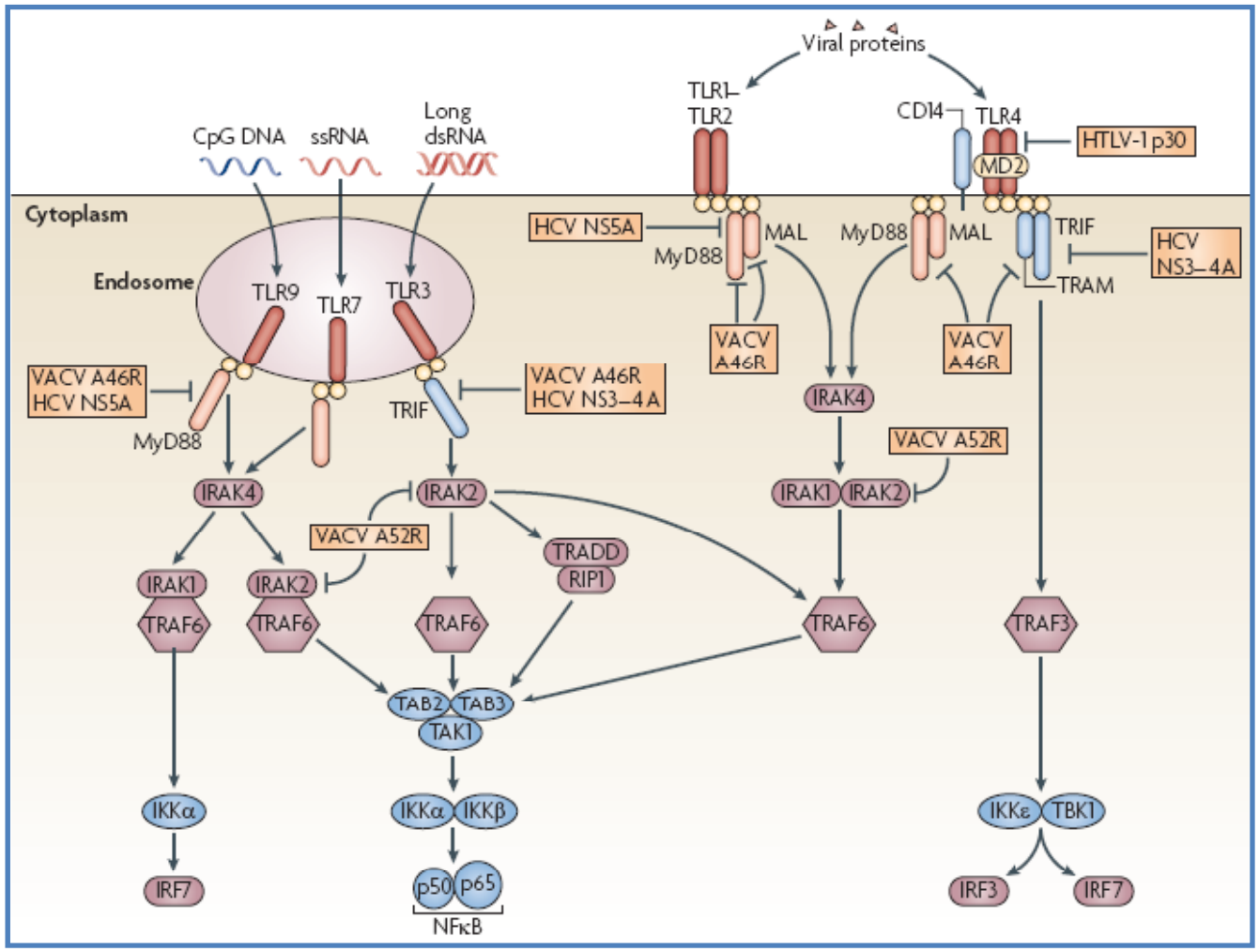


# 其中，I型干扰素是PRRs识别后介导抗病毒作用的重要因子



# 病毒亦可拮抗宿主的天然免疫应答

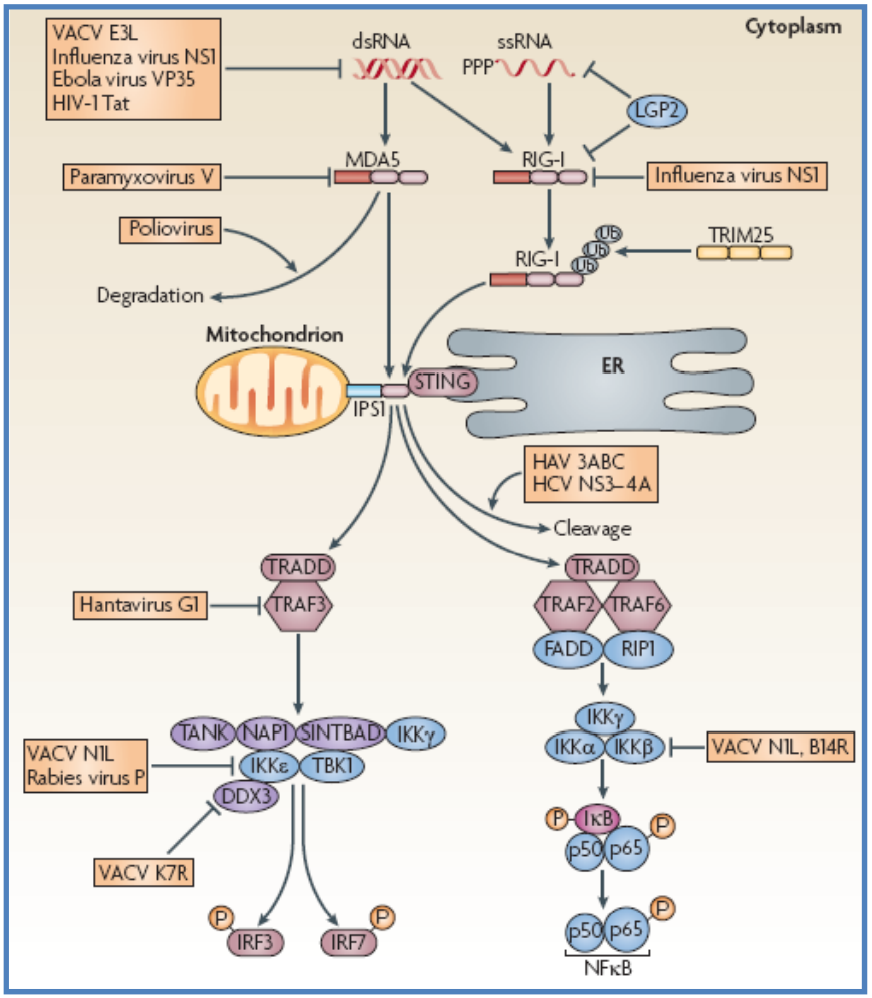
1. 多种病毒能够抑制宿主细胞中的TLR信号通路。




Andrew G. Bowie & Leonie Unterholzner. *Nature Reviews Immunology.* 2008.

# 病毒亦可拮抗宿主的天然免疫应答

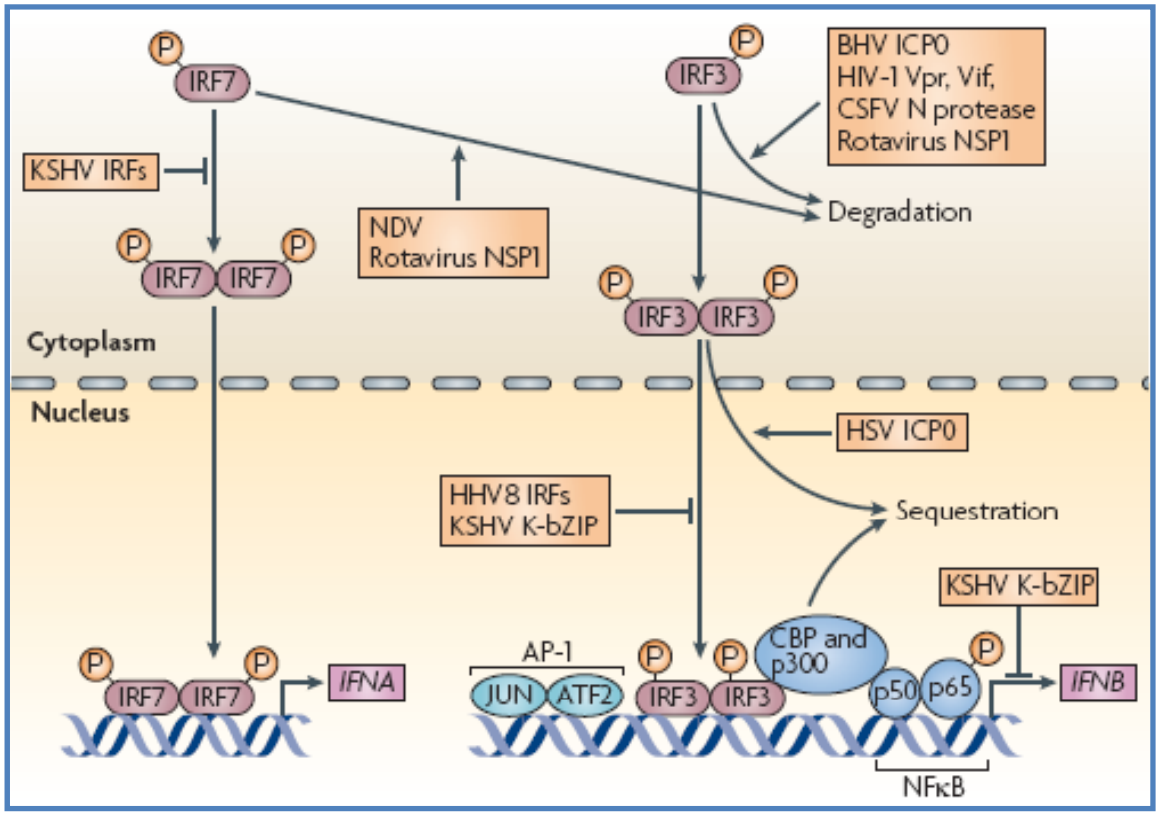
2. 多种病毒亦能够抑制宿主细胞中的RIG-I信号通路。



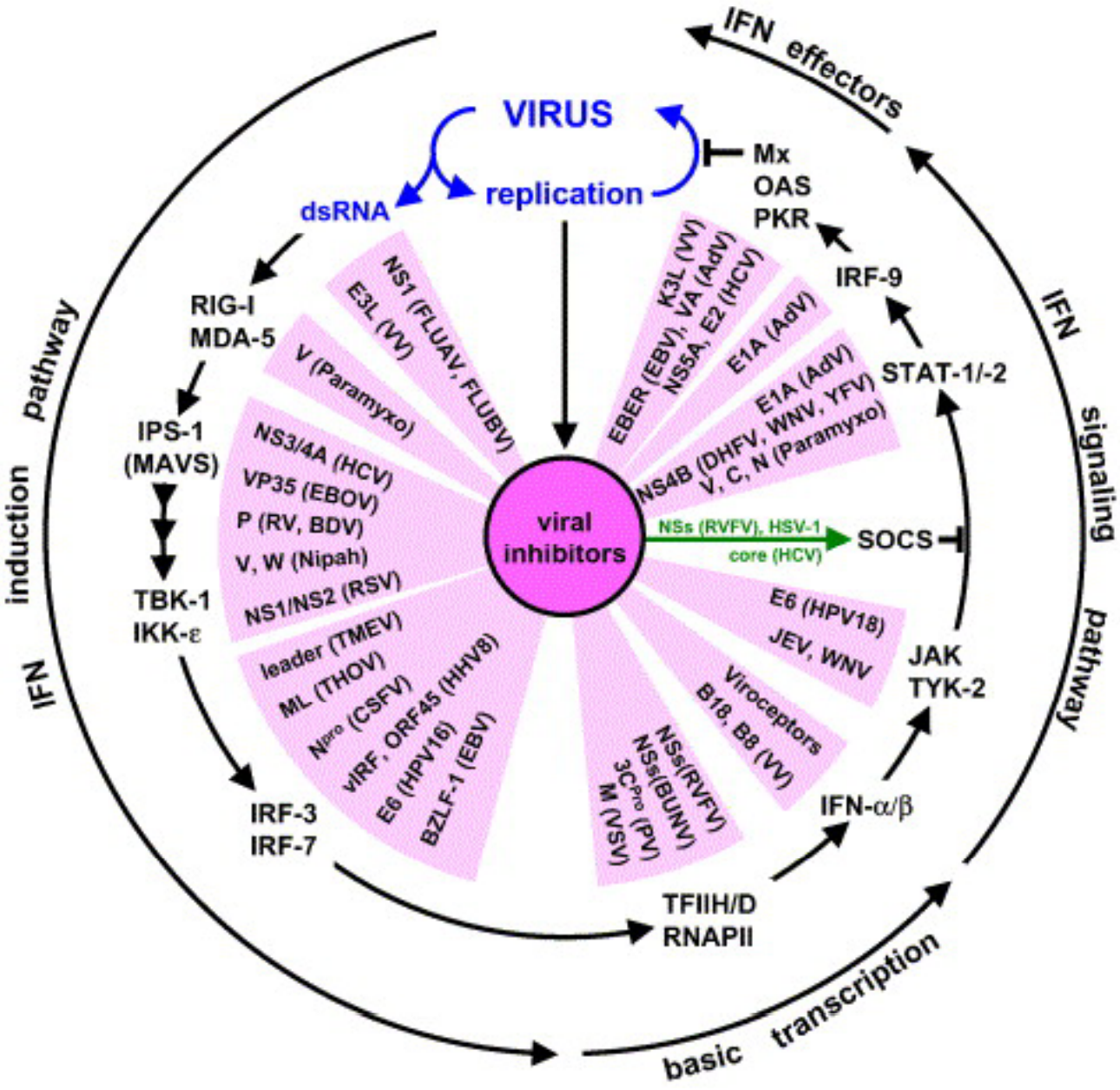

Andrew G. Bowie & Leonie Unterholzner. *Nature Reviews Immunology.* 2008.

# 病毒亦可拮抗宿主的天然免疫应答

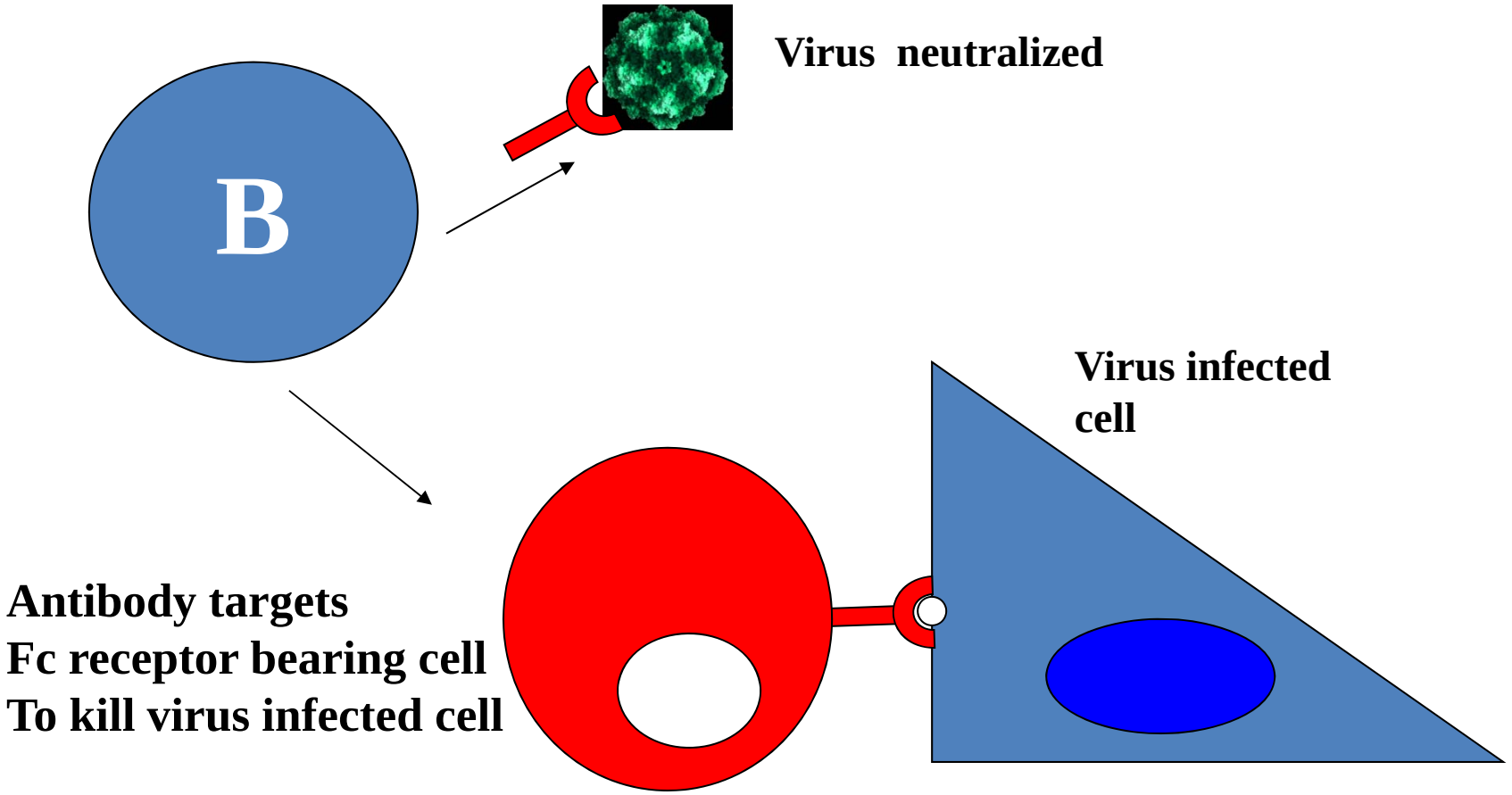
3. 多种病毒能够抑制宿主细胞中的PRR下游的信号通路。



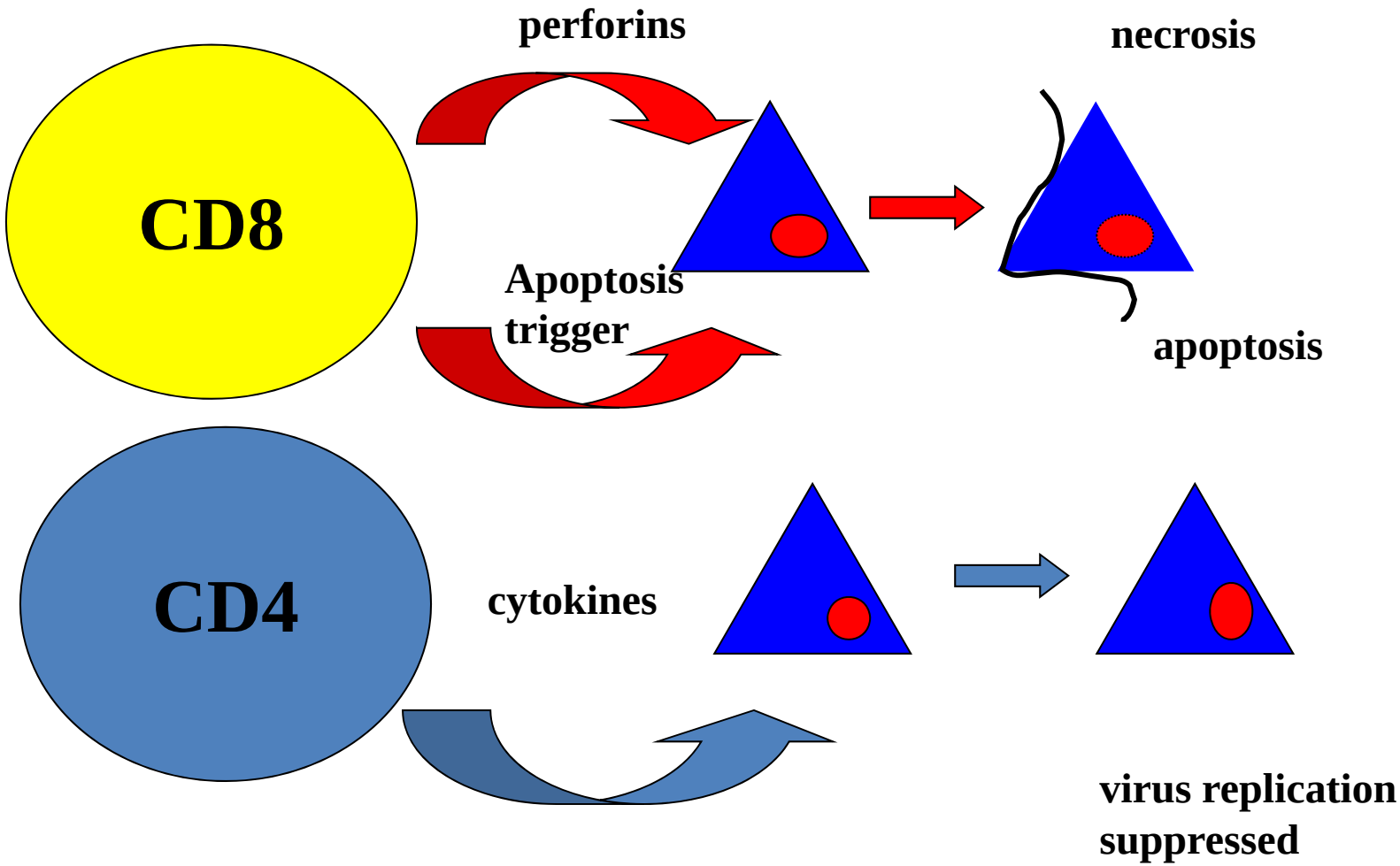

Andrew G. Bowie & Leonie Unterholzner. *Nature Reviews Immunology.* 2008.


IFN effectors
VIRUS
replication
dsRNA
Mx
OAS
PKR
IRF-9
STAT-1/-2
IFN signaling pathway
JAK
TYK-2
IFN-α/β
TFIIH/D
RNAPII
basic transcription
IRF-3
IRF-7
TBK-1
IKK-ε
IPS-1
(MAVS)
RIG-I
MDA-5
IFN induction pathway
viral inhibitors
SOCS
NSs (RVFV), HSV-1
core (HCV)
NS1 (FLUAV, FLUBV)
E3L (VV)
V (Paramyxo)
NS3/4A (HCV)
VP35 (EBOV)
P (RV, BDV)
V, W (Nipah)
NS1/NS2 (RSV)
leader (TMEV)
ML (THOV)
N^pro (CSFV)
vIRF, ORF45 (HHV8)
E6 (HPV16)
BZLF-1 (EBV)
NSs(RVFV)
NSs(BUNV)
3C^Pro (PV)
M (VSV)
Viroceptors
B18, B8 (VV)
E6 (HPV18)
JEV, WNV
NS4B (DHFV, WNV, YFV)
V, C, N (Paramyxo)
E1A (AdV)
EBER (EBV), VA (AdV)
NS5A, E2 (HCV)
K3L (VV)

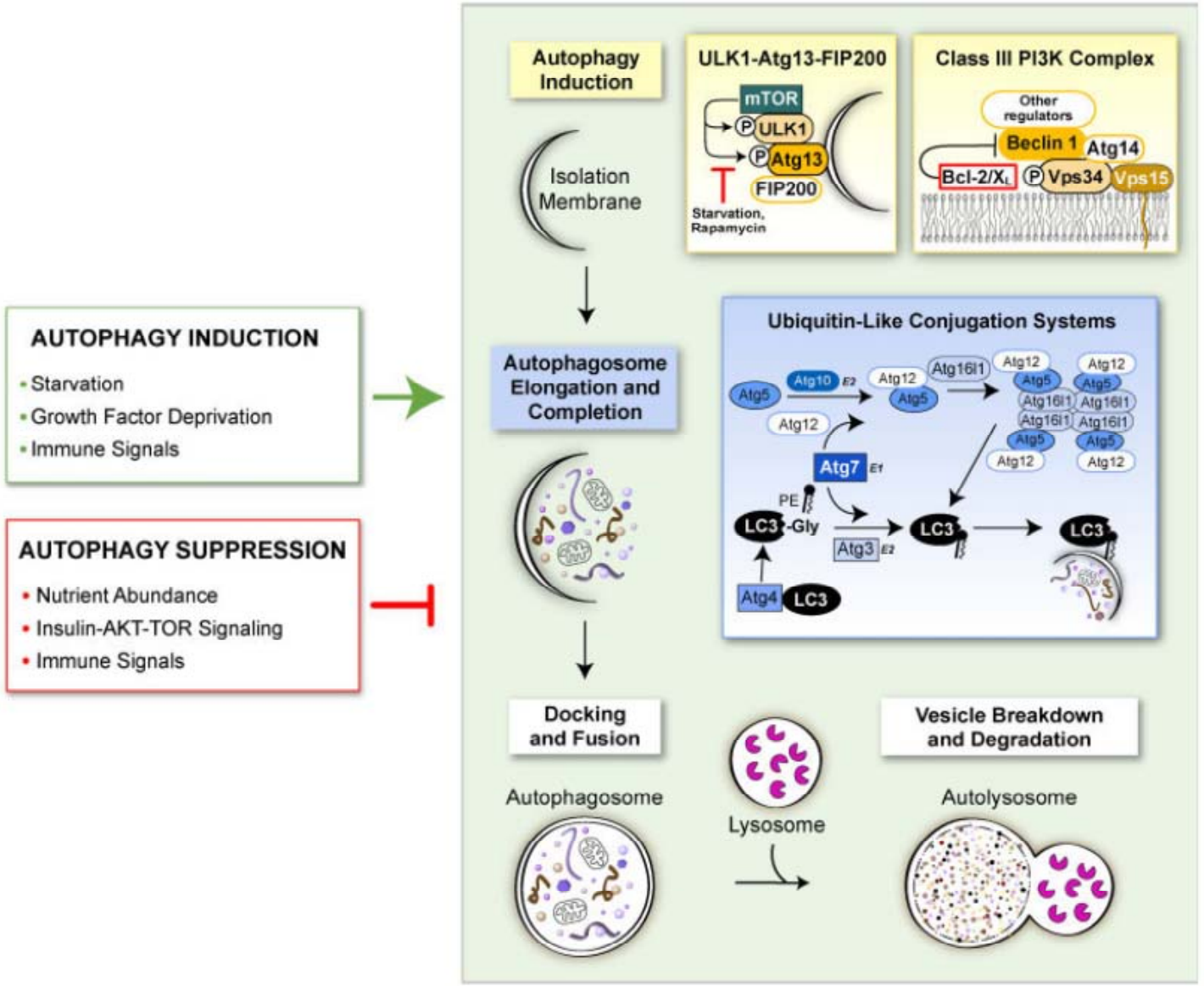
# Acquired anti-viral immunity (antibody)



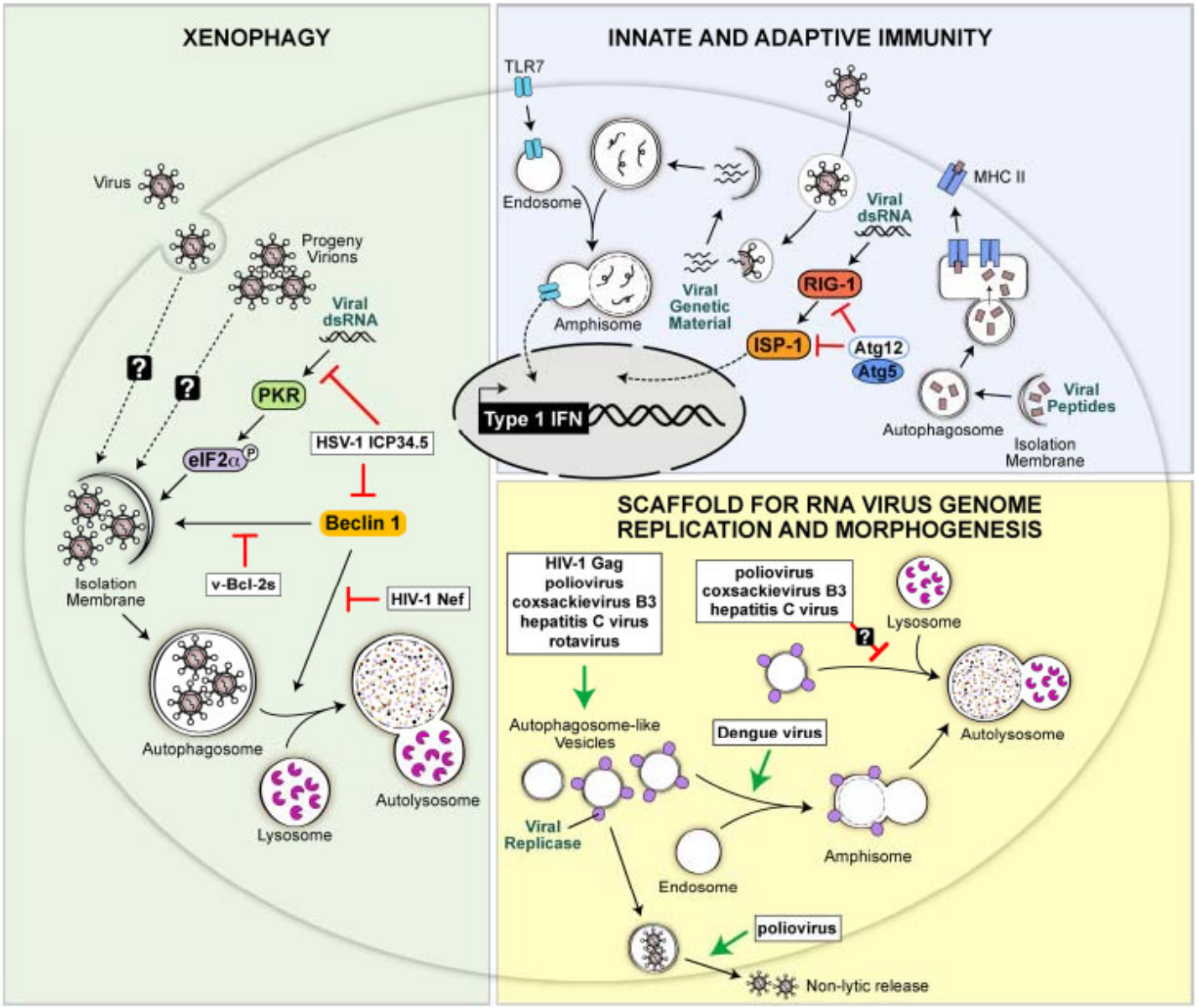
# Acquired immunity (CMI)



**Autophagy is an evolutionarily conserved intracellular process by which bulk cytoplasm is enveloped inside a double-membraned vesicle and shuttled to lysosomes for degradation.**



# Anti-viral and pro-viral functions of autophagy.



# 纲 要

- 病毒学发展史
- 医学分子病毒学研究重大事件和主要进展
- 医学分子病毒学研究热点和或重点
- 医学分子病毒学总结和展望

# 学习医学分子病毒学的内容

## 共性与个性

- 病毒的核酸，基因组的表达、调控
- 病毒基因的功能
- 病毒与宿主细胞的相互作用
- 病毒致病与免疫的机制（分子、细胞、整体）
- 控制病毒感染的策略

研究只能选择一个内容， 解决一个问题

# Viral Nucleic Acid（genome)

- 多样性: 大小悬殊(3000 nt- 200Kb)

  RNA,DNA,单链,双链,节段,环状,线形

- 基因重迭
- 基因结构与真核细胞相似(内含子,转录后拼接加工, 也有不同形式)
- 非编码区核酸的一,二级结构的意义
- 大病毒基因组中可插入外源基因

# Viral proteins

结构蛋白:衣壳 capsid(由亚单位capsomeres组成,螺旋 ( helical symmetry)对称,二十面体(icosahedral)对称, 复合对称 complex

包膜 envelope (蛋白主要由病毒基因编码,**寡糖 oligosaccharides** 组分由宿主细胞决定)多肽骨架在核糖体上合成,至高尔基复合体甘露糖**mannose** 修饰加入支链,糖蛋白分子与衣壳结合,包在其外,病毒体自细胞排出.

# Viral nonstructural proteins

- 复制酶 ( replicase)
- 多聚酶 ( polymerase)
- 与致病相关的酶或蛋白:流感,副流感病毒的HN, 疱疹病毒的TK酶,轮状病毒的类似肠毒素蛋白等
- 调控蛋白:EBNA, tet, rev,乙肝病毒X蛋白等

# 医学分子病毒学研究已经在控制病毒性疾病等方面发挥重要作用

- 病毒学研究取得长足的进步及作用：
  许多急性病毒感染在世界许多地方通过接种疫苗和其他公共卫生措施预防或控制。
  许多有效的抗病毒药物也广泛使用
  全球癌症负担相当大的一部分是由病毒感染，最常见的B型肝炎病毒和人乳头状瘤病毒感染引起的，既可以预防的疫苗。

  -------所有这些进步源自病毒的复制，传播，和发病机制的基础研究

# 新世纪有必要加强分子病毒学学习和研究

- New viruses periodically emerge and cause great personal and societal tragedy.
- AIDS, caused by human immunodeficiency virus type 1 (HIV-1), remains the defining epidemic of our time, the true cost of which cannot be calculated.
- Dengue and West Nile viruses continue to smolder, and Chikungunya virus, monkeypox virus, and Ebola and other hemorrhagic fever viruses crouch in the darkness. H5N1 avian influenza virus continues to sporadically infect humans in Southeast Asia and elsewhere. The emergence of a new influenza pandemic or a viral bioterrorism attack could have catastrophic consequences on public health, commerce, and civic discourse.

# 新世纪有必要加强分子病毒学学习和研究

Viruses and viral gene products have also emerged as valuable tools to study many aspects of biology and, potentially, to treat disease.

These tools include reverse transcriptase for the synthesis of cDNA, viral vectors for gene delivery and protein production, transgenic animal technology, vaccination, and oncolytic therapy, which attempts to harness the capacity of some viruses to specifically infect and kill cancer cells.

Studies to determine whether this approach has efficacy in the treatment of human cancers are under way.

# Emerging infections

- What will tomorrow bring? How will we deal with these new infections? Improved surveillance, more-rapid reagent sharing and information transfer, more-effective quarantine procedures, and various public health measures will undoubtedly contribute to controlling emerging diseases, but increasing attention and resources are likely to be devoted to maintaining, as well as expanding, the roster of antivirals and vaccines.
- Advances in genetics, biochemistry, structural biology, and computational biology provide a strong platform for the future development of additional antiviral drugs. Although we must certainly prepare for future threats, antiviral-drug development should not ignore viruses that currently account for a substantial burden of disease.

# Need for vaccines

- Why is it that many of our most successful vaccines were introduced 20 to 50 years ago (e.g., vaccines for hepatitis B, influenza, measles, mumps, and rubella viruses)?
- In addition to HIV, many globally important viruses (e.g., dengue virus, hepatitis C virus, human cytomegalovirus, and respiratory syncytial virus) still lack vaccines.

# Need for vaccines

Critical knowledge gaps must be filled before a “product” can be developed, and funding decisions must be tailored to these needs.

First, we must understand the basic biology of viral evolution and quasispecies.

Second, we need to define what constitutes a protective immune response.

Third, we have to acknowledge the economics of vaccine development and the risk to the private sector, recognizing that the necessity of immunizing a healthy naïve population to prevent a disease will be unacceptable if there are significant vaccine-associated adverse events.

# Viruses are great teachers and their lessons are not restricted to viral diseases.

- Viral replication is strictly dependent on cell structure, metabolism, and biochemical machinery. As a consequence, viral gene products interact with crucial regulatory nodes that control cell function, a situation that facilitates the identification and characterization of these nodes and the networks they control.
- The roster of important discoveries uncovered by studies of viral replication and transformation is long: the existence of mRNA and mRNA processing, including splicing, capping, and polyadenylation; transcriptional control elements and transcription factors; gene silencing mechanisms; cellular oncogenes and tumor suppressor proteins; and signal transduction pathways and tyrosine kinases, to name just a few

# Molecluarl Virolgy will play more important role in 21st Biology

- History has proven again and again that understanding the basic biology of viruses leads to new and often unexpected insights.
- We anticipate that studies of viruses will continue to yield surprising glimpses into the inner workings of their host cells.
- The knowledge, techniques, new ideas, and urgency to learn more are stronger than ever. The importance of studying the basic biology of viruses, even those that today may not seem relevant to human, animal, and plant disease, cannot be overstated

# 新世纪学习和研究分子病毒学的特点

- The general trends likely to drive virology research in the next decade include systems biology of virus-host interactions, viral ecology and the virosphere, evolution of viruses, and improved vaccines and therapeutics. Many of these advances will be accelerated by technologic innovations in high-throughput sequencing, the complete synthesis of viral genomes, small-molecule and shRNA screens, and the imaging of cells and whole organisms, in concert with traditional methods used by virologists.

# A systems approach to virology

- Instead of studying one gene or gene product at a time, examining large groups of genes or gene products allows the identification of fundamental biological networks.
- By networks, we mean complex and interconnected intracellular processes that control, for example, gene expression, organelle biogenesis, and metabolism, as well as networks of intercellular communication at the tissue, organ, and whole-organism level.

# A systems approach to virology

- Future studies of viral pathogenesis may be seen in terms of specific viral signatures of network imbalance that do not affect just one pathway but alter the fundamental homeostatic balance of a cell, organism, or population.

- Interactions of viral gene products with these networks will likely differ in different cell types and tissues. Technological advances in cell and organ culture will allow the in vitro study of viral infections under conditions that more precisely mimic the in vivo environment.

- This effort will extend our understanding of the interplay of microbial communities and host cells within an entire organism. Once such an understanding is achieved, we may be able to better identify cellular genes associated with disease risk and therefore predict which human or animal hosts should be vaccinated or prophylaxed.

# Interactomics

The process by which viral gene products interact with cellular gene products to affect different phenotypes. This field has flourished in studies of the oncogenic activity of DNA tumor viruses, but similar interactions surely underlie much of the cellular response to viral infection. Research on virus-host interactions and network dynamics will produce new insights into why some viruses can occasionally enter into new host species to cause new and unexpected diseases. In fact, the study of how zoonotic viruses become human pathogens has already become a major focus of 21st century virology.

# New and old: coupling new technology with established procedures

Experiments using cell culture, classical biochemistry, animal models, clinical trials, and population-based analyses will continue to be essential components of contemporary and future virology research.

The new technologies will not displace their predecessors but join and complement them.

For example, the characterization of new viruses discovered through deep sequencing will require cultured cells to investigate viral replication biology and host organisms to investigate viral pathogenesis and disease outcomes. The causal association of viruses with specific disease phenotypes will require experimental infections and intervention trials. Thus, the virology toolbox will be enhanced by technological innovations rather than replaced by them.

# 新世纪对病毒学工作者的要求

- Traditional teams of basic scientists working with clinicians will be augmented by mathematicians, physicists, and population biologists, among others.
- Training the next generation of scientists to be capable of undertaking this research will require more-diverse course offerings, enhanced training opportunities, especially involving interdisciplinary collaboration and computational approaches, and instruction in teamwork.
- To facilitate professional advancement, we will need to develop new strategies to recognize and reward individual contributions to group scientific efforts as team science becomes more and more prevalent.

# 新世纪病毒学研究工作的特点
# Call for collaboration

You will see not only authors with expertise in cell biology, immunology, and chemistry but also authors who are experts in ecology, computer and information science, mathematics, and physics, among others. Interdisciplinary research in virology is essential for future progress.

# PERSONAL CURIOSITY

- The power of a single mind to identify a problem and solve it is one of the greatest joys of humanity.
- The very meaning of the word is that:

We did not know what we would find when we started looking.

The process of discovery follows paths that often seem simple or elegant in retrospect but almost always reflect individual intellect, personal curiosity, and luck, which cannot be defined or packaged.

Although the world of science is changing in many ways, the role of the individual scientist in discovery cannot be underestimated.

# PERSONAL CURIOSITY

Someone has to see something no one else did. Many of the investigators who will make discoveries in the 21st century will do so because they want to understand how something works, not because something is trendy.

# Discussion of questions

**What do you think virus as a living molecular machinery can contribute t medical sciences and life sciences?**

**Do you think that molecular virology be the teacher for cell biology and immunology? Why?**

**Choose one and write a paragraph of words.**

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# Thank you ！

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