Parvoviridae: Second Report

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Knowledge on the unique characteristics of parvoviruses containing only one strand of DNA has expanded rapidly during the last years. The single-stranded DNA molecule reveals a hairpin structure both at the 5' and the 3' end, and about 1% of the nucleic acid of the viruses belonging to the parvovirus genus forms double strands similar to the self-complementary strands of adeno-associated virus (AAV) and densovirus species [3, 26]. The suggestion that parvovirus particles contain a polymerase seems to be very unlikely in the light of new data [2]. Further experience regarding the polypeptides shows that mature virus particles have three polypeptides: VP-1¹ Second report of the Study Group on Parvoviridae, Co-ordinating Subcommittee, International Committee on Taxonomy of Viruses (ICTV). The report and its proposals are presented unanimously by the Study Group. The revision and contribution of data by *G. Siegl* are gratefully acknowledged.

(mol.wt. 83,000), VP-2 (mol.wt. 65,000), and VP-3 (mol.wt. 60,000-62,000). All three polypeptides probably are derived from a common sequence during a late maturation step, as was shown for minute virus of mice (MVM) [31]. A number of other proteins ranging in size from 10,000 to 56,000 also are frequently detected in the gels. However, they seem to be nonessential for virus infection.

An important finding for rapid classification is the demonstration of a common FA polypeptide antigen in the genus *Adeno-associated virus*, which is shared by all AAVs [8].

Recent data suggest that the hepatitis A virus of man is not likely to be a parvovirus [21]. New candidate viruses which seem to have typical parvovirus properties, however, have been isolated and characterized. They include a parvovirus from rabbits [14], the Aleutian mink disease virus [23, 27] and an AAV from equines [7]. In addition, there are four new densovirus isolates from different *Lepidoptera* and *Orthoptera* species [11, 15-17].

References included in the report by *Bachmann et al.* [1] are not repeated in this review. For further information the reader is referred to the monograph by *Siegl* [28].

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1.1 Family: Parvoviridae

Taxonomic status: Family with three genera: *Parvovirus*, *Adeno-associated virus*, and *Demonavirus*.

Relationship with other groups: Unrelated to other families.

2 The virion

2.1 Chemical composition

2.1.1 Nucleic acid

DNA

Single-stranded

Linear

Number of pieces: Probably one.

Sedimentation coefficient: 15-18S (alkaline conditions).

Molecular weight: $1.5-2.2 \times 10^6$

Percentage weight of virion: 19-32%.

Base composition (%):

Genus *Parvovirus*: G, 19.5-22.8; A, 22-26.8; C, 21.4-22.9; T, 29-33.4. GC value: 40.9-45.2.

Hairpin structure both at the 5' and the 3' end of the otherwise single-stranded molecule (about 99% unique single-strand encapsidated) [3].

Genus *Adeno-associated virus*: Minus strand: G, 26.3; A, 20.5; C, 26.3; T, 26.5. GC value: 53.0. Plus strand: G, 26; C, 26.5; A, 25.2; T, 21.7. GC value: 53.1.

Genus *Densovirus*: GC value: 37-40. Minus and plus strands separately encapsidated [14].

Nearest neighbor analysis: Results from three species within the genus *Parvovirus* are available and are very similar (table I).

Homology studies: Hybridization experiments *in vitro* showed 60-70% homology among AAV types 1-4. Digestion of H-1, H-3 and Lu III

Table I. Nearest neighbor analysis

Sequence	MVM DNA	RV DNA	H-1 DNA
ApA	123 91	94 84	93 76
CnA	75 78	72 79	80 80
GnA	67 34	69 44	65 44
CnT	67 69	75 72	82 69
GnT	47 75	53 74	47 82
GnC	51 38	59 39	60 46
TnA	62	61	55
AnT	59	57	49
CnG	15	20	18
GnC	48	49	54

RF DNA with restriction endonucleases suggests that between H-1 and H-3 there is 95% homology, between H-1 and Lu III only about 80% [25]. The two densovirus viruses (Galleria and Junonia) show 87% homology in their DNA [12].

Infectivity has been shown for AAV-1 DNA.

Other features: AAV DNA and densovirus DNA contain single plus and minus self-complementary strands in separate virions which hybridize *in vitro* to form a double strand. About 1 % of MVM particles contain minus strands which, upon extraction, re-anneal with complementary plus strand to give double-stranded DNA [3,14].

2.1.2 Proteins

Percentage weight of protein: 63-81%.

Number of polypeptides: Three. Usually three polypeptides can be demonstrated in mature parvovirus and AAV virions, which probably are all derived from a common sequence : VP-1, VP-2 and VP-3. VP-3, the smallest polypeptide, is derived

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2.3 2.3.1 2.3.1.1 2.3.1.2

2.3.2

2.3.3

2.3.3.1

2.3.3.2

2.4

2.4.1

2.4.2

2.4.3

2.4.4

3 3.1

2.2 2.2.1

from VP-2 during the maturation process [5,21,31,32]. With members of the genus *Parvovirus*, VP-2 can be converted *in vitro* to VP-3 with proteases. Densoviruses seem to have four structural poly-peptides[12,34].

2.1.2.3

Molecular weights of polypeptides: Genus *Parvovirus*

	2.2.2		
	VP-1	VP-2	VP-3
Rat virus	81,00	64,00	60,000
H-1	86.00	66.00	62.000
MVM	83 00	64 00	61 000
Lu III	85.00	66.00	62.000
Bovine			
parvovir	86,00	77,00	67,000

2.2.4.5

Comparable data for all viruses. A variation of about 10% must be accounted for in the techniques used. All parvoviruses contain one major polypeptide (about 80% of the total protein). In RV, H-1, MVM, Lu III, and bovine parvovirus-1, VP-3 is the major polypeptide. Genus *Adeno-associated virus*: AAV types 1-3 contain VP-1, 87,000; VP-2, 73,000; and VP-3, 62,000m. Genus *Densovirus*: VP-1, 98,000; VP-2, 72,000; VP-3, 57,000; and VP-4, 46,000 [13,34].

2.1.2.4

2.1.2.5

2.1.3

2.1.4

Number of protein subunits: 62-72 molecules per virion. Enzymes: Probably lacking. Lipids: None. Carbohydrates: None.

² Since the major protein is found in the various species in different fractions, it should be indicated by addition of an 'm', e. g. VP-3m.

Physicochemical properties Density: Density of mature virions 1.39-1.42 g/cm³ in CsCl; particles with densities > 1.42 g/cm³ are probably 'dense' precursor particles; those banding between 1.34 and 1.38 g/cm³ most likely are defective particles [5, 19, 24, 28]. Empty particles have a density of 1.31 g/cm³. Sedimentation coefficient: 110-122S Weight in daltons: 5.5×10^6 to 6.2×10^6

Stability of infectivity pH: Stable between pH 3 and 9. Heat: Stable at 56° for 60 min (extremely stable at high temperatures). Lipid solvents: Stable. Radiation: Inactivation by UV light (single-hit kinetics). Other agents: Inactivated by formalin, β -propiolactone, hydroxylamine (single-hit phenomenon) and oxidizing agents. Structure Nucleocapsid Symmetry: Icosahedral. Diameter and number of capsomers in icosahedral form: 3-4 nm; probably 32.

Envelope: None. Cores

Dimensions: About 14-17 nm. Composition: Data not available. Morphology Overall shape: Cubic. Dimensions: 18-26 nm. Surface projections: None known. Special features in thin sections: None known. Replication

Site of accumulation of viral proteins 3 Nucleus.

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Nonstructural proteins: Two non-structural polypeptides (mol.wts. 24,900 and 15,800) were demonstrated during replication of AAV type 2 in KB cells [4].

Mode of nucleic acid replication

General account: Parental DNA is converted to linear duplex replicative forms (RF). One possible course is that RF DNA undergoes semiconservative replication at first. Following the burst of synthesis of viral proteins, the synthesis becomes asymmetric, favoring the production of single-stranded DNA and conserving the complementary strand in the RF. However, other modes of replication strategies are possible.

Effect of inhibitors: Any inhibitor of host cell DNA initiation or synthesis should inhibit parvovirus replication. Halogenated deoxyuridines, as well as cytosine arabinoside, actinomycin D, and α -amanitin, inhibit replication.

Site of maturation: Nucleus.

Other features:

Members of the genus *Parvovirus* require one or more cellular functions generated during the S or G2 phase of cell division, very probably during late S phase. One or more components of the cell DNA synthesizing apparatus probably required for the replication of viral DNA.

Members of the genus *Adeno-associated virus* require helper virus functions for measurable replication. Adenovirus coinfection leads to formation of new infectious virus. Co-infection with herpesviruses permits

formation of immunofluorescent antigens and infectious DNA.

4 Cooperative interactions

Recombination: No data available.

Multiplicity reactivation: No data available.

Phenotypic mixing: There is some indication of phenotypic mixing between H-1, H-3 and Lu III viruses [28].

Other interactions: No data available.

5 Host range

Genus *Parvovirus*: Cat, cattle, dog, goose, mink, mouse, pig, rabbit, rat. Genus *Adeno-associatedvirus*: Cattle, chicken, dog, horse, man, monkey. Genus *Densovirus*: Insects – Diptera, Lepidoptera, Orthoptera.

Experimental

In vivo: In homologous or closely related hosts. Rodent viruses and Lu III agent also replicate in Syrian hamsters [30].

In vitro:

Genus *Parvovirus*: Cell cultures of homologous or closely related original host. Some rodent viruses also grow in cells of human origin. Adaptation to other cell cultures (hetero-logous) may be possible under certain conditions (e.g., PPV to human cell lines).

Genus *Adeno-associated virus*: With adenovirus as helper in a variety of different cell species.

Genus *Densovirus*: Cell cultures of homologous and related hosts. Cyto-pathic effects in L cells.

6 Pathogenicity

6.1 Association with diseases:

Genus *Parvovirus*: Acute nonbacter-

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ial gastroenteritis in man. Hemor-rhagic encephalopathy in newborn rats. Acute hepatitis in goslings. Aleutian disease in mink [22]. Feline panleukopenia virus produces a sometimes fatal acute enteritis with leukopenia in adult cats, cerebellar hypoplasia with ataxia and death in 7 the fetus. The porcine virus induces fetal death and reproduction failures 8 [10, 18]. Bovine parvovirus-1 pro- 8.1 duces enteritis in calves. Rodent viruses and Lu III virus induce a mostly fatal disease in newborn rats and hamsters when inoculated experimentally. Cerebellar hypoplasia and dwarfism develop with low virus doses.

Genus *Adeno-associated virus*: None known.8.2

Genus *Densovirus*: Densonucleoses 8.3 fatal for several insect species. 9

6.2 Tissue tropism: 9.1

Genus *Parvovirus*: All fast-replicat ing tissues, especially the vascular epithelium, the external germinal layer of the cerebellum, the intestinal epithelium, the odontoblastic tissues, 9.2 the hepatic parenchyma, the sub-ependymal plate of the brain, fetal tissues, and tumors.

Genus *Adeno-associated virus*: All adenovirus-infected tissues.

Genus *Densovirus*: Multiply in most

of the tissues of larvae, nymphs and 9.3 adults.

6.3 Cytopathology: 9.4

Genus *Parvovirus*: Preference for actively dividing cell cultures. Granulation, rounding and eventually lysis

of cells. Nuclear inclusion bodies, margination of chromatin, nucleoli

always present, but slightly swollen. Genus *Adeno-associated virus*: Not detectable.

Genus *Densovirus*: Hypertrophy of the nucleus with accumulation of virions to form dense, voluminous, intranuclear masses. Geographic distribution: Worldwide among susceptible hosts.

Transmission

True vertical passage by ova is indicated for the goose parvovirus [6]. Transplacental transmissions have been detected for a number of species within the genus *Parvovirus*. The data on Lu III virus [30], Aleutian disease of mink [20] and the porcine parvo-viruses [10, 18] have been confirmed.

Horizontal: Yes. Vectors: Mechanically possible. Antigenic properties Number of distinct antigenic molecules in virion: The polypeptides of the virion are immunologically distinguishable; they are, however, anti-genically related.

Antigens involved in virus neutralization : No data available. AAV and bovine parvovirus polypeptide sera do not show neutralization or react with whole virion using hemagglutination inhibition, complement fixation or immune electrophoresis. Number of distinct nonstructural antigens : No data available. Specificity of different antigens: Genus *Parvovirus*: Hemagglutinating, complement-fixing and neutralizing antigens are type-specific without cross-reaction, except for minor cross-reactions between RV, H-1 and

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MVM in the fluorescent antibody (FA) test.

All AAVs share a common antigen, as can be demonstrated by FA [8].

9.5 Antigenic properties used for classification:

Demonstration of hemagglutinin, complement-fixing, immunofluorescing and neutralizing antigens.

10 Classification

Definition of family Parvoviridae: Contain linear, single-stranded DNA. The molecular weight of the DNA is between 1.5 and 2.2×10^6 . The virions are isometric, nonenveloped particles, 18-26 nm in diameter with icosahedral symmetry; probably 32 capsomers, 3-4 nm in diameter.

Buoyant density in CsCl gradients is between 1.39 and 1.42 g/cm^3 for the infectious particle. The viruses are ether- and chloroform-resistant, heat- and acid-stable (56° , pH 3 for 60min). The virion contains a hemagglutinin in many types, which has different activities for a variety of red blood cells. The virus multiplies in the nucleus, and the replication is dependent upon certain functions of the host cell or helper functions provided by other viruses.

Definition of genera:

Genus *Parvovirus*: Viruses replicate without a helper in susceptible cell cultures, mature virus particles only contain plus strands of DNA. Type species: Parvovirus r-1 (rat virus).

Other species belonging to genus *Parvovirus*:

Rat virus

H-1 virus

Minute virus of mice

Porcine parvovirus

Bovine parvovirus

Feline parvovirus [9]

Goose parvovirus

TVX

Lul[]

RT[11] Possible members:

Gastroenteritis virus of man (Norwalk and Norwalk-like agents)

Aleutian mink disease virus (differing polypeptide pattern) [23]

Lapine parvovirus [14]

Canine parvovirus Genus *Adeno-associated virus*: Replication dependent upon helper viruses (adenoviruses) for complete virion production, but infectious AAV, DNA and immunofluorescent antigens are made in the presence of herpesviruses. Mature virus particles contain either plus or minus strands of DNA that are complementary and come together *in vitro* to form a double strand. All AAVs share a common antigen demonstrable by fluorescent antibody techniques. Type species: AAV type 1 Other species belonging to genus *Adeno-associated virus*:

AAV type 1

AAV type 2

AAV type 3

AAV type 4

Bovine AAV (X7)

Canine AAV [33]

Avian AAV (AAAV) Possible member:

Equine AAV [7] Genus *Densovirus*: Arthropod parvo-viruses. Replicate without helper vi-

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rus; however, the single strands of the DNA are either plus or minus, are complementary, and come together *in vitro* to form double strands. Type species: *Densovirus* of *Galleria*. Other species belonging to genus *Densovirus*:

Densovirus of *Galleria* *Densovirus* of *junonia* Possible members: *Densovirus* of *Aedes*

Densovirus of *Acheta* [16] *Densovirus* of *Sibine* [15] *Densovirus* of *Bombyx* [11] *Densovirus* of *Diatraea* [17]

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