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TAILED BACTERIOPHAGES: THE ORDER *CAUDOVIRALES*

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I. Introduction

Tailed bacteriophages, or phages, are recognized immediately in the electron microscope by their polyhedral heads and tubular tails. Their special nature was acknowledged early in the history of virus taxonomy.

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The classification scheme of Lwoff *et al.* (1962) included two tailed phages, T2 and an unnamed phage of *Bacillus megaterium*. The scheme was based on a few basic criteria, mainly the nature of nucleic acids, capsid symmetry, and the presence or absence of an envelope. Most virus capsids were of cubic or helical symmetry. Tailed phages combined both types and were said to have "binary symmetry." The scheme was expanded later (Lwoff and Tournier, 1966), and viruses were classified into orders and families. Tailed phages constituted the order *Urovirales*, a term derived from the Greek word *uros* for tail, and a single family, the *Phagoviridae*. This hierarchical system did not survive the test of time, but its criteria and most proposed virus taxa did.

Subsequently, the International Committee on Taxonomy of Viruses (ICTV) classified viruses into families and genera, but refrained from creating higher taxa. In 1991, the order *Mononegavirales* was proposed to include paramyxoviruses, rhabdoviruses, and filoviruses (Pringle, 1991). The proposal, largely based on genome structure and strategy of replication, was adopted by the ICTV when relationships were confirmed by amino acid alignments (Murphy *et al.*, 1995).

A monophyletic origin of tailed phages had been advocated several times (Ackermann and DuBow, 1987a; Ackermann *et al.*, 1995a; Reanney and Ackermann, 1982). The apparent relatedness of these viruses made them prime candidates for classification as an order. At the 11th International Congress of Virology in Glasgow, tailed phages were presented as "*Caudovirales*: The Second Order in Virology" (Jarvis *et al.*, 1993). The name was derived from the Latin *cauda* for "tail." At the same time, amino acid alignments were becoming extremely important in virus classification, confirming the validity of established taxa and indicating possible phylogenetic relationships, especially between ssRNA viruses. Suddenly, a phylogenetic classification of viruses became a realistic goal. Because of the relative scarcity of amino acid sequence alignments in tailed phages, their classification as an order was postponed until more data would be available.

Since then, much more data on tailed phage morphogenesis, replication, and amino acid sequences have been obtained. In addition, the ICTV has recently classified coronaviruses and arteriviruses into a second order, the *Nidovirales* (Pringle, 1996). A discussion of the taxonomic status of tailed phages is thus timely.

This review is aimed at (1) identifying common properties of tailed phages and potential criteria for their classification as an order and (2) situating tailed phages with respect to other viruses. There are inherent difficulties in such an undertaking because the amount of literature on tailed phages is truly enormous; for example, the author's database

includes about 4500 tailed phages and 4500 references. It is not feasible to give exact references for countless observations made over and over again, for example, the presence of dsDNA or base plates. These data have already found their way into the 6th ICTV Report (Murphy *et al.*, 1995), which will also be used for comparisons with other viruses. References on many individual phages may be found in a monograph on viruses of prokaryotes (Ackermann and DuBow, 1987a,b). Fortunately, the profusion of phage data has generated a number of reviews dedicated to individual phages and specific aspects of phage biology. These reviews are an invaluable and indispensable basis for this paper which, in many ways, is a review of these reviews.

The reader will find a number of truisms like "all tailed phages adsorb to bacteria by their tails." This sounds like "all birds have wings," but must be said because the main purpose of this paper is to identify basic properties. In addition, there are observations or claims of a fundamental nature that require discussion, namely (1) the presence of tailed phages in eukaryotes, of tail-like appendages in other viruses, of ssDNA, circular DNA, or envelopes in tailed phages, and (2) the results of amino acid alignments. On the other hand, properties identified as species criteria (Ackermann *et al.*, 1992) are not discussed, namely the results of DNA–DNA hybridizations, serological data, restriction endonuclease and protein profiles, amino acid composition, host ranges, burst sizes, and latent periods. Properties of individual phages are likewise outside the scope of this review.

II. The World of Tailed Phages

A. Classification

Tailed phages are classified into three families, corresponding to the basic morphological types A to C of Bradley (1967), namely *Myoviridae* with long contractile tails (A), *Siphoviridae* with long noncontractile tails (B), and *Podoviridae* with short tails (C) (Murphy *et al.*, 1995). Each family can be divided according to head length into phages with isometric, moderately elongated, and very long heads (Fig. 1; Table I) (Ackermann and DuBow, 1987a). This subdivision is useful for identification because head shape is easily determined in the electron microscope, but it has little taxonomical value. Isometric heads clearly predominate; elongated heads are much rarer and derive probably from isometric capsids. The three families of tailed phages include 13 genera, mostly defined at the 10th International Congress of Virology (Pringle,

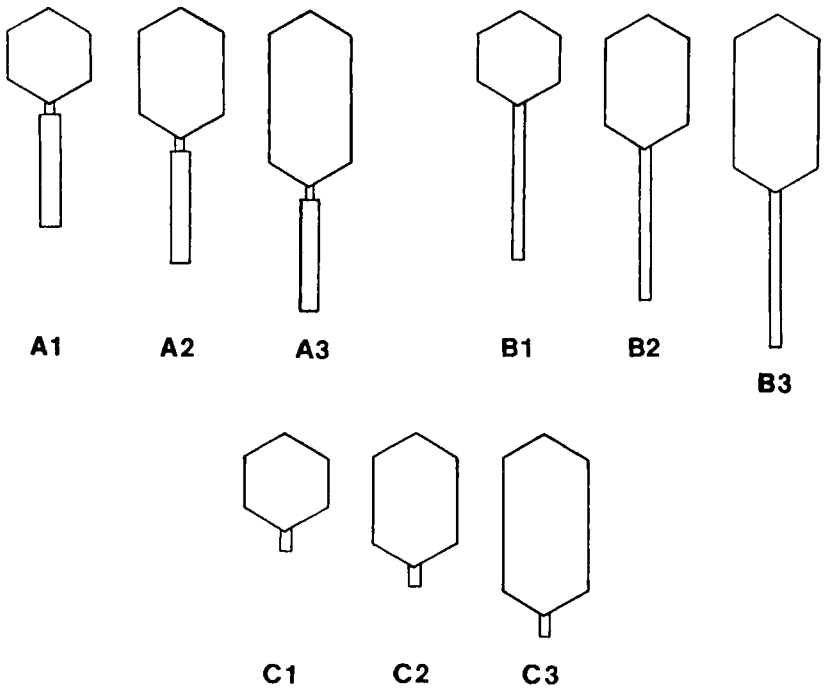

FIG 1. Morphotypes of tailed phages.

TABLE I

FREQUENCY OF MORPHOTYPES (in percent)

	Myoviridae type A	*Siphoviridae* type B	*Podoviridae* type C
1	21.0	50.9	12.7
2	3.2	9.5	1.3
3	0.2	0.8	0.3

1996), and about 250 species. This classification is rapidly evolving and more genera and species are likely to be defined in the near future.

B. Frequency and Distribution

About 4400 tailed phages have been observed in the electron microscope. Tailed phages comprise 96% of all phages and are by far the largest of all virus groups. Their numbers were computed by Ackermann (1996). Since the frequency of new phage descriptions has declined, this computation remains essentially valid at the time of this writing. A complete list of host genera may be found in the original reference. Table I shows the frequency of *Myoviridae*, *Siphoviridae*, and *Podoviridae* phages in the major bacterial groups of Bergey's Manual (Holt, 1984, 1986, 1989, 1989). *Siphoviridae* are by far the most frequent phage group (61.7%), followed by the *Myoviridae* (24.5%) and *Podoviridae* (13.9%).

Tailed phages occur in all parts of the bacterial world (see Table II). Most phages have been found in easily cultivated bacteria of medical or industrial importance, for example, enterobacteria, bacilli, clostridia, lactococci, pseudomonads, staphylococci, and streptococci. It is of particular interest that tailed phages occur in cyanobacteria and four genera of archaea, namely *Halobacterium*, *Methanobacterium*, *Methanobrevibacter*, and *Natronobacterium* (Stolt and Zillig, 1994; Witte *et al.*, 1997). These hosts are extreme halophiles or methanogens of the *Euryarchaeota* group.

C. Monophyletic Origin

There is no evidence for a polyphyletic origin of tailed phages. The probability of an independent appearance of proteins, or specification of the same message, has been estimated at $1/4^{-270}$ for a protein of 600 amino acids and at 2^{-620} for a protein of 150 amino acids (De Ley, 1968; States and Boguski, 1990). The structural complexity of tailed phages is such that this leaves little place for convergent evolution.

On the other hand, there is much evidence that morphological features of tailed phages may be conserved over long geological periods. A powerful mechanism of conservation, with the added benefit of protection against the environment, would be perpetuation of integrated prophages. Indeed, certain phages appear as living fossils antedating the separation of their hosts. A few examples may suffice:

TABLE II

FREQUENCY AND DISTRIBUTION

Bergey's Manual, part	Host group	*Myoviridae*	*Siphoviridae*	*Podoviridae*	Total
1	Spirochetes	8			8
2	Spirilla and vibrioids	10	14		24
4	G– aerobic rods and cocci	225	267	205	697
5	G– facultatively anaerobic rods	401	264	260	925
6	G– anaerobic rods	2	18	1	21
7	G– sulfate and sulfur reducers	1	1		2
8	G– anaerobic cocci		2	2	4
9	Rickettsias and chlamydias		1	1	2
10	Mycoplasmas	1	2	14	17
11	Endosymbionts			1	1
12	G+ cocci	39	1094	28	1161
13	Endospore formers	241	269	45	555
14	G+ nonsporing regular rods	67	201	2	270
15	G+ nonsporing pleomorphic rods		140	5	145
16	Mycobacterium	1	75		76
17, 26	Nocardioforms	1	95	1	97
18	Anoxygenic phototrophic bacteria	3	6	2	11
19	Cyanobacteria	14	5	13	32
20	Chemolithotrophs	1	1		2
21	Budding and appendaged bacteria	8	90	14	112
22	Sheathed bacteria		1		1
23	Nonfruiting gliding bacteria	30	2		32
24	Myxobacteria	11		5	16
25	Archaebacteria	6	7		13
28	Actinoplanetes	1	4		5
29	Streptomycetes	2	110	8	120
30	Maduromycetes		3		3
31	Thermomonosporae		27		27
32	Thermoactinomycetes		3	1	4
33	Other actinomycete genera		6		6
Totals		1073	2708	608	4389
Percent		24.5	61.7	13.9	

G+ = Gram-positive; G– = Gram-negative.

1. Tailed phages of eubacteria and archaea are of similar morphology; for example, the halobacterial phage ΦH resembles coliphage P2. In view of the different lifestyles and physiology of their respective host bacteria, a migration of eubacterial phages into archaea appears improbable, and it is likely that ΦH-like viruses existed before the separation of eubacteria from the archaeal lineage (Zillig *et al.*, 1996).
2. The "killer-particles" of the genus *Bacillus*, defective temperate phages well-known to lyse bacteria from without in a bacteriocin-like fashion and characterized by small heads containing fragments of bacterial DNA, are found not only in *Bacillus*, but also in the related bacterial genera *Actinomyces*, *Clostridium*, *Streptococcus*, and *Streptomyces* (Ackermann and DuBow, 1987a).
3. *Bacillus cereus* has a temperate phage with a head of about 92 nm in diameter and a giant contractile tail of 485 × 20 nm, provided with three long wavy tail fibers (Fig. 2). A defective phage with similar characteristics is harbored by *B. megaterium*. In addition, *Clostridium acetobutylicum* carries genes coding for the giant tail of the particle, but not its head (Ackermann *et al.*, 1995b).

D. Geological Age

Life probably started with undifferentiated prokaryotes. Paleontological evidence from microfossils suggests that the diversification of life into its principal phyla took place very early, possibly more than 3.8 billion years ago (Pflug, 1982). The first true microbial fossils were found in rocks from Western Australia dating back 3.4 to 3.5 billion years ago (Schopf, 1993; Schopf and Packer, 1987). Prokaryotes divided very early into eubacteria, archaea, and ancestors of eukaryotes (Gogarten *et al.*, 1989; Iwabe *et al.*, 1989; Koch, 1994). The eubacterial branch produced cyanobacteria and with them oxygen-producing photosynthesis. Cyanobacteria were apparently in existence 3.5 billion years ago (Schopf, 1993). The occurrence of tailed phages in all parts of the bacterial world and the antiquity of their hosts suggests that tailed phages emerged early in the history of life, perhaps at about the same time as bacteria themselves, long before eukaryotes, eukaryotic viruses, and prokaryotic viruses with limited host ranges such as RNA

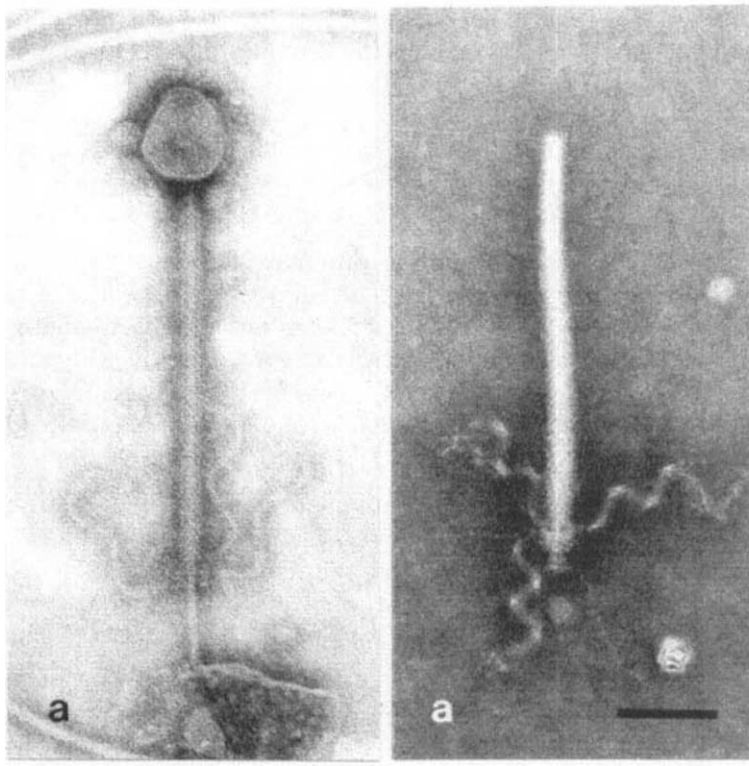

FIG 2. Intact particle with partially contracted tail (a, left) and headless tail (a, right) of *Bacillus cereus* phage Bace-11, phosphotungstate, ×148,500. Bar = 100 nm.

phages. Tailed phages may thus be as old as 3.5 to 3.7 billion years. It is safe to consider them as the oldest viral group in existence.

III. MORPHOLOGY

A. Uniqueness of the Tail

Tailed phages are conspicuously associated with prokaryotes. However, there are bacterial viruses with a special variety of a tail and reports of tailed viruses or nucleocapsids infecting eukaryotes. The obvious questions are: how true are these reports, and are the particles described true tailed phages? Before we answer, it is necessary to define

the tail of a common tailed phage: it is a hollow proteinic tube of constant length and width (with respect to species), built of subunits, regularly present in all members of a given species, and provided with conspicuous transverse striations in the *Myoviridae* and *Siphoviridae* families.

Tailed phages in eukaryotes and tail-like appendages in viruses of bacteria, algae, and insects were reported in the following circumstances:

1. T7-like particles were observed in cultures of the green alga *Chlorella pyrenoidosa* (Moskovets *et al.*, 1970; Tikhonenko and Zavarzina, 1966). A likely explanation is that the algal cultures were contaminated by bacteria and that the T7-like particles were bacterial viruses.

2. T7- and λ-like viruses from *Penicillium* and *Cephalosporium* cultures were grown on *E. coli* (Tikhonenko, 1978; Tikhonenko *et al.*, 1974). The circumstances of their isolation suggest that these entities were laboratory contaminants.

3. The *Tectiviridae** family of bacteriophages, which includes viruses of enterobacteria, pseudomonads, and bacilli, is characterized by an isometric capsid with an inner lipoprotein vesicle and a tail-like structure of extruded vesicle material, absent in quiescent particles and produced during infection. The structure is of variable length and has no transverse striations (Ackermann and Dubow, 1987b; Bamford *et al.*, 1995).

4. Arrowhead-like particles with thick stubby tails were observed in PEG precipitates of water samples from Icelandic solfataras. The particles seem to be associated with the archaeal bacterium *Thermoproteus* (Zillig *et al.*, 1994, 1996).

5. Four nonpropagated viruses of green algae with large isometric capsids show tail-like structures:

   a. *Aulacomonas* sp. viruses with a double capsid (?) have thick hollow tails that seem to be permanent and to consist of subunits (Swale and Belcher, 1973).

   b. *Brachiomonas* sp. viruses have very long inconstant tail appendages (Hoffman, cited by Van Etten *et al.*, 1991).

*The characteristics of virus groups cited for comparison are listed in Table XXI.

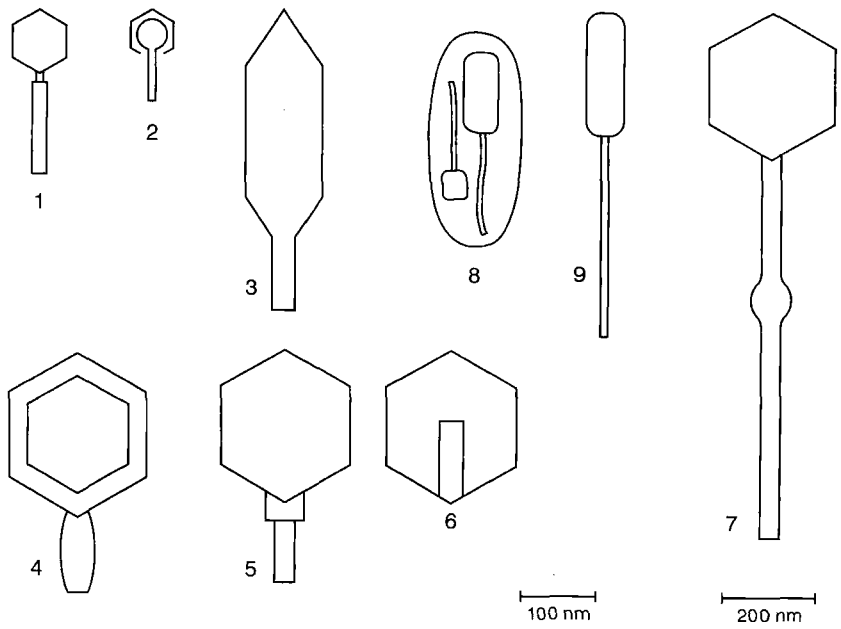

FIG 3. Viral particles with tails and tail-like appendages; scales are identical except for (7). 1: Idealized tailed phage of the *Myoviridae* family. 2: Tectivirus with extruded tail tube. 3: Archaeal virus of *Sulfolobus*? 4: *Aulacomonas* virus. 5,6: *Chlorococcum* virus with extruded and quiescent tail. 7: *Uronema* virus. 8: *Bracovirus* with two nucleocapsids. 9: *Oryctes* virus nucleocapsid.

c. *Chlorococcum minutum* viruses have preformed internal tails that are extruded for infection (Gromov and Mamkaeva, 1981).

d. *Uronema gigas* viruses with dsDNA. About 10% have enormous tails with a central swelling at midpoint (Dodds and Cole, 1980).

6. In the genus *Bracovirus* of the *Polydnaviridae* family, a virus group that infects parasitic wasps, part of the nucleocapsids are tailed. The "tails" may be extruded nucleoprotein (Murphy *et al.*, 1995; Stoltz and Vinson, 1977).

7. The enveloped virus of the beetle *Oryctes rhinoceros* has multiple elongated nucleocapsids with "tails" that are extruded when the envelope is removed (Murphy *et al.*, 1995; Payne *et al.*, 1977).

TABLE III

VIRUSES AND VIRUS-LIKE PARTICLES WITH TAIL-LIKE APPENDAGES

Host	Virus group	Capsid, nm	Tail length, nm	Tail permanent
Eubacteria	*Tectiviridae*	63	40–60	–
Archaea?	?	300 × 100[a]	100[a]	+?
Green algae:				
Aulacomonas	?	~215	~175	+?
Brachiomonas	?	~390	500	–
Chlorococcum	?	~200	90	+
Uronema	?	390	1000	–
Insects:				
Braconid wasps	*Polydnaviridae*, genus *Bracovirus*	30–150 × 40	140–220[a]	+?
Oryctes	?	160 × 50	270 × 10	–

nm, nanometers; +, present; –, absent; ~, approximately.

[a]Author's estimate.

Viruses and virus-like particles with tail-like appendages are listed in Table III. In a general way, their capsids are much larger than those of tailed phages, and their "tails" are or seem to be inconstant. The conclusion is that (1) true tailed phages have not been found in eukaryotes, and (2) the tail-like appendages of the *Tectiviridae*, putative archaeal viruses, and some viruses of green algae and insects are generally nonpermanent and, with the possible exception of the *Brachiomonas* virus, do not seem to have a subunit structure. In addition, it is unclear if the "tails" of *Bracovirus* and *Oryctes* virus nucleocapsids have any function. It appears that tailed phages, tectiviruses, and the *Aulacomonas* and *Chlorococcum* viruses have found independent solutions to the problem of infecting unicellular hosts from without. However, the tail of tailed phages is apparently unique in viruses.

B. Principles of Structure

The basic tailed phage has a head and a tail and is "naked," that is, devoid of an envelope. In contradiction with this tenet, two enveloped, chloroform- and ether-sensitive tailed phages were described. The particles showed a halo around the head after uranyl acetate staining that

was interpreted as an envelope (Hernández-Allés *et al.*, 1995; López *et al.*, 1977). In the author's experience, a halo is a common artefact after positive staining of phage heads with uranyl acetate. Enveloped tailed phages have yet to be found.

1. The Head

The head, or capsid, is an icosahedron or an elongated derivative of this body. Octahedral phage heads were repeatedly described in the sixties, soon after the introduction of negative staining. Upon reexamination, several of these "octahedral" heads turned out to be icosahedra. There is no proof that octahedral phage heads exist at all; if examined closely, most phages with isometric heads simultaneously show capsids with pentagonal and hexagonal outlines, indicating their icosahedral nature. Interestingly, the scaffold around which the T4 capsid is assembled has a sixfold symmetry (Engel *et al.*, 1982). The scaffold is regarded as an extension of the connector (see sections III.B.3 and VI.A.5).

The capsid generally appears very thin and smooth without visible capsomers. This contrasts with the appearance of adenoviruses, papovaviruses, and herpesvirus capsids, but recalls the smooth surface of iridoviruses. The smooth aspect of most tailed phage capsids may be attributed to tight fitting of protein subunits, presence of protein molecules in the center of capsomers (Yanagida, 1977), or cross-links between capsid subunits (Conway *et al.*, 1995; Hatfull and Sarkis, 1993; Popa *et al.*, 1991; Duda *et al.*, 1995a). Visualization of capsomers on mature capsids usually requires shadowing with or without freeze-etching.

Capsomers are nevertheless a basic feature of tailed phage capsids and appear, as in other viruses, as hexamers and pentamers with central cavities. They are frequently seen on proheads. Their arrangement is generally skew. Capsomer and triangulation numbers of a few well-studied tailed phages, including three phages with elongated heads, are shown in Table IV. In addition to these viruses, *Bacillus* phage SP50 and *Erwinia* phage 59 are reported to have 492 and 252 capsomers, respectively (Eiserling and Boy de la Tour, 1965; Kishko *et al.*, 1983). These numbers are unusually high for isometric capsids and need to be confirmed. In well-studied isometric phage heads, the preferred structure is a lattice of T = 7*l* (left-handed skewness), with 72 capsomers, corresponding to 12 pentamers, 60 hexamers, and 420 subunits. Papovaviruses also have a T = 7 lattice with 72 skewed

TABLE IV

CAPSOMER AND TRIANGULATION NUMBERS

Family	Phage	Capsid, nm	Capso-mers	T	References
Myoviridae	M	92		13?	Müller *et al.*, 1991
	P2	57[a]	72	7	Dokland *et al.*, 1992
	P4	45	42[b]	4	Dokland *et al.*, 1992
	SPO1	87	162[b]	16	Parker *et al.*, 1983
	T4	111 × 78[a]	152	13*l*	Baschong *et al.*, 1988 Branton and Klug, 1975; Yanagida, 1977
	λ	60[a]	72	7*l*	Williams and Richards, 1974
	HK97	54	72[b]	7	Xie and Hendrix, 1995
	φCbK	217 × 60	>200	7*l*	Lake and Leonard, 1974; Leonard *et al.*, 1972
Podoviridae	P22	65[a]	72	7	Prasad *et al.*, 1993
	T7	60[a]	72	7	Steven *et al.*, 1983
	φ29	52 × 38[a]	41	3	Anderson and Reilly, 1993
			16	1	Viñuela *et al.*, 1976.

nm, nanometers; T, triangulation number. Phage hosts: φCbK, *Caulobacter*; SPO1 and φ29, *Bacillus*; all others are enterobacteria.

[a]Measured by the author after catalase calibration.

[b]Inferred from triangulation numbers.

capsomers, but the latter are pentameric only (Cole, 1996; Murphy *et al.*, 1995). On the other hand, prolate phage heads have more capsomers than isometric capsids and may have different triangulation numbers. In the defective coliphage P4, a T = 4 capsid is produced by altering the arrangement of P2 subunits (Dokland *et al.*, 1992). For *Bacillus* phage φ29, a T = 1 capsid was postulated (Viñuela *et al.*, 1976), but a modified T = 3 structure with 11 pentamers and 30 hexamers seems to be more likely (Anderson and Reilly, 1993).

2. *The Tail*

The tail is organized along completely different principles. It is basically a hollow tube of fixed length and width built of a variable number of stacked rows of subunits. The tail generally has a sixfold symmetry, an apparently unique feature in viruses. In the myoviruses T4 and P2 and the siphovirus λ, tail tubes are built of disks of six

subunits (Eiserling, 1983; Katsura, 1983; Lengyel *et al.*, 1974; Mazza and Felluga, 1973). In *Caulobacter* phage ϕCbk, a siphovirus, the tail consists of stacked disks of three identical subunits and has a threefold symmetry (Leonard *et al.*, 1973). Many tailed phages are provided with six tail appendages (see section III.C). This suggests that disks of six subunits are a general feature of phage tails.

The contractile tail of the *Myoviridae* is a separate development. In all myoviruses, the tail tube or "core" is surrounded by a sheath that is separated from the head by an empty space or "neck." Although many phages have longer tail sheaths than T4, the principle of tail contraction is always the same. In T4 and its relatives, the sheath has a sixfold symmetry and consists of 24 rows of six subunits each, arranged in a helix and not simply stacked disks. Upon contraction, the subunits slide over each other and form a short cylinder with 12 rows of subunits (Eiserling, 1983). Similarly, the tail sheaths of coliphages Mu and P2 and *Bacillus* phages G and SPO1 exhibit a sixfold symmetry with six subunits per striation (Admiraal and Mellema, 1976; Donelli *et al.*, 1972; Lengyel *et al.*, 1974; Parker and Eiserling, 1983). A further general feature is that contracted sheaths may become loose and slide off the tail. Tail sheath length is regulated by the tail tube (Abuladze *et al.*, 1994; Ackermann and Gauvreau, 1972; Parker and Eiserling, 1983).

This contractile sheath must be a very old, strictly conserved organelle because it occurs in eu- and archaebacterial phages, is regularly present in all myoviruses, and is nearly always functional. However, rudimentary, permanently contracted tail sheaths have been observed in the CEβ species of *Clostridium* phages (Ackermann and DuBow, 1987a).

The short tails of the *Podoviridae* appear as derivatives of long, noncontractile tails. Their association with Gram-negative aerobic or facultatively aerobic rods and cocci and their relative rarity in other bacterial hosts (Table II) suggest that podoviruses arose independently several times, probably by alteration of a tail length ruler protein (see section III.D).

3. *The Connector*

Head and tail are held together by a small disk located inside the head at the site of tail attachment. The disk is called the connector or portal protein oligomer (reviewed by Valpuesta and Carrascosa, 1994). It corrects a mismatch between the fivefold symmetry of the head and

the sixfold (or threefold) symmetry of the tail and has important functions in head assembly and DNA encapsidation (see sections VI.A.5 and 6). The connector is a 14–17 nm wide disk with a central hole and 12-fold symmetry (12 subunits) in phages T4, T3, T7, P22, λ, and φ29, but has 13 subunits in *Bacillus* phage SPP1. Connectors are frequently seen in disrupted phages where they remain associated with the tail. It is unclear how the connector overcomes the symmetry mismatch between head and tail. It has been suggested that this is done mechanically, the connector being too large to pass through the hole in the capsid wall that corresponds to the site of tail attachment (Eiserling, 1983; Leonard *et al.*, 1973). The connector seems to be a universal component of tailed phages.

C. Facultative Structures

Tailed phages present an extraordinary collection of facultative structures. This appears as a consequence of their old age; more than any animal and plant viruses, tailed phages had time to evolve. Some structures are relatively frequent. Others have been observed in a single virus only, and part of them, for example, head appendages, collar fibers, or transverse tail disks with small fibers, are apparently useless for the phage and seem to exist for the sole delight of electron microscopists. The variety of facultative structures is illustrated in Fig. 3. Briefly, the following types have been observed (for references, see Ackermann and DuBow, 1987b):

1. More or less elongated heads.
2. Heads with knobs or fibers on the whole capsid or on vertices.
3. Collars with or without fibers.
4. Tails surrounded by spiral filaments or a mantle of "contraction fibers."
5. Tails with transverse disks provided or not provided with short fibers.
6. Base plates and spikes.
7. Tail fibers of many types: single or multiple, long or short, straight, kinked, wavy, or bifurcated.

Elongated capsids occur in about 15% of tailed phages (Table I), but are very rare among other viruses with cubic symmetry, occurring in

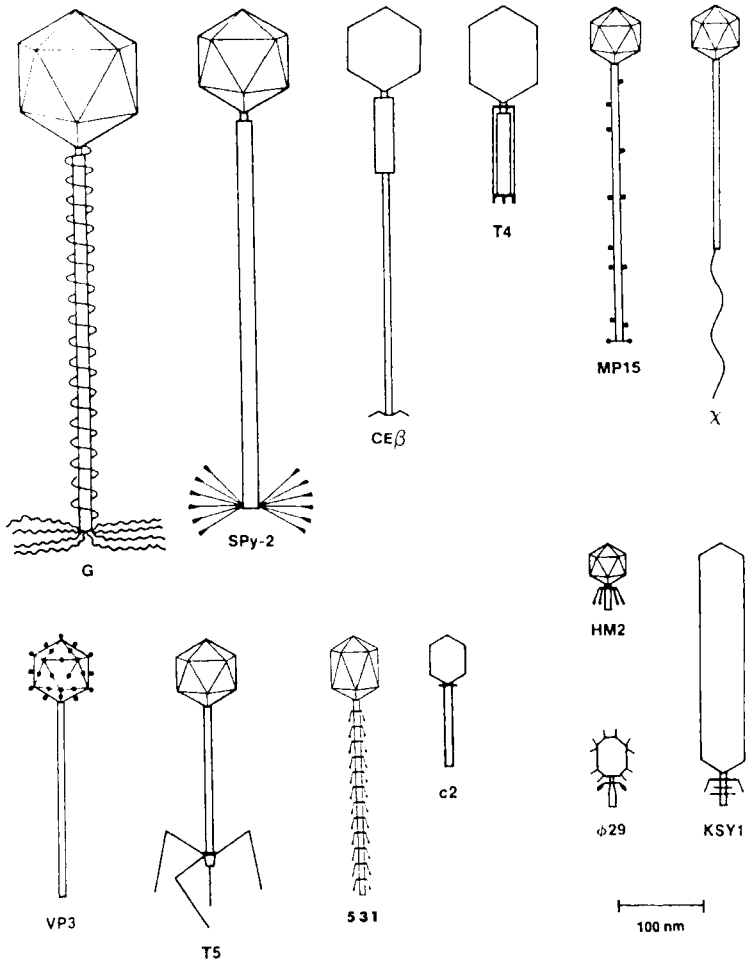

FIG 4. Morphology of selected tailed phages of *Acinetobacter* (531), *Bacillus* (G, MP15, SPy-2, φ29), *Clostridium* (CEβ), enterobacteria (T4, T5, χ), *Lactococcus* (c2, KSY1), and *Vibrio* (VP3). Assembled from Ackermann and DuBow (1987b). Phage 531 is from Ackermann and Berthiaume (1995) (Reprinted with permission from CRC Press, Boca Raton, Florida.)

two types of plant viruses only. The nonclassified *Badnavirus* genus, a group of dsDNA-containing plant viruses, comprises bacilliform particles of about 130 × 30 nm. Elongated capsids, of 30–57 × 18 nm, are also seen in the genus *Alfamovirus*, a member of the ssRNA-containing

TABLE V

SIXFOLD SYMMETRY IN TAIL APPENDAGES

Family	Host	Phage	Features	References
Myoviridae	*Bacillus*	SP50	6 spikes	Pohjanpelto and Nyholm, 1965
		SPO1	6-sided base plate	Parker and Eiserling, 1983
		ϕ25	12 spikes	Liljemark and Anderson, 1970
		Bace-11	13 long fibers	Ackermann *et al.*, 1995b
		PBS1	3 long fibers	Eiserling, 1967
	Enterobacteria	Mu	6 short fibers	Grundy and Howe, 1985
		P1	6 kinked fibers	Walker and Walker, 1983
		P2	6 short fibers	Lengyel *et al.*, 1974
		T4	6-sided collar & base plate, 6 spikes, 6 long kinked fibers	Eiserling, 1983; Kellenberger *et al.*, 1965
Siphoviridae	*Bacillus*	ϕ105	6 spikes	Ackermann *et al.*, 1994b
	Enterobacteria	T5	3+1 fibers	Bradley and Kay, 1960
		Ur-λ	3+1 fibers	Hendrix and Duda, 1992
Podoviridae	*Bacillus*	ϕ29	12 collar appendages	Anderson *et al.*, 1966
	Clostridium	HM2	12 collar appendages	Ogata *et al.*, 1969
	Enterobacteria	P22	6 spikes	Anderson *et al.*, 1960
		T7	6 kinked fibers	Matsuo-Kato *et al.*, 1981

Bromoviridae family (Murphy *et al.*, 1995). The production of elongated capsids is thus a characteristic of tailed phages.

Similarly, base plates, tail spikes, and the other types of facultative structures mentioned earlier are specific organelles of tailed phages (it would be a stretch of the imagination to link the head fibers of *Bacillus* phage ϕ29 to the penton fibers of the adenovirus capsid). Although present in a minority of tailed phages only, they add to the differences between tailed phages and other viruses. Tail appendages often have a sixfold symmetry. A few examples may suffice, for example, phage T4 with its hexagonal collar and base plate and its six tail fibers and spikes (Eiserling, 1983) and phage ϕ29 with its 12 collar appendages (Anderson *et al.*, 1966). There are many phages with six-sided base plates or six spikes, easily seen on avulsed, upturned base plates or on podoviruses turned upside down in negatively stained preparations. Spikes are often club-shaped. Some phages have single tail fibers, but multiple tail fibers are far more frequent. Insofar as these tiny and often entangled structures can be resolved, they often number six and sometimes three. A few phages seem to have 12 tail fibers, but this is difficult to ascertain. A particularly interesting variety, represented by

TABLE VI

MAIN DIMENSIONS OF PARTICLES

Family	Head diameter, nm		Tail length, nm	
	Average	Range	Average	Range
Myoviridae	85	53–160	167	80–485
Siphoviridae	55	40–97	191	79–539[a]
Podoviridae	58	38–75	19	3–40
All	66	38–160	154	3–539

nm, nanometers. Computed from 251 phage species with isometric heads. See legend of Fig. 5.

[a]Tails of 825 nm in length have been observed in nonclassified siphoviruses of *Thermus* (Yu *et al.*, 1996).

T5 and Ur-λ, has a pointed tail tip with a single fiber and a subterminal disk with three long kinked fibers. The general pattern is thus one of sixfold symmetry (or a fraction or a multiple of six). As the sixfold symmetry of the tail itself, it is unique in viruses.

D. Dimensions

Tailed phages are relatively large viruses with capsids of about the same size as adeno- and reoviruses (Murphy *et al.*, 1995) (Table VI). Despite a considerable overlap in dimensions, myovirus capsids are generally larger than those of sipho- and podoviruses. Overall, head diameters vary between 34 and 160 nm, but peak sharply at 60 nm (Fig. 5). The capsid diameter thus appears as a partially conserved property, again indicating a common origin of tailed phages or at least of capsid genes.

Myovirus and siphovirus tail lengths have a wide range, and their distribution does not show any peaks. In both T4 and λ, tail length is specified by a ruler protein that acts as a "tape measure" around which tail tube monomers polymerize. Duplications or deletions in ruler protein genes result in tails that are proportionally longer or shorter (Abuladze *et al.*, 1994; Katsura and Hendrix, 1984). At least in T4 they must be rare, because it has taken 40 years of countless observations to find tail length variants in this phage. Ruler proteins have not been found or sought in other tailed phages. However, (1) tail length is

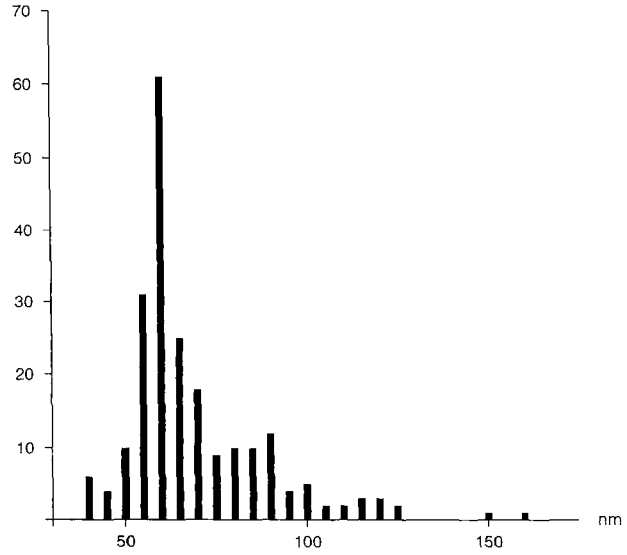

FIG 5. Size distribution of isometric phage heads. Computed from 251 phage species with isometric heads from *Acinetobacter*, actinomycetes, and related bacteria, *Aeromonas*, *Agrobacterium*, *Bacillus*, *Brucella*, *Clostridium*, cyanobacteria, enterobacteria, Gram-positive cocci, *Lactobacillus*, *Listeria*, mycoplasmas, *Pasteurella*, pseudomonads, *Rhizobium*, and *Vibrio* (Ackermann and DuBow, 1987b; Ackermann *et al.*, 1994a,b, 1995b, 1997; Bes, 1994; Jarvis *et al.*, 1991; Zink *et al.*, 1992). Modified from Ackermann and DuBow (1987b). (Reprinted with permission from CRC Press, Boca Raton, Florida.)

uniform in every individual tailed phage species, (2) there are no transition forms between sipho- and podoviruses, and (3) siphoviruses never produce aberrant particles of the podovirus type, and vice versa. This suggests that ruler proteins are common features of phage tails and that the principle of tail length regulation is highly conserved.

A close look at minor dimensions is particularly revealing (see Table VII). It appears that many dimensions, such as tail diameter and the width of tail striations, are very uniform. This is another strong argument for a common origin of tailed phages. It appears that tailed phages have conserved a great number of minor and seemingly unimportant dimensions, perhaps because, by contrast with capsid size, there is no evolutionary pressure to modify them. However, literature reports of base plate widths (12–48 nm) and length of tail fibers (10–350 nm) and spikes (8–32 nm) show considerable variations. This probably reflects some inaccurate measurements as, in the author's experience, the base plate is usually 20–28 nm wide and spikes are 14–16 nm long.

TABLE VII

CONSERVED MINOR DIMENSIONS

Phage part	Parameter	Dimensions, nm
Capsid	Thickness	2–3
Neck		10 × 8
Collar		12 × 2
Sheath, extended	Diameter	~18
	Striations, repeat	4
Sheath, contracted	Diameter	~24
	Lumen	8–9
	Thickness of wall	3
Tail tube	Diameter	7–10
	Striations, repeat	4
	Lumen	2–3
	Thickness of wall	3–4
Base plate	Width	20–28
	Thickness	3–5
Tail fibers	Diameter	2
Spikes	Length	14–16

nm, nanometers. Modified from Ackermann and DuBow (1987b). (Reprinted with permission from CRC Press, Boca Raton, Florida.)

IV. PHYSICOCHEMICAL PROPERTIES OF PARTICLES

Typically, tailed phages consist of DNA and protein only. The presence of 12–15% lipids has been reported in three mycobacterial phages (Gope and Gopinathan, 1982; Jones *et al.*, 1970; Soloff *et al.*, 1978), and traces of lipids have been described in a few other tailed phages. These reports have not been confirmed and may be explained, at least in part, by contamination of phage preparations by bacterial lipids. Despite the general absence of lipids, about 30% of tailed phages are inactivated by ether and chloroform (Ackermann and DuBow, 1987b). Sensitivity to lipid solvents does therefore not prove the presence of lipids (see section III.B). Small amounts of glycoproteins or hexosamine are present in some phages (Ackermann and DuBow, 1987b). In essence, tailed phages appear as devoid of lipids and carbohydrates.

TABLE VIII

PHYSICAL PROPERTIES OF PHAGE PARTICLES

Family	Particle weight, $\times 10^6$ daltons		Sedimentation velocity, S		Buoyant density in CsCl, g/ml	
	Ave.	Range	Ave.	Range	Ave.	Range
Myoviridae	140	58–208	702	283–1027	1.49	1.41–1.54
Siphoviridae	84	60–114	468	262–608	1.50	1.45–1.54
Podoviridae	88	29–204	582	429–910	1.45	1.44–1.52
All	100	29–208?	580	262–1027?	1.49	1.41–1.54
N	19		29		62	

N, number of species; S, sedimentation velocity at 20°C in water. Computed from classified phage species of actinomycetes and related genera, *Aeromonas*, *Agrobacterium*, *Bacillus*, cyanobacteria, enterobacteria, Gram-positive cocci, *Lactobacillus*, mycoplasmas, pseudomonads, and *Vibrio* (Ackermann and DuBow, 1987b; Ackermann *et al.*, 1994b).

Particle weights and sedimentation coefficients seem to be out of favor in virology, having been rarely determined during the last 10 years. As a consequence, there are few data available for comparison; it can only be said that tailed phages are heterogeneous with respect to both criteria, but rank far below pox- and phycodnaviruses and above papovaviruses (Cole, 1996; Moss, 1996; Murphy *et al.*, 1995). By contrast, the buoyant density in CsCl has been determined in many tailed phages (see Table VIII). It reflects their high DNA content (see section V.A) and is very high in absolute terms, comparable only to that of baculovirus nucleocapsids (1.48 g/ml) or a few small isometric viruses with ssRNA (comoviruses, 1.3–1.5 g/ml; leviviruses, 1.46 g/ml) (Murphy *et al.*, 1995).

The weight of capsids, tail sheaths, and tail tubes varies considerably. Large variations are also observed in the molecular weights of major head and tail sheath proteins (14–175 and 36–72 kDa, respectively) (see Table IX). Tail tube proteins are much more uniform in size. Indeed, regardless of the phage host, the major tube proteins of sipho- and podoviruses and the tail core proteins of myoviruses are generally small and have molecular weights of about 20 kDa. However, tail tube proteins of relatively high molecular weight are found in *Bacillus* phage

TABLE IX

MOLECULAR WEIGHT OF TAIL TUBES

Family	Host	Phage	kDa	References
Myoviridae	*Bacillus*	SPO1	12	Parker and Eiserling, 1983
	Enterobacteria	MM	15.7	Müller *et al.*, 1991
		Mu	12.5	Giphart-Gassler *et al.*, 1981
		P1	21.4	Walker and Walker, 1981
		P2	19.1	Temple *et al.*, 1991
		T4	19	Arisaka *et al.*, 1988
		Xenorhabdicin[a]	20	Thaler *et al.*, 1995
	Haemophilus	HP1	16.3	Esposito *et al.*, 1996
	Pseudomonas	PS17	18	Sasaki *et al.*, 1997
Siphoviridae	Enterobacteria	λ	31	Murialdo and Siminovitch, 1972
		T1	26	Ramsay and Ritchie, 1984
		T5	19	McCorquodale and Warner, 1988
	Lactobacillus	LL-H	16.8	Mikkonen and Alatossava, 1994
	Lactococcus	c2	29	Lubbers *et al.*, 1995
		r1t	24	Van Sinderen *et al.*, 1996
	Listeria	2671	21.7	Zink and Loessner, 1992
		2685	23.5	Zink and Loessner, 1992
	Mycobacterium	L5	25	Hatfull and Sarkis, 1993
Podoviridae	*Bacillus*	ϕ29	65	Martín *et al.*, 1996
	Enterobacteria	T7	22	Klaus *et al.*, 1992
	Streptococcus	Cp-1	65	Martín *et al.*, 1996
	Synechococcus[b]	LPP1	80	Sherman and Haselkorn, 1970

kDa, kilodaltons.

[a]Particulate bacteriocin of *Xenorhabdus nematophilus*.

[b]Cyanobacterium.

ϕ29, its close relative *Streptococcus* phage Cp-1, and a virus of cyanobacteria. They seem to represent separate developments within small phage groups.

V. THE GENOME

A. *General Properties*

The genome of tailed phages is typically a single molecule of linear dsDNA. The presence of ssDNA was recently reported in three tailed *Proteus* phages, two of which were of the T4 type (Sekaninová *et al.*, 1994); this conflicts with all previous observations and must represent

an experimental error. On the other hand, *Rhodopseudomonas* phage Rφ6, a siphovirus with an isometric head, contains circular supercoiled DNA, whereas the Rφ6 prophage carries a penicillin-resistant gene and behaves as a plasmid (Pemberton and Tucker, 1978; Tucker and Pemberton, 1978). Rφ6 may represent a natural plasmid–phage hybrid.

Although there is relatively little data, tailed phages seem to have the highest relative DNA content of all viruses. They contain about 50% DNA, whereas the highest DNA contents reported elsewhere in virology are 20% for adenoviruses and 14–15% for tectiviruses, respectively (Murphy *et al.*, 1995). The high DNA content of tailed phages evidently reflects the high buoyant density of phage particles.

The molecular weight of tailed phage DNA varies between 17 and about 725 kb. Its distribution shows a sharp peak at 50 kb (Ackermann and DuBow, 1987b), which corresponds to the preferred capsid diameter of 60 nm (see section III.D). The average genome size of tailed phages is in the middle range of dsDNA viruses, below that of herpesviruses and well above that of adenoviruses (Murphy *et al.*, 1995; Roizman and Sears, 1996; Shenk, 1996). The giant genome of 725 kb has been found in *Bacillus megaterium* phage G (Donelli *et al.*, 1975; Dore *et al.*, 1977; Fangman, 1987) and is the largest known genome in the viral world. As far as is known, large parts of tailed phage genomes are nonessential; for example, as much as 40% of the P22 genome is dispensable for both lytic growth and lysogeny (Poteete, 1988). This leaves many opportunities for the acquisition of foreign genes and suggests that tailed phage genomes developed from simple entities with few genes and limited coding capacity.

Guanine–cytosine (GC) contents of tailed phages usually parallel those of their hosts. Their range is as wide as that of herpesviruses (32–75%, Roizman and Sears, 1996). GC contents are obviously not criteria of order rank and are reported here only because of their general importance in virus taxonomy.

B. Facultative Properties

Tailed phage DNAs have numerous particulars of genome anatomy or composition, some of which are rare or absent in other viruses (see Table X). They include:

1. Circular permutations, terminal repeats, *pac* and *cos* sites, terminal proteins, single-stranded gaps, and RNA molecules.
2. Unusual bases and DNA-associated sugars.

TABLE X

GENERAL PROPERTIES OF DNA

Family	% of particle		MW, $\times 10^6$ kb		G + C, %	
	Ave.	Range	Ave.	Range	Ave.	Range
Myoviridae	43	30–55	155	33–725	40	28–61
Siphoviridae	48	34–62	60	12–133	51	32–72
Podoviridae	45	36–51	45	17–92	49	27–66
All	46	30–62	79	12–745	48	27–72
N	82		114		88	

G + C, guanine + cytosine; kb, kilobases; MW, molecular weigth; N, number of species. Computed from classified phage species of actinomycetes and related bacteria, *Agrobacterium*, *Bacillus*, *Brucella*, *Clostridium*, cyanobacteria, enterobacteria, Gram-positive cocci, *Lactobacillus*, *Listeria*, mycoplasmas, pseudomonads, *Rhizobium*, and *Vibrio* (Ackermann and DuBow, 1987b; Ackermann *et al.*, 1994a,b; Bes, 1994; Jarvis *et al.*, 1991).

The combination of circular permutation and terminal redundancy generally, but not always, indicates the presence of *pac* sites for initiation of DNA packaging. Phages with *pac* sites may be virulent or temperate. By contrast, phages with *cos* sites are generally temperate and never have circular permutations and terminal redundancies. Two phages, BT11 and H39, are reported to have circularly permutated and nonredundant genomes (Moynet *et al.*, 1985).

Cos sites or cohesive ends are single-stranded "sticky" DNA overhangs of 7 to 21 nucleotides (Lubbers *et al.*, 1994) that enable phage genomes to circularize after infection and are also involved in DNA packaging (see section VI.A.6). *Cos* sites seem to have common characteristics. A survey of 18 *cos*-type phages suggests (1) that cohesive ends protrude from the 5′ DNA end in phages of Gram-negative bacteria and from the 3′ end in phages of Gram-positives, and (2) that their DNAs have regions with dyad symmetry close to the cohesive ends (Lillehaug *et al.*, 1991; Lubbers *et al.*, 1994).

Pac and *cos* sites have been found in many tailed phages and are mutually exclusive, so that tailed phages have been divided into *pac* and *cos* types (Forsman and Alatossava, 1991). Their mutual exclusiveness and apparent ubiquity suggest that there is a basic ancient dichotomy in tailed phages. The author's database includes 22 tailed phages with *pac* sites, 17 phages with circular permutations and terminal

redundancies but no formally identified *pac* sites, and 120 phages with *cos* sites.

Terminal redundancies occur in the DNA of the African swine fever virus and of adeno-, herpes-, irido-, phycodna-, pox-, and tectiviruses (Murphy *et al.*, 1995). The equivalents of *pac* and *cos* sites seem to exist in other dsDNA viruses as well. Hepadnavirus DNA has cohesive ends, herpesvirus DNA has a single nucleotide extension at each 3′ end of the genome and circularizes after infection, replicated poxvirus DNA appears as a covalently closed circle, and the presence of circular permutations and terminal redundancies in iridovirus DNA suggests the presence of *pac* sites (Ganem, 1996; Moss, 1996; Murphy *et al.*, 1995; Roizman and Sears, 1996).

Terminal proteins occur in a few closely related phages of the *Podoviridae* family, namely *Bacillus* phage ϕ29 and *Streptococcus* phage Cp-1 (Salas, 1988). The proteins are covalently linked to both DNA ends, give the DNA molecule a circular appearance, prime DNA replication, and are correlated with the presence of virus-coded DNA polymerases of family B (see Table XI). Related terminal proteins are found in adenoviruses and tectiviruses. Their presence is attributed to horizontal gene transfer (see sections VI.A.4 and VII.C). In addition, terminal proteins have been found in streptococcal phages of the *Siphoviridae* family, but their physiological role is unclear (Romero *et al.*, 1990b).

Single-stranded gaps in the DNA filament, first observed in coliphage T5 (McCorquodale and Warner, 1988), exist in a few other tailed phages and also in badna-, caulimo-, and hepadnaviruses (Ganem, 1996; Murphy *et al.*, 1995). RNA in the size range of 80–700 nucleotides was found within the head of *Natronobacterium* phage ΦCh1. It is thought to be host and not phage RNA (Witte *et al.*, 1997). This is the first observation of simultaneous presence of DNA and RNA in mature viruses.

Unusual bases of tailed phages (reviewed by Ackermann and DuBow, 1987b) include analogues of:

1. Adenine (2,6-diaminopurine, 6-methyladenine).
2. Cytosine (5-hydroxycytosine, 5-hydroxymethylcytosine, 5-methylcytosine).
3. Guanine (7-7-methylguanine).
4. Thymine (5-dihydroxypentyl uracil, α-glutamylthymine, 5-hydroxymethyluracil, 5-oxymethyluracil, *N*-thyminylputrescine, uracil).

TABLE XI

DNA ENDS OF SELECTED TAILED PHAGES

	Terminally redundant	Nonredundant	References
Circularly permutated	LL-H, P1, P22, SPP1, T1, T4, ΦH	BT11, H39	Drexler, 1988; Forsman, 1994; Forsman and Alatossava, 1991; Morelli *et al.*, 1979; Moynet *et al.*, 1985; Schnabel *et al.*, 1982; Thomas, 1966; Yarmolinski and Sternberg, 1988
Nonpermutated	Mu, N4, T5, T7		Harshey, 1988; Hausmann, 1988; Kiino and Rothman-Denes, 1988; McCorquodale and Warner, 1988; Thomas, 1966
Cohesive		c2, HPl, P2, SPO2, λ, ϕC31, ϕ105	Bertani and Six, 1988; Chow *et al.*, 1972; Esposito *et al.*, 1996; Klaus *et al.*, 1992; Powell *et al.*, 1989; Thomas, 1966
Proteins	ϕ29, Cp-1		Salas, 1988

Phage hosts: *Bacillus* (SPO2, SPP1, ϕ29, ϕ105); enterobacteria (N4, P1, P2, P22, T1, T4, T5, λ), *Haemophilus* (HP1), *Halobacterium* (ΦH), *Lactobacillus* (LL-H), *Lactococcus* (c2), *Streptococcus* (BT11, Cp-1, H39), *Streptomyces* (ϕC31).

These modified bases may replace normal bases completely or in part. Their purpose is to protect infecting phage DNA against degradation by bacterial restriction endonucleases. The most famous example is T4 DNA, where all cytosine residues are replaced by 5-methylhydroxycytosine. The bases 2,6-diaminopurine, 6-methyladenine, and 7-methylguanosine occur in traces only. Interestingly, modified bases exist in two unrelated tailed archaebacterial phages. The genome of ΦH, a myovirus, codes for three cytosine methyltransferases and ϕN, a siphovirus, has all its cytosine residues replaced by 5-methylcytosine (Stolt *et al.*, 1994; Vogelsang and Osterhelt, 1987). Outside tailed phages, phycodnaviruses contain traces of 5-methylcytosine and 6-methyladenine (Murphy *et al.*, 1995; Van Etten *et al.*, 1991). DNA-associated sugars include glucose, fucose, mannose, and possibly gentiobiose (Ackermann and DuBow, 1987b). The presence of unusual bases and sugars is a criterion of species level only. However, the frequency and variety of unusual bases set tailed phages apart from other viruses.

C. Functional Genomic Maps

The basic difference between *pac*- and *cos*-type phages is reflected in their genomic maps. *Pac*-type phages, with their circularly permutated genomes, generally have circular maps. One exception is coliphage P1, which has a linear map with ends defined by a recombinational hot spot; however, the P1 prophage map is circular (Yarmolinski and Sternberg, 1988). On the other hand, all phages with nonpermutated genomes, which include the *cos* type, have linear maps. Functional genomic maps are presently available for 29 tailed phages (Table XII). Unfortunately, many of them are rudimentary and do not include structural genes or make no distinction between head and tail genes; further, the left ends of the maps of coliphage T1 and *Bacillus* phage SP82G are arbitrarily designed. The most complete maps are those of *Bacillus* phage ϕ29 and enterobacterial phages Mu, P1, P2, P22, T4, T7, and λ. any genomic maps have evolved over a long time. Only the latest references are given here.

The comparison of genomic maps is essential to an understanding of phage evolution. More than 25 years ago, Dove (1971) noted that the prophage maps of P2, P22, and λ were partially congruent. Further detailed comparisons of P22 and λ maps showed extensive similarities and wide divergences. Similarities included genome organization, regulation of lytic growth, and prophage integration. On the other hand, P22 and λ differed considerably in morphogenesis and DNA metabolism and encapsidation. Differences were of the all-or-nothing type. Based on these observations and the construction of viable hybrids between λ and P22, a theory of modular evolution of lambdoid phages was proposed, namely that lambdoid evolved through parallel evolution of largely interchangeable "modules" (Botstein and Herskowitz, 1974; Susskind and Botstein, 1978). Subsequently, similarities were found between the genomes of phages λ and Mu (Kemp, 1987) and phages P2 and HP1 (Esposito *et al.*, 1996). The theory of modular evolution has now been extended to other viruses and vindicated by numerous observations of horizontal gene transfer (see section VII). Evolution through exchange of "modules" of genes or blocks of genes now appears as a major mode, if not the principal one, of tailed phage evolution.

Many new insights were obtained by a detailed analysis of new data from lambdoid phages, especially of head and lysis genes (Casjens *et al.*, 1992). It was concluded that (1) genes with related functions clustered together; (2) genes of different phages could be homologous (related) or analogous (encoding different proteins with identical functions); (3) gene orders were more conserved than nucleotide sequences;

TABLE XII

FUNCTIONAL GENOMIC MAPS

Host	*Myoviridae*	*Siphoviridae*	*Podoviridae*	References
Bacillus	SPO1, SP82G		φ29	Hemphill and Whiteley, 1975; Murialdo and Becker, 1978; Stewart, 1993
Corynebacterium		β		Bishai and Murphy, 1988
Enterobacteria	Mu, P1, P2, P4, T4, 186	T1, λ	P22, T7	Bertani and Six, 1988; Casjens *et al.*, 1992; Daniels *et al.*, 1983; Drexler, 1988; Harshey, 1988; Hausmann, 1988; Kutter *et al.*, 1994; Poteete, 1988; Yarmolinski and Stern berg, 1988; Ziegelin and Lanka, 1995
Haemophilus	HP1			Esposito *et al.*, 1996
Halobacterium	ΦH			Stolt *et al.*, 1994
Lactobacillus		LL-H, φg1e		Kodaira *et al.*, 1997; Mikkonen, 1996
Lactococcus		bIL67, c2, r1t, Tuc2009		Arendt *et al.*, 1994; Lubbers *et al.*, 1995; Schouler *et al.*, 1994; Van Sinderen *et al.*, 1996
Mycobacterium		L5		Hatfull *et al.*, 1993
Pseudomonas	PS17	D3112		Bidnenko *et al.*, 1989; Shinomiya and Ina, 1989
Rhizobium		16-3		Sik and Orosz, 1971
Staphylococcus		φ11		Kretschmer and Egan, 1975
Streptococcus			Cp-1	García *et al.*, 1997
Streptomyces		φC31		Klaus *et al.*, 1992

(4) tailed phages could form "quasi-species" with little sequence similarity, but the same gene orders and transcription patterns; and (5) tailed phages routinely acquired genes from their hosts, phages, or other sources. It was also noted that the arrangement of portal, scaffolding, and capsid protein genes was similar in phages T4, T7, and φ29 and that a tailed *Staphylococcus* phage showed the same gene order of lysogeny sites (*att*, *int*, *xis*) as lambdoid phages. Finally, the question of a common origin for tailed phage capsid proteins was raised. The present survey of the available functional maps confirms or shows that:

1. Genes with related functions cluster together.
2. Roughly 50% of genes are morphopoietic (about 40% in the large phages P1 and T4 and 60–65% in the small phages Mu and ϕ29).
3. Morphopoietic genes are generally located at the left end or the center of the genome. Exceptions are phages Mu, P22, and T7.
4. Head genes precede tail genes. Exceptions are the arbitrary maps of phages T1 and SP82G, where this order is inversed and which should probably reoriented, and the genomes of T4 and T7, where tail genes are found in the middle between two head gene clusters.
5. Genes for lysis, integration-excision, or DNA replication, *pac* sites and origins of replication occupy variable positions with respect to morphopoietic genes. No pattern is apparent and no hypothetical "ur-genome" can be constructed with these elements.
6. DNA polymerases may be present or absent and are not a universal feature of tailed phages.

Phage Mu stands apart. In addition to ordinary phage genes, its genome has an invertible segment at the right end, a transposon module at the left end, and variable sequences at both. The invertible segment is also found in phage P1, and the variable sequences are pieces of host DNA acquired when Mu DNA is packaged into phage heads (Harshey, 1988; Kemp, 1987). It has been proposed that both the invertible segment and the transposon module derive from a Tn3-like transposon (Kemp, 1987). Transposable phages are also found in pseudomonads but, unlike Mu, are members of the *Siphoviridae* family (Bidnenko *et al.*, 1989; DuBow, 1987). Their ability to transpose could have been acquired as in phage Mu.

Clustering of related genes, already noted in the early sixties in phage T4 (Epstein *et al.*, 1963), is common in tailed phages, most of which have their genes arranged in an orderly fashion. A model example is phage T7, whose genome consists of three distinct blocks, namely genes for early functions, DNA metabolism, and virion structure and assembly (Hausmann, 1988). Notable exceptions are phages P1 and T4, whose genomes appear almost disorganized and as mixtures of structural and other genes. This is probably due to intragenomic rearrange-

ments and extensive horizontal gene transfer; indeed, phage P1 has been called a pastiche (Yarmolinski and Sternberg, 1988), cobbled together from many elements of different origin. However, even P1 and T4 show blocks of related morphopoietic genes; for example, T4 has four separate assemblies for head, tail, base plate, and tail fiber genes. At least in lambdoid phages, the functional clustering of genes provides finer levels of regulation because genes whose products interact with each other occupy adjacent positions (Campbell, 1994). This would constitute a powerful mechanism to ensure evolutionary stability.

In conclusion, tailed phage genomic maps are distinguished from those of other viruses with dsDNA by their large stretches of morphopoietic genes and, insofar as this has been investigated, the presence of lysis genes. No genomes of other dsDNA viruses (Cole, 1996; Ganem, 1996; Moss, 1996; Murphy *et al.*, 1995; Roizman and Sears, 1996; Shenk, 1996) are remotely comparable to those of tailed phages.

VI. Lifestyles

A. The Lytic Cycle

1. Overview

Phage replication generally leads to lysis of the host, and the vegetative or productive cycle is therefore also called the *lytic cycle*. As in other viruses, this cycle includes the steps of infection, transcription, translation, DNA replication and encapsidation, particle assembly, and release. Contrary to eukaryotic viruses, the vegetative cycle of most phages, tailed or not, is measured not in hours, but in minutes. Relatively long latent periods of 5 to 17 hours are common in phages of cyanobacteria (Ackermann and DuBow, 1987a), but the typical phage cycle is very short and often in the order of 20 minutes. Despite important exceptions, the multiplication of tailed phages has many common features indicating that these viruses have a common origin or are at least compatible with it.

Most data for steps 2 to 5 are from a few well-studied phages and have already been reviewed, sometimes repeatedly. There are specific reviews for *Bacillus* phage ϕ29 (Salas, 1988; Salas *et al.*, 1995) and enterobacterial phages Mu (Harshey, 1988; Howe, 1987a,b), N4 (Kiino and Rothman-Denes, 1988), P1 (Yarmolinski and Sternberg, 1988), P2

and P4 (Bertani and Six, 1988; Lindqvist *et al.*, 1993; Ziegelin and Lanka, 1995), P22 (Poteete, 1988; Susskind and Botstein, 1978), T1 (Drexler, 1988), T4 (Mosig and Eiserling, 1988; Mosig *et al.*, 1995), T5 (McCorquodale and Warner, 1988); T7 (Hausmann, 1988), and λ (Campbell, 1994; Enquist and Skalka, 1978; Furth and Wickner, 1983). In addition, all these phages have been reviewed by Klaus *et al.* (1992), and the replication strategy of the best-known phages has been reviewed by Keppel *et al.* (1988).

2. *Infection*

All tailed phages adsorb to bacteria by their tails and infect them from the outside. Most adsorb to the cell wall. There are tailed phages whose primary receptors are located on pili, flagellae, or capsules, but all reach eventually the cell wall by pilus retraction, sliding along the flagellae, or digestion of the capsule. Tailed phages of the wall-less mycoplasmas probably attach to the plasma membrane of their hosts.

Once adsorbed, tailed phages digest the cell wall using specialized enzymes (see sections VI.A.7 and VII.E) located at the tail tip and "inject" their DNA through the cytoplasmic membrane. The contractile tail of the *Myoviridae* is a special injection device. After the cell wall is digested, the tail sheath contracts and the central tail tube is lowered into the hole in the cell wall to make contact with the plasma membrane. This mode of infection is a unique feature in viruses. The DNA enters the cell and the empty capsid remains outside. No tailed phage enters its host.

This behavior contrasts with that of viruses of multicellular organisms, all of which enter host cells. However, infection from without accompanied by nucleic acid injection is seen in many tail-less phages with cubic symmetry (*Cortico-*, *Levi-*, *Micro-*, and *Tectiviridae*) and in the *Phycodnaviridae* family of algal viruses (Murphy *et al.*, 1995); it is also likely that some nonclassified algal viruses with tail equivalents infect their hosts from without (see section III.A).

3. *Transcription*

The infecting phage DNA is characteristically transcribed in three partially overlapping stages: early, middle, and late. Early (immediate-early) genes (1) prepare the host for the phage, mediating shutoff of host syntheses, degradation of host DNA, protection against restriction

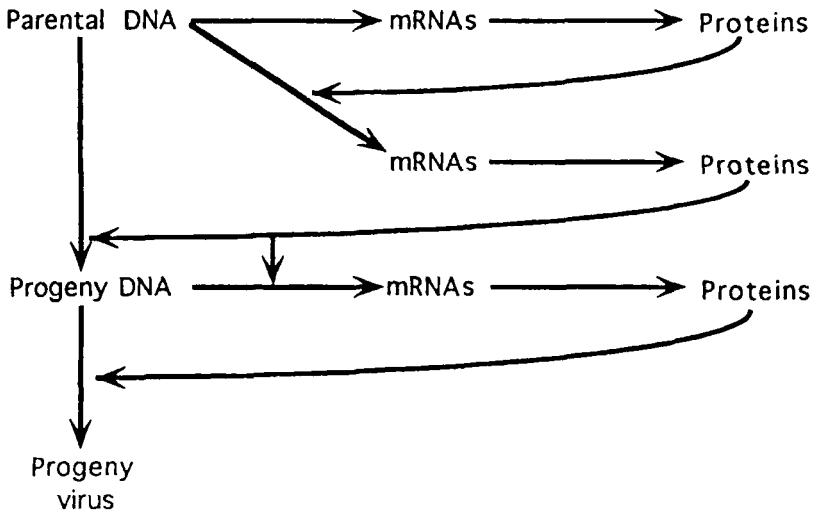

FIG 6. Flow of herpesvirus replication. From Roizman and Palese (1996). (Reprinted with permission from Lippincott-Raven, Philadelphia.)

enzymes, and takeover of host metabolism, (2) initiate DNA replication, and (3) induce the synthesis of regulatory proteins. Typically, lytic phages use host RNA polymerases for transcription of their early genes. Exceptions are coliphages T7 and N4, both podoviruses. T7 codes for an RNA polymerase transcribed immediately after infection (Hausmann, 1988), and N4 contains a phage-coded RNA polymerase that is injected into the host for early transcriptions (Falco *et al.*, 1977). Middle (intermediate) genes for DNA synthesis start to operate 3 to 8 minutes after infection and continue to function during the late phase. Middle genes are under phage control to the extent that phages code for DNA polymerases (see section VII.C). Finally, late genes, starting as early as 8 minutes after infection, code for structural and lysis proteins and DNA packaging. There is no obvious pattern in the direction of transcription.

In a general way, the transcription pattern of tailed phages resembles that of herpesviruses (Fig. 6). Transcription in three and sometimes two waves is also found in adeno-, baculo-, irido-, papova-, and poxviruses and in the African swine fever virus (Cole, 1996; Moss, 1996;

TABLE XIII

CHARACTERISTICS OF DNA REPLICATION

Phage	DNA ends	Cyclization	Origin	Direction	Θ rings	Rolling circle	Concatemers
P2	cos	+	Single	Uni		+	+
P4	cos	+	Single	Bi	+		+
λ	cos	+	Single	Bi	+	+	+
P1	CP-TR	+	Single[a]		+	+	+
P22	CP-TR	+	Single	Uni	+	+	+
T4	CP-TR	–	Multiple	Bi	–	–	+
T7	TR	–	Multiple	Bi	–	–	+
T1	CP-TR	+					
T5	TR		Multiple?	Bi?			+
N4	TR		Both ends	To center			+
Mu	T site	–	Either end	End-to-end	+?		–?
ϕ29	Protein	–	Either end	End-to-end			–

Bi, bidirectional; CP, circularly permutated; cos, cohesive ends; T site, transposition site; TR, terminally redundant, Uni, unidirectional; +, yes or present; –, no or absent.

[a]For lytic growth.

Murphy *et al.*, 1995, Roizman and Sears, 1996; Shenk, 1996). It seems to be a universal feature of dsDNA viruses.

4. *DNA Replication*

After entering the bacterial cell, the DNA of tailed phages assumes a circular state or remains linear. Cyclization is achieved by two mechanisms. DNA replication usually results in the formation of concatemers, long DNA molecules made up of multiple copies of unit-length DNA (see Table XIII). According to their replication strategy, tailed phages can be divided into four major categories (Keppel *et al.*, 1988):

1. Phages with genomes cyclized by joining cohesive ends (λ, P2, P4) or site-specific recombination of terminally redundant ends (P1, P22).

2. Phages with linear genomes, terminal repeats, and internal origin of replication (T4, T7).

3. Phages with replication initiated at the ends of the genome (N4, ϕ29).
4. Phages replicating via transposition into the host genome (Mu).
   a. In the λ-P2-P4 group, replication starts at a single site and moves in one or two directions. In phage λ, Θ or Cairns rings are produced in the first rounds of replication and the genome is amplified by rolling-circle replication. In P4, Θ rings seem to be the end-products of replication.
   b. In P1, the viral DNA probably cyclizes via the phage-coded *lox-cre* system of site-specific recombination (Yarmolinski and Sternberg, 1988); however, no such system is available in P22.
   c. T4, although its genome is both circularly permutated and terminally redundant, has no recombination system as phage P1. The T4 genome remains linear; replication is bidirectional and characterized by multiple recombinations with secondary origins of replication and production of branched concatemers.
5. T7 has the same type of replication, but concatemers are linear due to end-to-end joining of daughter molecules. Phages T1 and T5 may belong here, but their replication is poorly known. Cyclization has not been observed, and the phages may replicate either as T4 or T7. T5 concatemers are branched.
6. In N4, replication starts at both ends of the genome and moves towards the center. It has been suggested that N4 uses a discontinuous mode of DNA replication (by lagging-strand synthesis?) with 3′ single-stranded ends as substrates for the addition of nucleotides. Concatemers are of the head-to-tail type.
7. In ϕ29 and its relatives, a phage-coded protein, gp3, is covalently linked to the DNA ends. This protein acts as a primer for a phage-coded DNA polymerase. Replication starts at either DNA end, uses strand displacement, and yields unit-length molecules.
8. In Mu, replication starts at one end of the genome. In transposing into host DNA, Mu DNA becomes accessible to the host metabolic machinery and replicates. Mu genomes are unit-length, and concatemers are not formed. (This is not completely true; see section VI.A.6)

At first glance, tailed phages exhibit a bewildering variety of replication strategies (see Table XIII). However, most tailed phages produce DNA concatemers; those which do not are exceptional. Further, since all phage genomes with cohesive ends and at least part of genomes with terminally redundant ends are able to form circles, it seems that cyclization after infection is the norm. This provides some advantages for phages because it offers protection against host-coded exonucleases and facilitates integration into the bacterial chromosome to achieve a temperate stage (Keppel *et al.*, 1988).

Rolling-circle replication is a basic mechanism of circular replicons, also found in the *Microviridae* and *Inoviridae* families of phages with ssDNA, gemini- and herpesviruses, a large number of plasmids, and viroids. The initiator protein of rolling-circle replication (Rep protein) has been found in phage 186, a relative of P2 (Ilyina and Koonin, 1992). It remains to be seen whether all phages of the *cos* type use the rolling-circle mechanism and have Rep proteins.

Phages N4, ϕ29, and Mu are not typical tailed phages. The reason for the special behavior of N4 is not understood. The replication mode of ϕ29 and its relatives is attributed to simultaneous acquisition of type B DNA polymerase and a replication-priming protein through horizontal gene transfer. Both type B DNA polymerase and protein-primed replication are also found in adenoviruses and phage PRD1 of the *Tectiviridae* family (see section V.B) (Bamford *et al.*, 1995; Salas, 1988; Shenk, 1996). In addition, there are extensive sequence homologies between the DNA polymerases of phages ϕ29 and PRD1 (Savilahti and Bamford, 1987), and the protein-priming terminal proteins of PRD1 and ϕ29 have similar hydropathy profiles (Hsieh *et al.*, 1987). The particular lifestyle of Mu is probably due to the acquisition of a transposon (see section V.C).

Tailed phages share certain features of replication with other dsDNA viruses. As ϕ29, adenoviruses replicate by strand displacement and do not form concatemers. Papovavirus replication is bidirectional and yields Θ rings. Herpesvirus replication resembles that of phage λ in featuring cyclization after infection, Θ rings followed by rolling circles, and concatemers. Among the other large dsDNA viruses, African swine fever virus and irido- and poxviruses also produce concatemers (Cole, 1996; Moss, 1996; Murphy *et al.*, 1995), Roizman and Sears, 1996; Shenk, 1996).

5. *Particle Assembly*

The literature on phage morphogenesis is abundant and has been the subject of a book (DuBow, 1981) and many excellent reviews,

a few of which are cited here (Berget and King, 1983; Black *et al.*, 1994; Casjens and Hendrix, 1988; Casjens and King, 1975; Hendrix, 1985; Hendrix and Garcea, 1994; Kellenberger, 1990; Kellenberger and Wunderli-Allenspach, 1995; Murialdo and Becker, 1978; Wood and King, 1979).

The assembly of the important phages P2, P4, P22, T4, T7, λ, and φ29 is essentially understood. The study of phage T4 has been particularly rewarding. In a general way, a capsid is assembled, filled with DNA, and provided with a tail. The process is highly ordered, characterized by sequential interaction of proteins, intervention of nonstructural catalytic assembly factors, and proteolytic cleavage of capsid proteins. Many structural proteins are synthesized and assembled simultaneously. The morphogenesis of individual phages is so similar that a general assembly pathway for tailed phages has been proposed (Casjens and Hendrix, 1988), of which a modified version is presented here. The general sequence of tailed phage assembly is as follows:

1. Phage heads:
   a. A ring of portal protein (connector) is formed.
   b. Scaffold and capsid protein assemble on the portal ring.
   c. Capsid proteins form a rounded procapsid around the scaffold.
   d. Scaffold proteins exit to be recycled or are digested.
   e. The mature procapsid (prohead) expands by proteolytic cleavage of subunits and becomes angular. Simultaneously, DNA enters through the portal ring.
   f. The head is completed by addition of stabilizing and "decoration" proteins.
2. Long tails (*Myo-* and *Siphoviridae*):
   a. A base plate or a tail tip are made.
   b. The tail is built from the distal end upwards. Tube and sheath proteins are added sequentially.
   c. The tail is joined to the head by the connector.
3. Short tails (*Podoviridae*): tail subunits are added to the completed head.

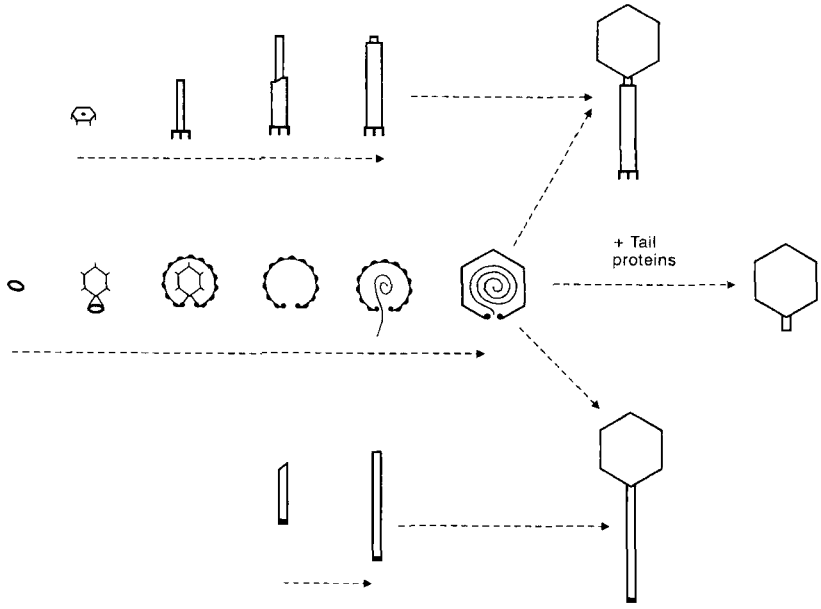

FIG 7. Generalized assembly pathway for tailed phages (after Casjens and Hendrix, 1988). Head assembly starts with the connector, to which a scaffold is added. A procapsid assembles around the scaffold. The latter leaves or is digested and the procapsid expands as DNA enters. Tail assembly starts at the distal end of the tail (the base plate in some phages). In myoviruses and siphoviruses, the tail is joined to the head; in podoviruses, tail proteins assemble to the head. The process is completed by addition of tail fibers or spikes.

The general assembly pathway (see Fig. 7) is thus branched in the *Myo-* and *Siphoviridae*. In T-even phages, tail fibers are assembled in a third pathway and joined to the completed particle. The complexity of tail fibers in many other phages suggests that a similar third pathway exists elsewhere. Fibers may be joined to the tail before or after head–tail joining. Head shape is determined by the scaffolding protein and/or interactions between vertex and main capsid proteins (Haynes and Eiserling, 1996; Kellenberger, 1990). Tail length is regulated by ruler proteins (see section III.D).

The start of capsid assembly with a connector and use of an internal scaffold were thought to be mandatory features of tailed phage morphogenesis and still appear typical for these viruses. However, there are exceptions: (1) in P22, the portal protein is added to an already assembled procapsid (Prasad *et al.*, 1993), (2) P4 has no internal scaffold and

its capsid is constructed within an external dodecahedral scaffold (Marvik *et al.*, 1995), and (3) coliphage HK97 has no scaffold, and its portal protein, although present, is not required for procapsid assembly (Duda *et al.*, 1995b).

Proheads are always rounded, thick-walled, and relatively small. After proteolytic cleavage of the major capsid protein, proheads expand by 15 to 20% and assume the typical thin-walled angular aspect of the normal phage head. On the other hand, P22 and T7 do not show any protein processing, λ shows fusion of capsid proteins, and in other phages, for example, coliphage HK97 and mycobacterial phage L5, proteolytic cleavage of the capsid protein is followed by cross-linking of subunits (see section III.B.1). The purpose of cross-linking is probably further stabilization of the capsid (Popa *et al.*, 1991). Despite these variations, the morphogenetic pathways of tailed phages are very similar.

In addition to mature virions and proheads, tailed phages produce a wide range of abnormal particles that are useful in mapping morphopoietic genes and determining morphogenetic pathways. Those produced by phage T4 have received particular attention. There are giant, dwarf, or monster heads with or without DNA, heads with multiple tails, double heads, regular or irregular polyheads, tail length variants (especially in siphoviruses), tails with multiple sheaths, polytails and polytubes, and several types of polysheaths (Ackermann and DuBow, 1987a; Black *et al.*, 1994). The great variety of abnormal structures is evidently related to the complexity of tailed phages and sets them further apart from other virus groups.

With respect to other viruses, proteolytic cleavage of virus proteins is an integral part of the morphogenesis of many viruses of vertebrates, insects, and plants (adeno-, como-, herpes-, noda-, picorna-, poty-, retro-, and togaviruses) (Hellen and Wimmer, 1992). Adenovirus morphogenesis apparently involves a scaffolding protein that is eliminated from the maturing capsid (Shenk, 1996). In herpesviruses, the procapsid is built around a scaffolding protein (Roizman and Sears, 1996) and matures by becoming angular and increasing in size (Homa and Brown, 1997). In the *Microviridae* (the ΦX174 group), a procapsid is built with the help of external and internal scaffolds and matures by DNA encapsidation and removal of the scaffolds (Dokland *et al.*, 1997). Despite these similarities, the complex branched assembly line of tailed phages has no equivalent in virology, indicating again that tailed phages are descendants from a common ancestor.

6. *DNA Packaging*

The subject of DNA packaging in phages has been reviewed repeatedly (Black 1988; 1989, 1995; Casjens, 1985; Catalano *et al.*, 1995; Valpuesta and Carrascosa, 1994). In addition, the packaging of phage DNA is usually discussed in the same reviews that provide information on phage replication and particle assembly (see sections VI.A.4 and 5).

DNA encapsidation is the last step in tailed phage DNA maturation and is linked to prohead expansion. It is a complex, efficient process in which DNA is introduced at high speed into preformed procapsids, involving the connector, the procapsid, and a DNA-cutting enzyme called "terminase." The actual manner of DNA packaging has not been established. Three basic models have been proposed, suggesting that packaging is driven by prohead expansion, rotation of the connector, or the terminase. The process is energy-consuming and powered by ATP hydrolysis (Black, 1988, 1995).

Terminases are phage-coded proteins that bind to and cut DNA concatemers, generally at *pac* or *cos* sites (see section V.B). They consist of a large and a small subunit with molecular weights of 44–73 and 10–45 kDa, respectively. The small subunit is generally responsible for DNA recognition and binding. The larger subunit ensures DNA cutting, binding of the terminase to the connector, and, possibly, DNA translocation into the phage head. Terminases generally have ATPase and endonuclease activity (Black, 1988; 1989; Valpuesta and Carrascosa, 1994). It has been proposed that terminase and portal vertex form a complex or "packasome" that translocates DNA by hydrolyzing ATP (Black, 1988, 1995).

DNA packaging is directional and processive. The DNA enters through a unique opening, the portal vertex. The DNA end packaged last is the first to be ejected. Inside the phage head, the packaged DNA assumes a condensed and ordered state; however, it is not clear whether it is laid down in concentric shells or as folded rods, as a toroid or as a spool. The prohead expands during packaging. This complex process is apparently common to all tailed phages and highly conserved (Black, 1995).

Tailed phages differ by the nature of the packaging substrate (concatemers or unit-length DNA), the starting point of DNA packaging, and the site of DNA cutting. The "headful hypothesis" (Streisinger *et al.*, 1967) specifies that the DNA of certain phages is cut when the head is filled. Phages using the headful mechanism generally receive an excedent (terminal redundancy) of circularly permutated DNA. In *pac*-type phages, packaging of phage DNA starts at the *pac* site and contin-

ues until the empty prohead is filled. In *cos*-type phages, DNA packaging starts at a *cos* site and ends at a *cos* site. Consequently, tailed phages have been grouped into four categories (Black, 1988, 1989):

1. Phages with unique DNA ends and sequence-specific cuts, either at cohesive sites (λ, P2) or nonpermutated redundancies (T3-T7, T5).
2. Phages with variable ends, using a processive headful mechanism with DNA cut at specific *pac* sites (P1, P22, T1).
3. Phages with random cutting of concatemers, using a pure headful mechanism (T4).
4. Phages packaging nonconcatemeric DNA (P2, ϕ29, Mu).

Because of the high frequency of phages with proven *cos* and *pac* sites, a slightly different arrangement seems to be more appropriate:

1. Phages with concatemeric DNA:
   a. Cut at fixed sites:
      α. *Cos*-type phages (λ, P2).
      β. *Pac*-type phages (P1, P22, T1; Mu).
      γ. Nonpermutated redundancies (T3, T7, T5).
   b. Cut at variable sites (T4).
2. Phages with nonconcatemeric DNA (ϕ29).

P2 and Mu are basically *cos*- and *pac*-type phages with concatemeric DNA and special properties. P2 replicates via a modified rolling-circle mechanism and produces concatemers with *cos* sites, but the novel DNA is cyclized before packaging. However, P2 also packages linear DNA (Pruss *et al.*, 1975). Mu apparently linked up with a transposon (see section V.C). Novel Mu DNA is not exclusively unit-size, but exists in several forms including Θ rings and long filaments (Howe, 1987b). T3–T7, T4, and T5 possibly derived from *pac* type phages by loss of *pac* sites. Phage ϕ29 and its relatives separated from mainstream phages by acquiring a replication-initiating protein and replication by strand displacement (see section VI.A.4). Phage ϕ29 is also unusual because its DNA codes for a-174 base "packaging RNA" that binds to procapsids at the portal vector and has an essential role in the encapsidation of ϕ29 DNA (Guo and Trottier, 1994).

Tailed phages share certain features of DNA packaging with other dsDNA viruses. Hepadnaviruses have *cos* sites, herpesviruses possess a recognition site for headful packaging, and iridoviruses may have *pac* sites (see section V.B). Packaging of DNA into preformed capsids seems to be a common feature of most dsDNA viruses with cubic symmetry (Hendrix and Garcea, 1994). It is seen in the African swine fever virus and in adeno-, herpes-, irido-, and tectiviruses; in addition, poxvirus DNA enters immature envelopes and the viral RNA of hepadnaviruses, once described from DNA, is packaged into novel capsids and there reverse transcribed into DNA (Ganem, 1996; Murphy *et al.*, 1995; Moss, 1996; Roizman and Sears, 1996; Shenk, 1996). Only papovavirus capsids seem to co-assemble with their DNA (Cole, 1996). It is clear that different viruses have found similar solutions to the problem of DNA encapsidation.

7. Lysis

All tailed phages are released by a single event called "lysis," in which the cell bursts suddenly and is destroyed. There is no gradual release by transport vesicles, budding, or extrusion. Lysis is achieved by many viruses. In tailed phages, lysis is mediated by a combination of peptidoglycan hydrolases and holins. It seems that every tailed phage is equipped with these two types of enzymes and that they cluster together in a "lysis cassette" (Gasson, 1996; Young, 1992). At least in some phages, both enzymes are encoded by the same gene (Hertwig *et al.*, 1997).

Peptidoglycan hydrolases, also called "endolysins," attack the murein layer of the bacterial cell wall and comprise four classes: muramidases or "lysozymes," amidases, peptidases, and transglycosylases (glucosaminosidases) (Ghuysen *et al.*, 1966; Vasala *et al.*, 1995; Young, 1996). All four types occur in tailed phages and are irregularly distributed (section VII.E), but have analogous functions.

Holins are small proteins that cause nonspecific lesions in the bacterial plasma membrane, allowing the peptidoglycan hydrolases to reach the peptidoglycan. Holins fall into at least 12 families, but have a common architecture (Bläsi and Young, 1996; Young and Bläsi, 1995). It seems that holin genes code for proteins with opposite functions, the holin itself and an inhibitory protein (Bläsi and Young, 1996).

There is little comparable in the release of other viruses. Eukaryotic cells have no peptidoglycan and their viruses evidently need no peptidoglycan hydrolases. Among cubic, filamentous, and pleomorphic

TABLE XIV

TEMPERATE VERSUS VIRULENT PHAGES

Category	Examples	*Cos* site	Integration	Plasmid stage	Transposition	*Pac* site
Temperate	λ	+	+	(–)	–	–
	P22	–	+	–	–	+
	P1	–	(–)	+	–	–
	Mu	–	+	–	+	+
Virulent	T1	–	–	–	–	+
	T4	–	–	–	–	–

Cos, cohesive; *pac*, initiation of DNA packaging; +, yes or present; –, no or absent; (–), normally not.

phages, the *Fusello-*, *Ino-*, and *Plasmaviridae* do not lyse their hosts (Murphy *et al.*, 1995). The phages of the *Cortico-*, *Levi-*, *Lipothrix-*, and *Microviridae* families are lytic, but their molecular mechanisms of lysis, apparently very different from those of tailed phages, have not been identified, and holins have not been reported there (Young, 1992). Phages of the *Cystoviridae* and *Tectiviridae* families have a peptidase and a muramidase, respectively (see section VII.E). It remains to be seen if algal viruses have a dual lysis system.

B. The Temperate Cycle

In the temperate or lysogenic cycle, bacteriophage genomes enter a state of latency that may break down spontaneously or under the effect of inducing agents, for example, UV light. The phage genome then reverts to a vegetative state with production of progeny phages. The latent phage genome is called a prophage. The genomes of temperate tailed phages comprise three groups: (1) genomes with cohesive ends and the ability to integrate, (2) genomes with *pac* sites, able to integrate or to persist as plasmids, and (3) Mu-type DNAs with a transposition site. Integrating phages may become virulent if their integrase gene is deleted or damaged by mutation (Mikkonen, 1996). The complex relationship between temperate and virulent phages, *cos-* and *pac*-sites, integration and plasmid stages appears as follows (see Table XIV).

Prophages are maintained by integration into the bacterial genome or as plasmids. Integration provides a handy way of preserving phage

genes over enormous time spans because the phage DNA is replicated as part of the bacterial genome and protected against the environment (see section II.C). Integration normally requires cyclization of the phage genome and is a crossing-over process (Campbell, 1962) mediated by specific enzymes called "integrases." The integrated phage genome is maintained by repressors and becomes independent again only after excision by special enzymes or "excisionases." The sites for integrase, prophage attachment, and excisionase (*int*, *att*, *xis*) cluster together to form a "lysogeny cassette." The integrases belong to one and the same family of site-specific recombinases (see section VII.D). Excisionases have not always been found and may be absent in some cases. Phage Mu stands apart because it integrates using a different type of enzyme, a transposase, and by transposition into the host genome. In the plasmid mode, prophages persist as circular entities within the cytoplasm.

It is interesting that both plasmid mode and integration occur in archaeal phages. In particular, *Halobacterium* phage ΦH has a plasmid stage, and the prophage of the recently described *Natronobacterium* phage ΦCh1 integrates into the host genome (Schnabel *et al.*, 1984; Witte *et al.*, 1997). Does the plasmid stage represent a major avenue in evolving lysogeny? It does not seem so.

First, integrated prophages seem to be far more frequent than plasmid prophages. Second, many plasmid prophages are able to replicate alternatively as integrated prophages, notably P1, P4, and λ (Table XV). Phage P1 and its relatives, although able to integrate (Yarmolinski and Sternberg, 1988), prefer the plasmid mode of prophage replication. On the other hand, λ prefers integration and replicates as a plasmid, λ*dv*, only if early replication genes, especially *N*, are eliminated (Enquist and Skalka, 1978; Kleckner and Signer, 1977). Overall, integration is the prevailing mode of prophage maintenance, and the plasmid mode appears as a separate development due to deletions or the occasional acquisition, by individual phages, of a plasmid origin of replication. The integrative mode is widespread in tailed phages, but irregularly distributed. In addition, integrases occur in other types of phages, plasmids, and transposons (see section VII.D). It is concluded that neither type of lysogeny, the integrative and the plasmid mode, are basic features of tailed phages (see Table XVI).

It is remarkable that latency and temperate cycles are widespread in the viral world, occurring not only in tailed phages, but also in 10 more families of bacterial and vertebrate viruses. In the *Fuselloviridae* family (archaea) and the *Plasmaviridae* (mycoplasmas), the viral genome is integrated by enzymes of the λ integrase family (Maniloff *et*

TABLE XV

OCCURRENCE OF PLASMID PROPHAGES

Family	Host	Phage	Plasmid	Integration	References
Myoviridae	Enterobacteria	P1, others	+	+	Yarmolinski and Sternberg, 1988
		P4	+	+	Bertani and Six, 1988
	Halobacterium	ΦH	+		Schnabel *et al.*, 1984
Siphoviridae	*Agrobacterium*	ψ	+	+	Expert *et al.*, 1982
	Azospirillum	Al-1	+	+	Elmerich *et al.*, 1982
	Bacillus	TP-21	+		Walter and Aronson, 1991
	Bordetella	134, others	+	+	Holzmayer *et al.*, 1988
	Enterobacteria	λ, others	+	+	Enquist and Skalka, 1978; Kleckner and Signer, 1977; Matsubara and Otsuji, 1978
	Rhodospseudomonas	Rφ6	+		Tucker and Pemberton, 1978
	Streptomyces	φFS1	+		Chung, 1982
Podoviridae	*Pseudomonas*	F116	+		Miller *et al.*, 1977
Fuselloviridae	*Sulfolobus*	SSV1	+	+	Martin *et al.*, 1984; Reiter *et al.*, 1989
Nonclassified	*Methanococcus*	A3	+	+	Wood *et al.*, 1989

+, yes or present.

al., 1994; Reiter *et al.*, 1989). The ability of inoviruses to integrate is little known and the nature of their integrases has not been investigated. Retrovirus integrase is a transposase-like entity and unrelated to the λ-type integrase family. It is not site specific, the integration mechanism is very similar to that of phage Mu (Baker, 1995), and the integrase itself is a protein with a central five-stranded β-sheet and six helices that structurally resembles RNase H and the core domain of Mu transposase (Dyda *et al.*, 1994). Latency in herpesviruses may be due to plasmid formation or integration. In conclusion, it appears that latency evolved at least three times in viruses: by integration via integrases, integration via transposases, and recruiting of (or becoming) plasmids.

TABLE XVI

LYSOGENY IN VIRUSES

Host group	Virus group or family	Integration mode	Plasmid mode	References
Bacteria	Tailed phages	+	+	
	Fuselloviridae	+	+	Reiter *et al.*, 1989
	Inoviridae	+	+	Dai *et al.*, 1987; Lin *et al.*, 1994; Kuo *et al.*, 1987; Waldor and Mekalanos, 1996
	Lipothrixviridae	?	?	Zillig *et al.*, 1988
	Plasmaviridae	+		Maniloff *et al.*, 1994
Vertebrates	*Hepadnaviridae*	+		Ganem, 1996
	Herpesviridae	?	?	Roizman and Sears, 1996
	Papovaviridae	+	+	Cole, 1996; Howley, 1996
	Parvoviridae	?	?	Murphy *et al.*, 1995
	Retroviridae	+		Coffin, 1996
Insects	*Polydnaviridae*	?	?	Murphy *et al.*, 1995

+, yes or present.

VII. AMINO ACID SEQUENCE ALIGNMENTS

A. General Observations

Alignments of amino acid sequences from plant and vertebrate viruses had shown surprising relationships, for example, between togaviruses and isometric or filamentous viruses of plants. It permitted notably the identification of the Sindbis-like and picornavirus-like supergroups of (+) strand RNA viruses (Goldbach and Wellink, 1988; Goldbach *et al.*, 1991) and raised hopes that sequence alignments, whether of amino acids or nucleic acid bases, would solve many fundamental problems of virus phylogeny and taxonomy.

These high hopes have been frustrated with respect to tailed phages. So far, amino acid sequence alignments have not detected any proteins common to all tailed phages (Ackermann *et al.*, 1995a). Instead, they are essential for identification of genes in newly sequenced phage DNAs, for example, of DNA polymerases or muramidases. They also

indicate or confirm relationships between a few individual phages and provide ample evidence for horizontal gene transfer: not only between phages, but also between phages, plasmids, transposons, other viruses, bacteria, and eukaryotes. Some phage proteins can even be traced to humans. These alignments shed light on the evolution of tailed phages, not on their origin.

The list of sequenced tailed phage proteins is now considerable; to name a few examples, it includes DNA ligases and topoisomerases, dUTPases, exonucleases, introns, methyltransferases, NTP-binding proteins, origins of replication, primases–helicases, promoters, protein phosphatases, repressors, and sigma factors. This review will concentrate on frequently sequenced proteins, namely structural proteins, DNA and RNA polymerases, integrases, and lysis-related proteins.

B. Structural Proteins

With respect to major capsid and tail proteins, sequence alignments have essentially confirmed relationships between phages known to be related, for example, the phage pairs of coliphages λ and 21, *Bacillus* phage φ29 and *Streptococcus* phage Cp-1, and lactococcal phages c2 and bIL67 (Casjens *et al.*, 1992; Lubbers *et al.*, 1995; Martín *et al.*, 1996). Other reports indicate relationships between phages or structures formerly thought to be unrelated; for example, *Haemophilus* phage HP1, *Pseudomonas* phage PS17, and coliphage P2 (Esposito *et al.*, 1996), or between tail tube and sheath proteins of PS17 and T4 (Sasaki *et al.*, 1997). However, most major head and tail proteins appear to be unique. Database print-outs typically state that these proteins "belong to their own superfamily."

There is no apparent relationship between connector proteins (Ackermann *et al.*, 1995a; Eppler *et al.*, 1991), although the 12-fold symmetry of connectors is very unusual in biology. There are relationships between the connector, lower collar, scaffolding, and encapsidation proteins of phages φ29 and Cp-1 (Martín *et al.*, 1996), respectively, but this is hardly surprising and only confirms again that these two phages are related.

The situation is different in tail fibers. Indeed, the tail fiber genes of numerous enterobacterial phages (e.g., myoviruses Mu, P1, P2, T2, T4; siphovirus λ; podoviruses T3 and T7) appear as mosaics composed of multiple domains that are shared to variable extents, found in this or that phage and not in another. This is interpreted as evidence

for illegitimate recombination at previously unsuspected levels and of repeated horizontal gene transfer, resulting in host range modification and scrambling of motifs (Cerritelli *et al.*, 1996; Haggård-Ljungquist *et al.*, 1992; Hendrix and Duda, 1992; Sandmeier, 1994; Xue and Egan, 1995). Since scrambling of motifs probably produces new host ranges, one can expect that the tail fiber genes of other tailed phages are equally scrambled.

C. DNA and RNA Polymerases

Two families of DNA polymerases, termed A and B and exemplified by *E. coli* DNA polymerases I and II, respectively, are found in viruses (Braithwaite and Ito, 1993; Jung *et al.*, 1987). Family A is found in several tailed phages and in bacteria. Family B is ubiquitous in nature, occurring in tailed phages T4 and φ29, adeno-, herpes-, pox-, and other dsDNA viruses, bacteria, and an extraordinarily wide range of eukaryotes including humans (Braithwaite and Ito, 1993; Forterre *et al.*, 1994; Jung *et al.*, 1987). Viral DNA polymerases of the B family are protein- or RNA-primed, allowing for a further subdivision. In addition, tailed phages of the T7 group code for an RNA polymerase (Hausmann, 1988), a poorly known RNA polymerase is present within the head of podovirus N4 (Iino and Rothman-Denes, 1988), and "retronphage" φR73, a defective phage and relative of P4, carries a retroelement (Sun *et al.*, 1991). RNA polymerases with T7-like sequences are found in a wide variety of eukaryotes (Cermakian *et al.*, 1996).

Because RNA polymerases had been so useful in identifying relationships between (+)-stranded ssRNA viruses, it was attempted to classify dsDNA viruses into high-level taxa according to the presence of A- or B-type DNA polymerases and RNA or protein priming (Ward, 1993). Seven tailed phages with DNA polymerases were classified into two phyla, at least two classes and two orders, and six families. The possibility of horizontal gene transfer and the complete absence of DNA polymerases in well-known phages, for example, λ, were overlooked. This approach illustrates the dangers of classification by a single criterion and is not acceptable. In fact, everything indicates that DNA and RNA polymerases colonized tailed phages through horizontal gene transfer: their widespread occurrence in nature, their variety and irregular distribution in some tailed phages, and their absence in others (see Table XVII).

TABLE XVII

DNA AND RNA POLYMERASES

Family	Host	DNA polymerases		Reverse transcriptase	RNA polymerase
		Family A	Family B		
Myoviridae	*Bacillus*	SPO2			
	Enterobacteria		T4 (RB69[a])	ϕR73	
Siphoviridae	*Bacillus*	SPO1			
	Enterobacteria	T5			
	Mycobacterium	L5			
Podoviridae	*Bacillus*		ϕ29 (M2)		
	Enterobacteria	T7			N4,[c] T7 (K11, SF6, Kvp1[d])
	Streptococcus		Cp-1[b]		

Computed from Ackermann *et al.*, 1995a; Braithwaite and Ito, 1993; Forterre *et al.*, 1994; and Jung *et al.*, 1987. Related phages are listed in parentheses.

Additional references: [a]Wang *et al.*, 1997. [b]Martín *et al.*, 1996. [c]Kiino and Rothman-Denes, 1988. [d]Gadaleta and Zorzopoulos, 1997.

D. Integrases

Except for the transposase of phage Mu, all known phage integrases mediate site-specific recombination and belong to the same family, termed the λ or tyrosine recombinase family. Its members can be aligned in their C-terminal halves. A region of 40 amino acid residues near the C terminus appears being particularly well conserved (Argos *et al.*, 1986). A further conserved region is situated in the center of the molecule (Maniloff *et al.*, 1994). The preferred integration sites seem to be tRNA genes (Reiter *et al.*, 1989).

Integrases of the λ family occur in temperate tailed phages and the pleomorphic phages of the *Fusello-* and *Plasmaviridae* families (Table XVIII). Interestingly, the host of the fusellovirus is an archaebacterium. The impression that data are plentiful is misleading because many data are from related phages (e.g., P2-HP1-186-P4-ϕR73). In addition, λ-type integrases are found in some defective phages of unknown morphology, about 50 plasmids of yeasts or bacteria, transposons, and segments of bacterial chromosomes (Abremski and Hoess, 1992; Esposito and Scocca, 1997; Fremaux *et al.*, 1993; Lee *et al.*, 1991; Mercier *et al.*, 1990; Yagil *et al.*, 1989). They appear to be descendants from a common

TABLE XVIII

INTEGRASES IN TAILED AND OTHER PHAGES

Family	Host	Phages[a]	References[b]
Myoviridae	Enterobacteria	P1, P2 (P4, 186, φR73)	1–9
	Haemophilus	HP1	1, 2, 4, 9
	Pseudomonas	φCTX	4, 10
Siphoviridae	Enterobacteria	λ (DLP12, HK22, 21, 434, φ80)	1–7, 11
	Lactobacillus	LL-H (mv4), φadh, φgle	4, 5, 12, 13, 14
	Lactococcus	r1t, φLC3	2, 4, 15
	Leuconostoc	φ10Mc	16
	Mycobacterium	L5 (D29)	2, 4
	Staphylococcus	L54a (φ11)	1, 2, 4, 5, 6
Podoviridae	Enterobacteria	P22 (SF6)	1–4, 6, 7, 9
Fuselloviridae	*Sulfolobus*	SSV1	4, 17
Plasmaviridae	*Acholeplasma*	L2	4, 18

[a]Related phages are listed in parentheses.

[b]References: 1 = Abremski and Hoess, 1992. 2 = Ackermann *et al.*, 1995a. 3 = Argos *et al.*, 1986. 4 = Esposito and Scocca, 1997. 5 = Fremaux *et al*, 1993. 6 = Lee *et al.*, 1991. 7 = Mercier *et al.*, 1990. 8 = Sun *et al.*, 1991. 9 = Yagil *et al.*, 1989. 10 = Wang *et al.*, 1995. 11 = Campbell *et al.*, 1992. 12 = Dupont *et al.*, 1995. 13 = Kodaira *et al.*, 1997. 14 = Mikkonen, 1996. 15 = Van Sinderen *et al.*, 1996. 16 = Gindreau *et al.*, 1997. 17 = Reiter *et al.*, 1989. 18 = Maniloff *et al.*, 1994.

ancestor (Campbell *et al.*, 1992; Esposito and Scocca, 1997), but are also highly diversified. In multiple alignments of complete sequences, they show only 8–23% amino acid identity. These low percentages would normally be considered as negative or borderline (Ackermann *et al.*, 1995a).

The occurrence of λ-type integrases in tailed and pleomorphic phages, plasmids, and transposons suggests that they did not originate in tailed phages. Since plasmids often contain transposons, it is possible that these integrases originated there and spread to phages from bacteria or plasmids via horizontal gene transfer. Their extensive diversification indicates that they were acquired in the distant past.

E. Peptidoglycan Hydrolases

The four peptidoglycan hydrolases of tailed phages, namely muramidases, lysozymes, amidases, peptidases, and transglycosylases, are dis-

TABLE XIX

PEPTIDOGLYCAN HYDROLASES

Family	Enzyme[a]	Host	Phages	Refs.[b]
Myoviridae	Lysozymes	Enterobacteria	T4	1
	Transglycosylases	Enterobacteria	P2	1, 2
		Haemophilus	HP1	2
	Amidases	*Bacillus*	Bastille, PBSX	3, 4
		Streptococcus	EJ-1	1
Siphoviridae	Lysozymes	*Bacillus*	SF6	1
		Enterobacteria	PA-2	1
		Lactobacillus	LL-H (mv-1), φadh	1, 5, 6
		Lactococcus	Tuc2009, φLC3?, φvML3 (bIL67?)	1, 7, 8, 9, 10
	Transglycosylases	Enterobacteria	λ (HK22, 82), HK97	1, 11
	Amidases	*Bacillus*	P21	3
		Lactococcus	r1t?, φUS3	1, 12
		Streptococcus	Dp-1, β-3	1, 13
	Peptidases	Enterobacteria	λ (21, 434)	11
		Methanobacterium	ψ1	14
Podoviridae	Lysozymes	*Bacillus*	φ29 (PZα)	1, 10
		Enterobacteria	P22 (ES18)	1, 15
		Streptococcus	Cp-1	1
	Amidases	Enterobacteria	7	1
	Peptidases	Enterobacteria	P22 (ES18), T7	1, 15

[a]Due to unclear nomenclature and conflicting data, lysozymes and muramidases had been regarded as separated entities, and the latter were regarded as synonyms for amidases. This is corrected here. Related phages are listed in parentheses.

[b]References: 1 = Ackermann *et al.*, 1995a. 2 = Esposito *et al.*, 1996. 3 = Loessner *et al.*, 1997. 4 = Longchamp *et al.*, 1994. 5 = Henrich *et al.*, 1995. 6 = Vasala *et al.*, 1995. 7 = Arendt *et al.*, 1994. 8 = Birkeland, 1994. 9 = Schouler *et al.*, 1994. 10 = Shearman *et al.*, 1994. 11 = Casjens *et al.*, 1992. 12 = Van Sinderen *et al.*, 1996. 13 = López *et al.*, 1992. 14 = Stax *et al.*, 1992. 15 = Schicklmaier and Schmieger, 1997.

tributed without apparent regularity (Table XIX). Lysozymes are the prevailing type (14 phages), followed by amidases (9 phages). Lysozymes seem to be heterogeneous, comprising a core group of lysozymes from phage T4 (gpe and gp5), P22, φ29, lambdoid phage PA-2, and *Lactococcus* phage φvML3 (Ackermann *et al.*, 1995a). Besides their irregular distribution, many lines of evidence indicate that phage peptidoglycan hydrolases are spread by horizontal gene transfer:

1. Lysozymes are widespread in nature; for example:

a. T4 lysozyme resembles in its tertiary structure hen and goose egg lysozymes (Grütter *et al.*, 1983) and plant chitinases (Holm and Sander, 1994).

b. Tailed phage lysozymes have equivalents in streptomycetes and a fungus, *Chalaropsis* sp. (García *et al.*, 1988; Henrich *et al.*, 1995).

c. *Tectiviridae* phages have a lysozyme (Bamford *et al.*, 1995).

2. Endopeptidases occur in tailed phages and cystovirus φ6 (Caldentey and Bamford, 1992).

3. Transglycosylase-like proteins are found in phages and bacteria (Koonin and Rudd, 1994).

4. Several tailed phages have two or more muralytic enzymes:

a. Phage T4 has at least two lysozymes (gpe and gp5, possibly gp25) (Kutter *et al.*, 1994).

b. Phage λ and some of its relatives have a transglycosylase, gpR, and a putative endopeptidase, gpRz (Casjens *et al.*, 1992; Young, 1992).

c. Phages P22 and its relative ES18 have a lysozyme and a mosaic-like endopeptidase (Schicklmaier and Schmieger, 1997).

d. Phage T7 has an amidase, gp3.5, and an endopeptidase, gp18.5 (Ackermann *et al.*, 1995a; Hausmann, 1988).

5. In streptococcal phage Cp-1, the lytic enzyme derives from the fusion of two different modules. The N-terminal half is a lysozyme and the C-terminal moiety, responsible for the attachment to choline residues of the cell wall, is amidase-related. The amidase domain resembles that of an autolysin of *S. pneumoniae* (García *et al.*, 1988; López *et al.*, 1992). Apparently, phage Cp-1 has the best of two worlds.

6. The amidases of lactococcal phage φUS3 and streptococcal phages EJ-1 and HB-3 have many similarities to a major pneumococcal autolysin (Díaz *et al.*, 1992; Platteeuw and de Vos, 1992; Romero *et al.*, 1990a).

TABLE XX

THYMIDYLATE SYNTHASES IN PRO- AND EUKARYOTES[a]

Organism	Ec	Lc	H	M	Hs	Lm	T4	ϕ3T
Gene	*thyA*						*td*	*thyp3*
Ec	100	60	54	55	50	49	49	36
Lc		100	44	44	50	42	44	36
H			100	89	70	56	45	38
M				100	70	55	44	38
Hs					100	59	40	39
Lm						100	42	35
T4							100	33
ϕ3T								100

Ec, *Escherichia coli*; H, Human; Hs, *Herpesvirus saimiri*; Lc, *Lactobacillus casei*; Lm, *Leishmania major*; M, mouse; T4, phage T4; ϕ3, *Bacillus subtilis siphovirus* ϕ3.

[a]Origins of protein sequences and programs used are indicated elsewhere (Ackermann *et al.*, 1995a). Courtesy of G. Stewart.

7. The amidases of three *Bacillus* phages are constructed of modules with high homology to major autolysins of bacilli and *E. coli* (Loessner *et al.*, 1997).

In conclusion, no enzyme lysing bacterial cell walls can be considered as specific to tailed phages. The question of whether tailed phages acquired their lytic enzymes from bacteria or vice versa cannot be answered at this time.

F. Interpretation

The evidence for horizontal gene transfer in tailed phages extends to other functional proteins, for example, exonucleases and DNA topoisomerases. This is apparent in a pioneering study on phage T4, which shows relatives of T4 proteins surfacing in yeasts and other unsuspected entities (Bernstein and Bernstein, 1989). Further examples can be given at will. Amusingly, phage T4 appears as 2% human. Its approximately 200 genes code for at least five proteins with counterparts in humans: DNA polymerase, lysozymes (also present in human tears), and thymidylate synthase. If one aligns the amino acid sequences of a series of thymidylate synthases with a common tertiary

structure (Hardy *et al.*, 1987), human and T4 thymidylate synthases show 44.8% amino acid sequence identity (Table XX).

Thus, it appears that (1) the structural genes of tailed phages, which are likely to be indigenous and where relationships must be sought in the first place, seem to be essentially unrelated, (2) lysis- and lysogeny-related genes, the next best candidates, were acquired from the outside, and (3) their functional genes, for example, of DNA metabolism, are scattered over the living world. However, it cannot be excluded that the major capsid and tail proteins of tailed phages are ultimately related; indeed, it is likely that the evidence for relatedness has not yet been found and that amino acid sequence alignment is just the wrong technique for addressing the question:

1. Tailed phages are so much older than plant or animal viruses that relationships were erased and are no longer detectable by amino acid sequence alignments (see Doolittle, 1981, 1995).

2. Relationships (e.g., between capsid proteins) may be so weak that they are detectable only through examination of missing links; for example, proteins A, B, and C may be related, but may appear unrelated if A and C only are compared.

3. Relationships may be limited to specific domains (as in enterobacterial tail fibers or integrases) and will normally go undetected in amino acid alignments of whole proteins. For example, some ORFs of *Listeria* phage A511, a myovirus, show partial amino acid identity to tail protein A of coliphage T7, the upper collar protein of ϕ29, and a shape-determining protein of *E. coli* (Loessner and Scherer, 1995).

4. Relationships may be preserved in the three-dimensional structure of tailed phage capsid and tail proteins (see Doolittle, 1995). Unfortunately, not a single one of these proteins has been studied for its spatial structure.

VIII. Summary and Conclusions

Tailed bacteriophages have a common origin and constitute an order with three families, named *Caudovirales*. Their structured tail is unique. Tailed phages share a series of high-level taxonomic properties and show many facultative features that are unique or rare in viruses,

for example, tail appendages and unusual bases. They share with other viruses, especially herpesviruses, elements of morphogenesis and lifestyle that are attributed to convergent evolution. Tailed phages present three types of lysogeny, exemplified by phages λ, Mu, and P1. Lysogeny appears as a secondary property acquired by horizontal gene transfer. Amino acid sequence alignments (notably of DNA polymerases, integrases, and peptidoglycan hydrolases) indicate frequent events of horizontal gene transfer in tailed phages. Common capsid and tail proteins have not been detected.

Tailed phages possibly evolved from small protein shells with a few genes sufficient for some basal level of productive infection. This early stage can no longer be traced. At one point, this precursor phage became perfected. Some of its features were perfect enough to be transmitted until today. It is tempting to list major present-day properties of tailed phages in the past tense to construct a tentative history of these viruses:

1. Tailed phages originated in the early Precambrian, long before eukaryotes and their viruses.
2. The ur-tailed phage, already a quite evolved virus, had an icosahedral head of about 60 nm in diameter and a long noncontractile tail with sixfold symmetry. The capsid contained a single molecule of dsDNA of about 50 kb, and the tail was probably provided with a fixation apparatus. Head and tail were held together by a connector.
    a. The particle contained no lipids, was heavier than most viruses to come, and had a high DNA content proportional to its capsid size (about 50%).
    b. Most of its DNA coded for structural proteins. Morphopoietic genes clustered at one end of the genome, with head genes preceding tail genes. Lytic enzymes were probably coded for. A part of the phage genome was nonessential and possibly bacterial. Were tailed phages general transductants since the beginning?
3. The virus infected its host from the outside, injecting its DNA. Replication involved transcription in several waves and formation of DNA concatemers. Novel phages were released by burst of the infected cell after lysis of host membranes by a peptidoglycan hydrolase (and a holin?).

a. Capsids were assembled from a starting point, the connector, and around a scaffold. They underwent an elaborate maturation process involving protein cleavage and capsid expansion. Heads and tails were assembled separately and joined later.

b. The DNA was cut to size and entered preformed capsids by a headful mechanism.

4. Subsequently, tailed phages diversified by:

a. Evolving contractile or short tails and elongated heads.

b. Exchanging genes or gene fragments with other phages.

c. Becoming temperate by acquiring an integrase-excisionase complex, plasmid parts, or transposons.

d. Acquiring DNA and RNA polymerases and other replication enzymes.

e. Exchanging lysin genes with their hosts.

f. Losing the ability to form concatemers as a consequence of acquiring transposons (Mu) or protein-primed DNA polymerases (ϕ29).

Present-day tailed phages appear as chimeras, but their monophyletic origin is still inscribed in their morphology, genome structure, and replication strategy. It may also be evident in the three-dimensional structure of capsid and tail proteins. It is unlikely to be found in amino acid sequences because constitutive proteins must be so old that relationships were obliterated and most or all replication-, lysogeny-, and lysis-related proteins appear to have been borrowed. However, the sum of tailed phage properties and behavior is so characteristic that tailed phages cannot be confused with other viruses.

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TABLE XXI

APPENDIX: VIRUS FAMILIES OR GROUPS CITED IN THE TEXT

Family or group	Host	Nucleic acid	Symmetry	Envelope	Family or group	Host	Nucleic acid	Symmetry	Envelope
Adenoviridae	V	dsDNA	C	–	*Lipothrixviridae*	B	dsDNA	H	+
ASFV[a,b]	V	dsDNA	C	+	*Microviridae*	B	ssDNA	C	–
Baculoviridae	I	dsDNA	H	+	*Nodaviridae*	I	ssRNA	C	–
Badnavirus[b]	P	dsDNA	C	–	*Oryctes* virus[c]	I	dsDNA	H	+
Bromoviridae	P	ssRNA	C	–	*Papovaviridae*	V	dsDNA	C	–
Caulimoviridae	P	dsDNA	C	–	*Parvoviridae*	V, I	ssDNA	C	–
Comoviridae	P	ssRNA	C	–	*Phycodnaviridae*	A	dsDNA	C	–
Corticoviridae	B	dsDNA	C	–	*Picornaviridae*	V, I?	ssRNA	C	–
Cystoviridae	P	dsRNA	C	+	*Plasmaviridae*	B	dsDNA	Pleo	+
Fuselloviridae	B	dsDNA	Pleo	+	*Polydnaviridae*	I	dsDNA	H	+
Geminiviridae	P	ssDNA	C	–	*Potyviridae*	P	ssRNA	H	–
Hepadnaviridae	V	dsDNA	C	+	*Poxviridae*	V, I	dsDNA	H	+
Herpesviridae	V	dsDNA	C	+	*Reoviridae*	V, I, P	dsRNA	C	–
Inoviridae	B	ssDNA	H	–	*Retroviridae*	V	ssRNA	C	+
Iridoviridae	V, I	dsDNA	C	+ or –	*Tectiviridae*	B	dsDNA	C	–
Leviviridae	B	ssRNA	C	–	*Togaviridae*	V, I	ssRNA	C	+

A, algae; B, bacteria; C, cubic; H, helical; I, invertebrates; P, plants; Pleo, pleomorphic; V, vertebrates; +, present; –, absent.

[a]African swine fever virus.

[b]Genus unassigned to a family.

[c]Nonclassified, baculovirus-like.

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