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丝状病毒——被忽视的原核生物病毒“潜伏者”

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摘要 丝状病毒是一类具有极高多样性和普遍分布性的病毒类群, 它们可以侵染几乎所有的细菌门类, 甚至包括一些古菌. 经过将近60年对以M13, CTXΦ等为代表的丝状病毒的研究, 已经发现它们具有独特的生物学特征(如慢性感染)和重要的应用价值. 本文综述了丝状病毒的分离及分布状况、基因组特征及颗粒结构、生命周期、诱导调控机制、对宿主生理功能的影响、应用领域等, 并在此基础上对未来的研究方向做出展望. 本文认为今后对丝状病毒的深入研究, 将对进一步理解原核生物病毒-宿主相互作用关系和潜在的生态功能, 以及拓展这一重要病毒群系在环境和健康领域的应用深度和范围, 都具有重要的推动作用.

关键词 丝状病毒, 慢性感染, 生命周期, 诱导调控机制, 病毒-宿主相互作用

丝状病毒(*Inovirus*), 也常被称为丝状噬菌体(filamentous phage), 是丝状病毒科(Inoviridae)中最早被命名的一个属. 根据国际病毒分类委员会(International Committee on Taxonomy of Viruses, ICTV)的最新报告(2021年5月公布), 丝状病毒科包含21个属以及27个种. 其颗粒呈长细丝状, 直径6~10 nm, 长度800~4000 nm[1]. 病毒颗粒由蛋白外壳和一条呈双股螺旋的环状闭合单链DNA组成. 丝状病毒基因组的大小在5~15 kb[2]之间, 可编码10余种蛋白[3]. 目前已分离的大多数丝状病毒侵染革兰氏阴性细菌, 仅有个别以革兰氏阳性菌为宿主. 与其他病毒不同, 丝状病毒最独特的特征之一是它们具有建立慢性感染(chronic infection)的能力, 即病毒基因组以完全游离状态或整合到宿主染色体中的方式长期存在于宿主细胞内, 病毒颗粒大量释放时不会导致细胞裂解死亡[3,4]. 由于独特的病毒颗粒形态及生命周期, 丝状病毒的研究极大地丰富了人们对原核生物病毒多样性的认识. 此外, 丝状病毒还有重要的应用价值, 例如用于高通量蛋白质筛选的噬菌体展示技术(phage display), 以及作为药物运送的纳米载体等[5].

1 分离及分布情况

19世纪60年代, 丝状病毒f1, fd, M13(即Ff类丝状

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病毒)首次从大肠杆菌(*Escherichia coli*)中分离获得[6~9]. 随后, 丝状病毒陆续从其他细菌中被分离, 如侵染霍乱弧菌(*Vibrio cholerae* O139)的VSK[10]、侵染铜绿假单胞菌(*Pseudomonas aeruginosa* PAK)的Pf1等[11]. 目前已经分离的丝状病毒有62种(表1[12~64]), 它们主要以革兰氏阴性菌为宿主. 其中有17种侵染弧菌属(*Vibrio*)的细菌[10,18~28,60~62], 其他分离较多的丝状病毒宿主包括劳尔氏菌属(*Ralstonia*)[34~40]、黄单胞菌属(*Xanthomonas*)[41~47]的菌株(图1A).

由于已分离的绝大多数丝状病毒仅侵染属于γ变形菌门(γ-Proteobacteria)中的一些革兰氏阴性细菌, 丝状病毒长期以来被认为是一类很小的病毒群系. 然而, 近年来针对丝状病毒的(宏)基因组学分析完全改变了这一认知. Hay和Lithgow[1]通过对丝状病毒中包含Zot保守结构域的DNA复制蛋白pI的分析, 将丝状病毒属扩展为5个新的属(*Inovirus*, *Habenivirus*, *Fibrovirus*, *Lineavirus*, *Saetivirus*)及一个unclassified属. 进一步地, Roux等人[2]通过机器学习的方法, 利用保守蛋白和基因组特征的组合分析, 从全球微生物基因组和宏基因组中鉴定了10295个丝状病毒基因组, 它们被归为6个不同的科(Vespertilinoviridae, Amplinoviridae, Paulinoviridae, Densinoviridae, Photinoviridae和Protoinoviridae), 包含212个亚科(subfamily). 宿主预测分析表明, 它们可以侵染来源于多种生态环境的几乎所有的细菌门, 特别值得注意的是, 基因组分析和PCR验证首次发现丝状病毒可侵染部分古菌(图1B和C). 海洋作为地球

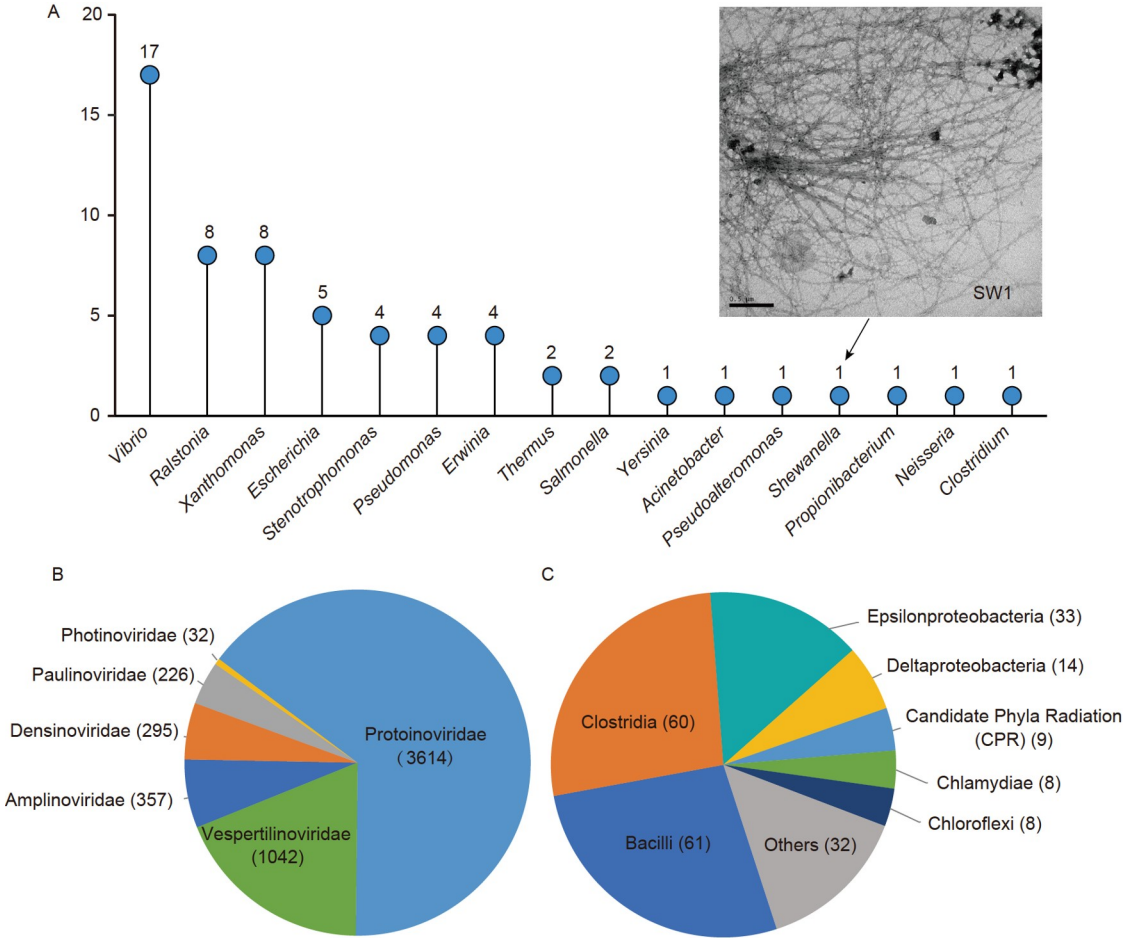

图 1　可培养和未培养丝状病毒及其宿主的分类. A: 目前可培养的丝状病毒的宿主菌分类(按照属水平划分)以及分离自*Shewanella piezotolerans* WP3的丝状病毒SW1电镜图(标尺为0.5 μm); B: 未培养丝状病毒分类及数量[2]; C: 未培养丝状病毒的宿主菌分类[2]

Figure 1　The distribution of cultivable and uncultivable *Inoviruses* as well as their hosts. A: The host taxonomy of currently cultivable *Inoviruses* (grouped by genus) and transmission electron microscopy image of *Shewanella piezotolerans* WP3 phage SW1; B: the viral taxonomy of uncultivable *Inoviruses*; C: the host taxonomy of uncultivable *Inoviruses*

表 1 目前已分离可培养丝状病毒

Table 1 The information of cultivable *Inoviruses* that have been isolated

丝状病毒	分类(属)[a)]	宿主	Accession number[b)]	基因组大小 (kb)	开放阅读框	GC(%)	分离时间	参考文献
f1	*Inovirus*	*Escherichia coli* X12	J02448	6.4	10	41	1960	[6,12]
M13	*Inovirus*	*Escherichia coli* K12	V00604	6.4	10	40.7	1968	[9]
fd	*Inovirus*	*Escherichia coli*	J02451	6.4	10	40.9	1963	[8]
If1	*Infulavirus*	*Escherichia coli*	U02303	8.4	10	43.7	1967	[13]
Ike	*Lineavirus*	*Salmonella typhimurium*	X02139	6.8	10	40.5	1972	[14]
I2-2	*Lineavirus*	*Salmonella typhimurium* JE2571	X14336	6.7	9	42.7	1982	[15]
CUS-1	–	*Escherichia coli* RS218	–	9.5	13	–	2002	[16]
Ypfφ	–	*Yersinia pestis* CO92	–	8.7	13	–	2007	[17]
CTXφ	*Affertcholeramvirus*	*Vibrio cholerae*	HQ224500	10.6	13	44.6	1996	[18]
VFJφ	*Saetivirus*	*Vibrio cholerae* ICDC-4470	KC357596	8.5	12	44.3	2013	[19]
VCYφ	*Vicialiavirus*	*Vibrio cholerae* 7D07PW5	JN848801	7.1	11	41.4	2011	[20]
KSF1φ	*Capistrivirus*	*Vibrio cholerae*	AY714348	7.1	14	44.4	2005	[21]
VfO3K6	*Versovirus*	*Vibrio parahaemolyticus*	AB043678	8.7	10	45.2	2000	[22]
VfO4K68	*Versovirus*	*Vibrio parahaemolyticus*	AB043679	6.8	8	47.2	2002	[23]
Vf33	*Villovirus*	*Vibrio parahaemolyticus*	AB012573	7.9	7	45.7	1984	[24]
fs1	*Fibrovirus*	*Vibrio cholerae* AI1855	D89074	6.3	15	43.4	1997	[25]
fs2	*Saetivirus*	*Vibrio cholerae* MDO14	AB002632	8.6	9	44.5	1997	[25]
ND1-fs1	*Fibrovirus*	*Vibrio cholerae* ND1	AB572858	6.8	12	42.9	2012	[26]
VSK	*Fibrovirus*	*Vibrio cholerae* PO7、B04	AF453500	6.8	14	43.7	1996	[10]
VEJφ	*Fibrovirus*	*Vibrio cholerae* MO45	FJ904927	6.8	11	43	2010	[27]
VGJφ	*Fibrovirus*	*Vibrio cholerae* SG25-1	AY242528	7.5	13	43.4	2003	[28]
Pf1	*Primolicivirus*	*Pseudomonas aeruginosa*	X52107	7.3	14	61.5	1966	[29]
Pf3	*Tertilicivirus*	*Pseudomonas aeruginosa*	M11912	5.8	10	45.4	1974	[30,31]
Pf4	–	*Pseudomonas aeruginosa* PAO1	–	–	–	–	2003	[32]
Pf5	–	*Pseudomonas aeruginosa* PA14	–	–	–	–	2007	[33]
φRSM1	*Habenivirus*	*Ralstonia solanacearum* M4S	AB259123	9	15	60	2007	[34]
φRSM3	*Habenivirus*	*Ralstonia solanacearum* MAFF730139	AB434711	8.9	15	59.7	2009	[35]
φRS603	*Habenivirus*	*Ralstonia solanacearum*	AB937974	7.6	13	59.4	2014	[36]
φRs551	*Habenivirus*	*Ralstonia solanacearum* UW551	KX179905	7.9	14	60.8	2017	[37]
φRSS1	*Restivirus*	*Ralstonia solanacearum* C319	AB259124	6.6	12	62.6	2007	[34]
φRSS0	*Restivirus*	*Ralstonia solanacearum*	JQ408219	7.2	12	62.1	2013	[38]
φRS611	*Restivirus*	*Ralstonia solanacearum*	AB931172	6.3	11	62.1	2010	[39]
φPE226	*Parhipatevirus*	*Ralstonia solanacearum*	HM064452	5.4	9	61.7	2010	[40]
φLf	–	*Xanthomonas campestris*	MH206184.1	6	10	59.9	1990	[41]
Xf	–	*Xanthomonas oryzae*	–	–	–	–	1969	[42]
Cf	–	*Xanthomonas campestris*	–	–	–	–	1980	[43]
Cflt	–	*Xanthomonas campestris*	–	7.6	–	–	1987	[44]
Cf1c	*Coriovirus*	*Xanthomonas campestris*	M57538	7.3	9	58.1	1991	[45]
Cf16	–	*Xanthomonas campestris*	–	–	–	–	1987	[46]

(表1续1)

丝状病毒	分类(属)[a)]	宿主	Accession number[b)]	基因组大小(kb)	开放阅读框	GC(%)	分离时间	参考文献
XacF1	*Coriovirus*	*Xanthomonas campestris*	AB910602	7.3	13	58.1	2014	[47]
Ngoφ6	–	*Neisseria gonorrhoeae*	–	–	–	–	2014	[48]
CRAφ	–	*Acinetobacter baylyi* ADP1	–	–	–	–	2016	[49]
φSHP1	*Psecadovirus*	*Stenotrophomonas maltophilia* P2	–	6.8	10	61.1	2012	[50]
φSHP2	–	*Stenotrophomonas maltophilia* P28	NC_015586	5.8	9	61.5	2013	[51]
φSMA6	*Scuticavirus*	*Stenotrophomonas maltophilia* Khak-84, Khak-94, Bor-40, Bor50	HG315669	7.6	10	62.6	2013	[52]
φSMA7	*Subteminivirus*	*Stenotrophomonas maltophilia* Khak-84, Bor-40	HG007973	7	10	62.3	2013	[52]
f327	–	*Pseudoalteromonas* BSi20327	–	–	–	–	2015	[53]
SW1	–	*Shewanella piezotolerans* WP3	–	7.7	9	–	2007	[54]
φOH3	–	*Thermus thermophilus* HB8	NC_045425	5.6	8	58	2016	[55]
PH75	–	*Thermus thermophilus*	–	–	–	–	2006	[56]
B5	–	*Propionibacterium freudenreichii*	NC_003460	5.8	10	64.3	2002	[57]
CAK1	–	*Clostridium acetobutylicum* NCIB 6444	–	6.6	–	–	1991	[58]
WW-nAnB	–	unknown	NC_026582	4.8	8	44.4	2011	[59]
vB_VpaI_V-P-3218	–	*Vibrio parahaemolyticus* VN-3218	–	11	14	44.2	2020	[60]
VAIφ	–	*Vibrio anguillarum* Ba35	–	6.1	11	–	2020	[61]
VALGΦ6	–	*Vibrio alginolyticus*	MN719123.1	8.5	13	44.3	2020	[62]
VALGΦ8	–	*Vibrio alginolyticus*	MN690600	7.3	10	46.3	2020	[62]
PEar1	–	*Erwinia amylovora*	MT901797	6.6	10	41.7	2020	[63]
PEar2	–	*Erwinia amylovora*	MT901798	6.6	10	41.7	2020	[63]
PEar4	–	*Erwinia amylovora*	MT901799	6.8	10	41.7	2020	[63]
PEar6	–	*Erwinia amylovora*	MT901800	6.6	11	41.7	2020	[63]
Xf109	*Xylivirus*	*Xanthomonas oryzae*	KX181651	7.2	12	59.6	2017	[64]

a) 根据ICTV对Inoviridae的分类报告(2021年5月公布). b) 来自NCBI数据库

最大的生态系统, 其中丝状病毒被发现在某些区域中大量存在. 2015年, 丹麦Jørgensen教授团队[65]通过对陆架边缘海洋深部沉积物宏转录组的分析, 发现丝状病毒科是高度活跃病毒中的优势类群. 同年, Dell'Anno等人发现丝状病毒在北冰洋深海沉积物中的丰度接近病毒总量的50%[65]. 近期, 本团队[66]宏基因组分析结果也显示, 丝状病毒在太平洋的表层和深层海水中均大量分布.

除了基于序列的组学证据外, 其他研究手段提供的实验证据也为丝状病毒在环境中的广泛分布提供了证据. 2003年, Middelboe等人[67]在丹麦Niva Bay近海沉积物中, 通过形态学分析发现潮间带的病毒群落以丝状病毒为主. 2014年, Wang等人[68]对珠江口水体的71株*V. cholera*进行分析, 发现大量pro-CTXΦ的(CTXΦ的原噬菌体形式)存在, 表明丝状病毒在河口环境中进行着活跃的基因水平转移. 同年, Pan等人[69]在美国普拉特河平原(Platte River floodplain)地下沉积物中, 利用丝裂霉素C诱导和透射电镜检测发现其中的优势菌株*Pseudomonas* sp. Alda10被丝状病毒侵染.

2　基因组特征及病毒颗粒结构

与大部分原核生物病毒相比, 丝状病毒的基因组较小, 编码基因的数量在7~13个之间. 作为丝状病毒的代表, Ff类病毒的基因组编码的蛋白可分为三类功能, 包括DNA复制(pI)、衣壳蛋白(pVIII, pIII, pVI,

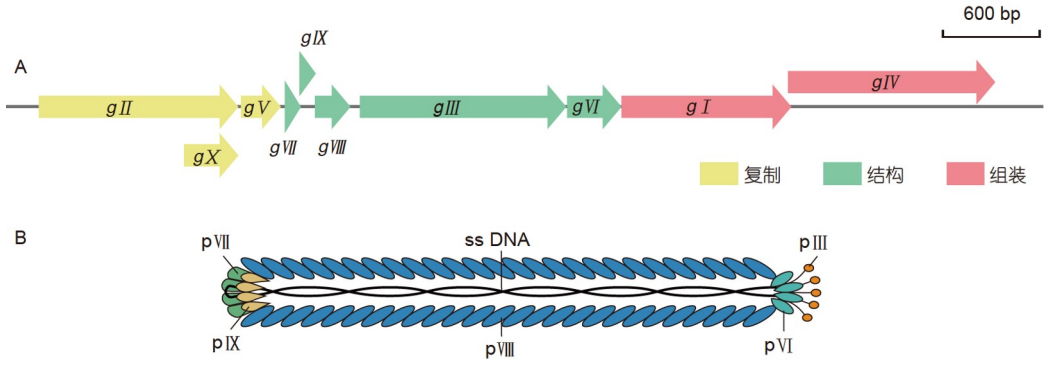

图 2 丝状病毒Ff的基因组(A)及病毒颗粒结构(B)
Figure 2 The schematic representation of genome (A) and virion structure (B) of the Ff phage

pVII, pIX)以及装配蛋白(pI, pIV)[4](图2A).

除了以上功能基因外，一些丝状病毒还携带与基因组整合、毒力因子等相关的基因，具有参与原病毒整合，影响宿主的致病性以及病毒-宿主相互作用等重要功能. 如ΦRSM1和ΦRSM3具有编码丝氨酸重组酶的基因[70]，Pf4和Pf5则可分别产生功能性切离酶XisF4和XisF5，参与丝状病毒从宿主基因组上的切离(excision)过程[71]. 而其他的丝状病毒, 如CTXΦ和VEJΦ等本身并不编码整合酶，而是借助于宿主的位点特异性重组酶XerC/XerD进行整合[72]. CTXΦ基因组中还包含编码霍乱毒素的基因*ctxAB*, 可能增加其宿主的致病性[73].

典型Ff类丝状病毒颗粒的主体由约2700个拷贝的主要衣壳蛋白pVIII构成(图2B)，它是一种具有α螺旋的小分子蛋白，其C末端带有正电荷. 次要衣壳蛋白pVII，pIX，pIII和pVI位于丝状病毒的两端，其中pVII和pIX封闭病毒颗粒的顶端, 而pIII和pVI封闭尾端[4,74]. 次要衣壳蛋白pIII的C末端结构域与疏水蛋白pVI相互作用，这对于病毒的稳定性和子代病毒颗粒的释放是必需的；而N末端分为N1和N2结构域，分别与宿主的受体蛋白TolA和菌毛结合，在侵染宿主的过程中发挥重要作用[75]. 蛋白pVII和pIX都是具有α螺旋结构的疏水小分子蛋白，二者形成的蛋白质复合物与病毒DNA相互作用，在病毒颗粒的前端形成包装信号发夹结构以启动病毒的组装[76].

3 生命周期

对于丝状病毒生命周期，目前研究得最清楚的是侵染*E. coli*的Ff类病毒，其生命周期可大致分为侵染、基因组复制和病毒颗粒组装及释放三个阶段(图3).

3.1 侵染

当Ff的衣壳蛋白pIII的N2结构域末端与侵染宿主菌毛的末梢接触后，pIII蛋白构象发生变化，诱导菌毛收缩，病毒穿过宿主外膜. 随后含有pIII蛋白的病毒末端进入周质间隙，暴露的pIII的N1结构域与锚定在内膜上的受体TolA蛋白结合. TolA是TolQRA复合物的一部分，可控制细胞分裂过程中膜的完整性和内陷[77~80]. pIII与TolA结合后, 病毒DNA穿过宿主细胞内膜，被注入宿主细胞质中，而主要衣壳蛋白嵌入内膜，用于之后包装形成新的病毒颗粒[7].

3.2 基因组复制

Ff的基因组以侵染型(infective form, IF)的单链环状DNA注入宿主细胞质后，会招募宿主RNA聚合酶结合于负链复制起始位点的发夹结构，开始以ssDNA为模板生成约18~20 nt的RNA引物，再在宿主DNA聚合酶III作用下延伸产生负链，形成复制型(replicative form, RF)的双链DNA[81,82]. RF DNA可以作为病毒正链DNA合成以及病毒mRNA转录的模板. 随后，pII蛋白与新合成的RF DNA正链结合，在特定位置(正链复制起始位点)剪切正链并附着于5′端[83,84]，而游离的3′端作为引物，以滚环形式合成新的正链. 当复制完成产生一个完整的环，pII蛋白切割并环化自由末端，同时产生IF和RF结构[84].

在侵染早期，丝状病毒基因组复制按照上述步骤进行, 随着RF DNA拷贝数的增加, 宿主细胞中病毒蛋

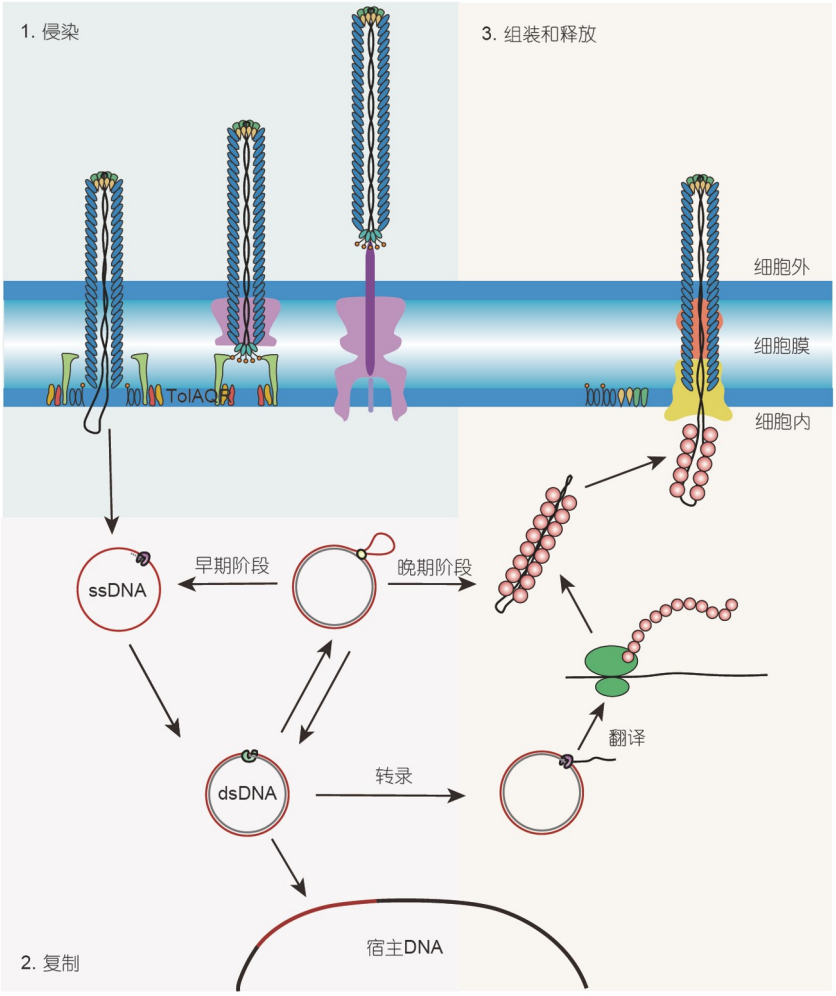

图 3　典型丝状病毒Ff的生命周期
Figure 3　The life cycle of Ff phage

白质的数量也逐渐增加[85]. 在侵染后期, pV蛋白水平达到临界值, 形成二聚体并开始覆盖整条IF DNA, 同时抑制负链的合成以及pII和pX蛋白的翻译, 导致IF DNA的大量累积[86,87].

3.3　组装和释放

在丝状病毒组装过程中, 新复制生成的ssDNA被pV所包裹, 并在“包装信号”的引导下, 将DNA-蛋白质复合物靶向宿主细胞的内膜. 在细胞外膜上分泌蛋白pVI的协助下, 由pI提供能量[88], 合成的病毒颗粒由锚定在细胞内膜上的pI和pXI分泌复合物排出[89,90], 最终成熟的病毒颗粒以非裂解方式通过细胞膜主动分泌到细胞外.

除了利用自身编码的分泌蛋白, 一些丝状病毒的释放需要宿主细胞的分泌系统或蛋白协助来完成. 例如, CTXΦ从*V. cholera*中释放时, 利用了内源性II型分泌系统的EspD蛋白[91]; MDAΦ从*N. meningitidis*中释放时, 则利用了内源性VI型分泌系统PilQ蛋白[92].

4　诱导调控机制

作为一类温和型病毒, 基因开关(genetic switch)的诱导与调控是丝状病毒生命周期转换的关键. 目前, 对于这一关键过程研究最多的是侵染*V. cholerae*的丝状病毒CTXΦ(图4). CTXΦ的基因转录由两个主要的启动子P_A和P_R控制, 其中P_A起始转录噬菌体的结构基因,

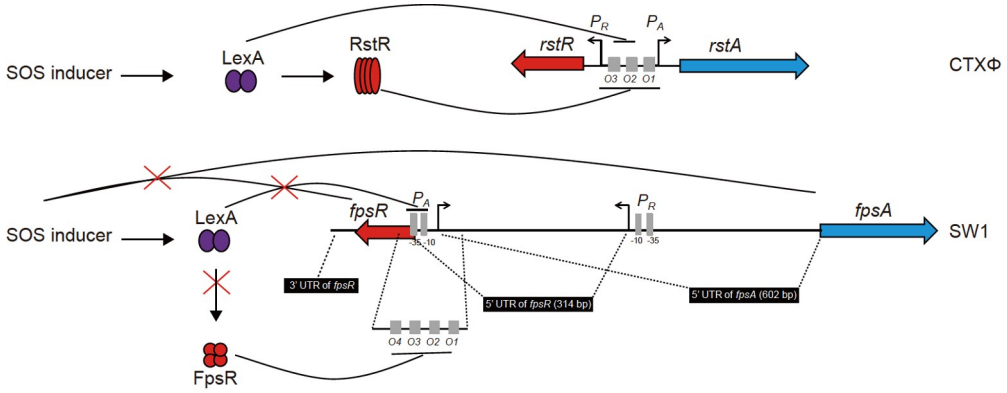

图 4 丝状病毒CTXΦ和SW1的基因开关对比
Figure 4 The comparision of genetic switch between CTXΦ and SW1

而P_R起始转录CTXΦ自身调控蛋白的编码基因*rstR*, RstR在*rstA*和*rstR*的基因间区包含3个结合位点(*O1*, *O2*, *O3*). 在溶原状态下, RstR结合在*O1*区域, 抑制由P_A起始的转录[93,94]; SOS途径的抑制因子LexA蛋白结合于*O2*区域[95,96]; P_R则处于开启状态, 不断合成RstR. 当原噬菌体诱导因素(如紫外照射等)出现时, RecA与ssDNA结合致使其激活, 激活态的RecA使LexA发生自我剪切, 则RstR结合于*O2*, *O3*, 阻碍了由P_R起始的转录, 从而降低RstR的水平. 在这种情况下, 启动子P_A的转录量增加, 病毒合成所需的基因得以大量表达. 随着RstR水平的降低, P_R途径又会被开启[96], 最终CTXΦ基因的转录又会被新合成的LexA和RstR所抑制[73].

与受SOS应激反应机制调控的CTXΦ不同, 分离自深海的丝状病毒SW1的基因开关受到低温的显著诱导[54,97]. SW1的基因开关具有独特的结构, 它包含两个反向交叉转录的启动子P_A和P_R, 分别控制一系列结构基因和自身调控因子的转录, 与CTXΦ不同, 它们之间没有共用的间隔区作为调控区域. 有趣的是, 这两个启动子之后均包含了很长的5′非翻译区(5′untranslated region, UTR), 它们在SW1的低温诱导中发挥者显著的作用; 此外, 在*fpsR*基因的下游还存在3′ UTR, 但其功能还不清楚[98]. SW1自身编码的FpsR蛋白可与相应的操纵子结合, 通过“空间位阻”和“路障”两种机制调节病毒基因的转录, 从而在SW1基因开关的转换中发挥关键作用[99]. 最近, 本团队[66]研究发现, SW1基因开关的诱导依赖于一个阈值温度(4℃), 且这种依赖性并不与温度梯度成反比(图4).

除了转录水平, 对丝状病毒基因开关的调控还可发生在原病毒的切离过程. 分离自*P. aeruginosa* PAO1和PA14的Pf4和Pf5分别编码的切离酶XisF4和XisF5都能显著提高原噬菌体的切离频率, 但只有XisF5是Pf5切离所必需的. XisF4具有非常精妙的调控方式: 一方面, *xisF4*和邻近的噬菌体抑制基因*pf4r*具有不同的转录方向, 但两者有重叠的5′ UTR. XisF4和Pf4r不仅会激活自身的表达, 还会相互抑制. 另一方面, *xiF4*基因的表达受到宿主蛋白MvaT和MvaU的协同抑制[71]. 另外, Pf4还可以编码生成Ⅱ型毒素-抗毒素(PfiT/PfiA), 其中毒素蛋白PfiT通过对*xisF4*和噬菌体复制起始因子(replication initiation factor)的抑制作用, 实现对Pf4切离和复制的调控[100].

5 丝状病毒对宿主生理功能的影响

由于独特的建立慢性感染的特征, 丝状病毒与宿主之间的关系除了寄生, 还体现了显著的合作关系[2], 它们对宿主的影响体现在多个方面(图5).

5.1 致病性

研究表明, 一些丝状病毒可直接或间接地影响宿主的毒力因子和致病性. 从*V. cholera*中分离的CTXΦ携带霍乱毒素基因(*ctxA*, *ctxB*)[73], 从*V. parahaemolyticus*中分离的VfO4K68和VfO3K6携带封闭带毒素基因(*zot*)和辅助霍乱肠毒素基因(*ace*)[23], 它们与弧菌的致病性(如人类急性腹泻等)密切相关. 侵染*R. solanacearum*的ΦRSS1通过改变宿主细胞膜的疏水性使得局部产生高细胞密度, 导致毒力基因*phcA*激活, 从而诱发宿

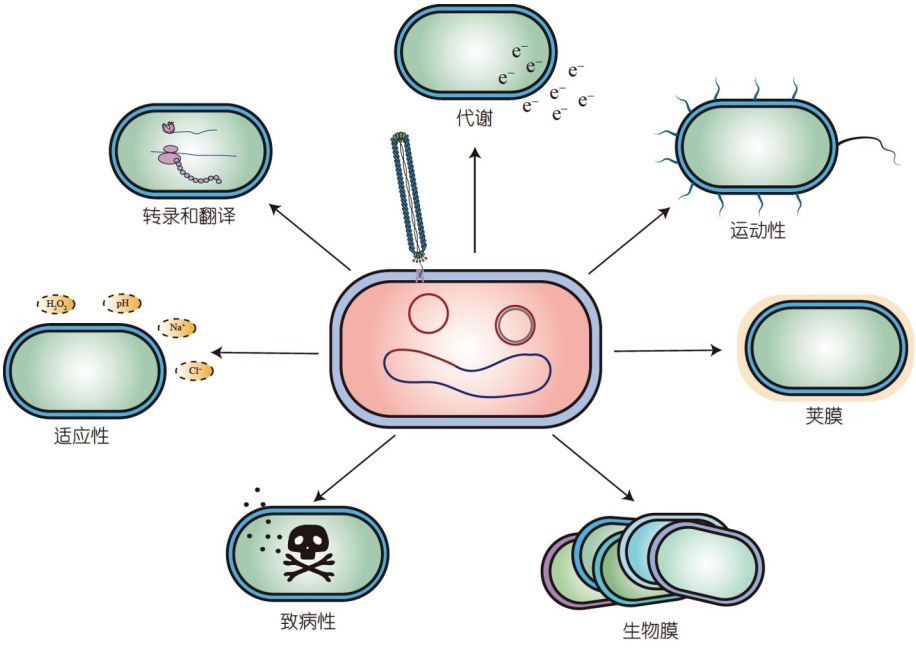

图 5　丝状病毒对宿主生理功能的影响
Figure 5　The influence of *Inoviruses* on the physiology of their hosts

主的毒性，这也是许多经济作物发生病害及产量降低的重要原因[101].

5.2　环境适应性

以两株来源于海洋的丝状病毒为例：分离自北极海冰中*Pseudoalteromonas* sp. BSi20327的丝状病毒f327，虽然降低了宿主对H_2O_2以及NaCl的耐受能力，但使得BSi20327的运动性和趋化性增强，从而有助于宿主对海冰环境的适应[53]. 在对深海沉积物来源的丝状病毒SW1的研究中发现，SW1的缺失导致其宿主*Shewanella piezotolerans* WP3在低温下侧生鞭毛数量明显增多，由侧生鞭毛介导的涌动能力(swarming motility)显著增强[102]. 进一步地，在模拟深海原位的条件下，SW1可显著影响WP3的生长以及众多与转录和翻译等基础生命活动相关基因的表达[103]. 这些结果提示在深海环境中，丝状病毒能显著调节深海细菌的转录和翻译元件的合成，以及群体性运动能力，从而降低能量消耗，这可能是其在深海环境中维持物质能量平衡的重要适应性策略之一[103].

5.3　生物膜

在自然环境中，微生物常以生物膜的形式存在[104]，已有多项研究表明丝状病毒对其宿主(如*P. aeruginosa*)形成生物膜的过程具有显著的影响[105~107]. 研究者对以游离状态和生物膜状态生长的*P. aeruginosa* PAO1进行差异表达基因分析发现，丝状病毒Pf1的所有基因的转录水平在生物膜中显著升高[108]；进一步的研究表明，丝状病毒Pf4的缺失会减缓宿主PAO1生物膜的形成并降低生物膜的结构完整性[107]，同时，Pf4的产生会使得生物膜基质形成高度有序的液晶结构，增强其黏附性以及抗生素耐受性，从而增强生物膜抵抗环境胁迫的功能[105].

6　应用

丝状病毒由于其基因组小且易于操作、独特的复制和整合模式、易于培养等特点，在生物技术、生态环境以及新型材料等方面都有诸多应用[5].

在生物技术方面，1985年Smith[109]首次实现利用丝状病毒M13表达外源基因，自此开启了噬菌体展示技术(于2018年获诺贝尔化学奖)的研究. 相比于烈性病毒，丝状病毒在增殖期间不会裂解宿主，极大地简化了淘选过程中纯化病毒的步骤，因此以M13，f1，fd为代表的丝状病毒被广泛用于噬菌体展示技术. 这项

技术从起初用于蛋白的展示以及筛选，现已拓展至疾病的诊断和治疗等多个领域. 其衍生品在生物制剂、载体疫苗和生物医疗支架等方面也有广阔的应用前景. 此外, 利用丝状病毒在宿主细胞内存在多拷贝质粒形式的特点，可将其改造成克隆/蛋白表达载体. 基于丝状病毒SW1的RF DNA构建的蛋白表达载体pSW2, 可以在*E. coli*和*S. piezotolerans*中稳定复制, 并成功实现蛋白在低温条件下的高效表达[110]. 在pSW2基础上进一步优化改造获得的表达载体pSW4，可用于高效表达深海来源的DNA聚合酶[111].

在环境治理方面，研究发现用丝状病毒共接种细菌可以增加退化土地中微生物群落的多样性，从而改善土壤微生态环境. 此外，丝状病毒较小的基因组非常便于操作改造，可用于表达特定蛋白作为特异性受体或探针，从而作为检测环境样品中极低水平的病原体和污染物的实时传感器. 这不仅有助于做出更明智的环境修复决策, 而且还能显著节省时间和资源[112].

在材料科学方面，丝状病毒具有均匀的粒子长度, 为理解胶体液晶的特性提供了有用的模型，可广泛应用于软物质物理学方面的研究[113]. 另外，丝状病毒还可用作新型纳米材料，大大降低生产成本. Sattar等人[114]构建了一种方法, 该方法能产生不包含功能基因及抗性基因的极短的Ff来源的纳米颗粒.

7 总结及展望

长期以来，丝状病毒的分布普遍性及生态功能重要性都被忽略. 近年来，基于宏基因组的生物信息学分析获得了大量的丝状病毒基因组序列，极大地改变了人们对丝状病毒的认识. 丝状病毒在多种地球环境中均大量存在，且具有广泛的宿主侵染范围. 值得注意的是，研究表明丝状病毒群体携带了庞大的功能基因库[2]，考虑到它们与宿主长期和平共处的生存策略, 人们有理由推断丝状病毒在调控微生物代谢活性和适应性, 影响物种多样性与演化过程, 调节生物地球化学循环等方面都具有重要的生态功能.

需要指出的是，目前已分离的丝状病毒数量还非常有限，且集中在少数类群. 考虑到已被揭示的丝状病毒极大的多样性，更多代表性的丝状病毒的分离和鉴定工作亟待开展. 同时，应该注意到丝状病毒独特的生物学特征: (ⅰ) 较小的基因组且缺少保守的病毒特征蛋白; (ⅱ) 以ssDNA作为基因组，不易被SYBR-Green等常用核酸染料检测; (ⅲ) 长丝状的病毒颗粒形态使得其易与鞭毛、菌毛等细菌表面附属物混淆，这些特点使得丝状病毒在研究中通常被忽视. 此外，已有实验数据表明，一些丝状病毒并不受实验室中常用的DNA损伤试剂如UV、丝裂霉素C的诱导(本实验室未发表结果), 这也进一步增加了其被分离的难度.

进一步来说, 受没有可操作的遗传体系的限制, 多数已分离的丝状病毒生命周期的分子机制研究仍然非常欠缺. 例如, 侵染过程中, 除了已知的少数丝状病毒以细胞表面的菌毛作为识别受体，大多数丝状病毒的侵染受体仍然未知; 关于丝状病毒复制机制的研究仅限于侵染*E. coli*的Ff病毒，其他宿主来源的丝状病毒的复制机制研究极少; 除了宿主的调控因子，病毒自身蛋白在复制过程中发挥了怎样的作用尚不清楚; 在病毒组装和释放过程中，侵染时保留于内膜上的衣壳蛋白如何释放参与新的病毒颗粒组装，又如何分泌到外膜等内在的分子机制仍有待研究.

综合来看，半个多世纪以来对少数可培养丝状病毒的研究已经揭示了其独特的生命周期和特征，进而推动了它在多个领域的重要应用. 近年来，飞速发展的宏基因组及宏病毒组分析将丝状病毒从“小众”推向“大众”. 本文认为, 在各类人工和自然环境中, 这一原本“隐匿”的病毒类群如何调节其宿主的生理代谢和种群结构，进而如何影响人类和环境健康，是有待解答的重要科学问题. 同时, 在最新提出的“微生物群系”的理念和框架下深入理解丝状病毒这一广泛分布的原核生物病毒的生态功能与地位，将是今后值得探究的方向.

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Inoviruses—the neglected "lurkers" in prokaryotic viruses

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Inoviruses are highly diverse and globally prevalent, and the prokaryotes they infect cover almost all bacteria phyla, even including some archaea. After nearly sixty years of study on the typical *Inoviruses* represented by M13 and CTXΦ, it has been shown to possess unique biological characteristics (e.g. chronic infection) as well as important application value. In this reviews, we firstly summarized the isolation and environmental distributions of *Inoviruses*, and then described their genome feature, virion structure, life cycle, induction and regulatory mechanism, the physiological influences on their hosts, and their applications in different fields. Finally, prospects and directions for future investigation are proposed based on current situation of *Inovirus* research. We believe that further study on *Inoviruses* will significantly promote the understanding of prokaryotic virus-host interactions and their potential ecological functions, Meanwhile, these efforts will undoubtedly expand the depth and range of the application of *Inoviruses* in the field of human health and environmental science.

***Inoviruses*, chronic infection, life cycle, induction and regulatory mechanism, virus-host interaction**

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