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The Genome Structure of a New Chicken Virus Identifies It as a Parvovirus

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SUMMARY

The nucleic acid of chicken parvovirus-like particles showed sensitivity to DNase and S1 nuclease treatment and resistance to digestion with RNase. Viral DNA readily served as a template for self-primed conversion *in vitro* into a double-stranded form of about 5200 base pairs. There was no evidence for encapsidation of strands of opposite polarities. These findings confirm the taxonomic classification of chicken parvovirus-like particles as fowl parvovirus type 1 within the *Parvovirus* genus of the Parvoviridae.

It has been recently reported that small hexagonal viral particles of 19 to 24 nm in diameter and with a buoyant density in CsCl of 1·43 g/ml are present in the intestines of chickens showing the symptoms of stunting disease (Kisary *et al.*, 1984). When day-old broiler chickens are infected per os with purified virus, significant growth retardation accompanied by poor feathering and bone disorders develop at the age of 4 weeks (Kisary, 1985*a*). In addition, specific nuclear fluorescence has been seen in the epithelial cells in the small intestine of chickens infected experimentally at 1 day of age (Kisary, 1985*b*). Altogether, these observations strongly suggest that the newly recognized small viral particles of chicken origin belong to the *Parvovirus* genus of the family Parvoviridae.

In the present paper, we confirm this classification by showing that the genome of chicken parvovirus-like particles displays properties characteristic of parvovirus DNA. We found that the virions contain a single genome which served as a template *in vitro* for second-strand synthesis in the presence of DNA polymerase and appropriate precursors without prior denaturation or addition of an exogenous primer. The converted double-stranded form was resistant to RNase and S1 nuclease treatment but sensitive to digestion with DNase. After conversion it migrated in agarose gel as a linear double-stranded DNA of about 5200 base pairs. The *in vitro* conversion could be prevented by treatment of extracted nucleic acid with DNase and S1 nuclease but not with RNase treatment. The size and single-strandedness of the virion DNA and the fact that it contains a primer for its conversion into a full-length double-stranded form unambiguously classify the newly identified chicken virus as a member of the *Parvovirus* genus in the Parvoviridae.

The chicken parvovirus-like strain designated ABU was propagated in broiler chickens and purified as described elsewhere (Kisary, 1985*a*). The virions (Fig. 1) were disrupted with SDS and proteinase K treatment essentially according to the method used by Summers *et al.* (1983). The nucleic acid was extracted with phenol, precipitated by ethanol and finally dissolved in 10 mM-Tris–HCl, 1 mM-EDTA (pH 7·5). The DNA of minute virus of mice (MVM), a well-characterized member of the Parvoviridae (Ward & Tattersall, 1982) was used in some of these experiments as a reference.

The genome of parvoviruses consists of a single-stranded DNA molecule containing a 3′ end palindrome which serves as a natural primer for the synthesis of a complementary strand in

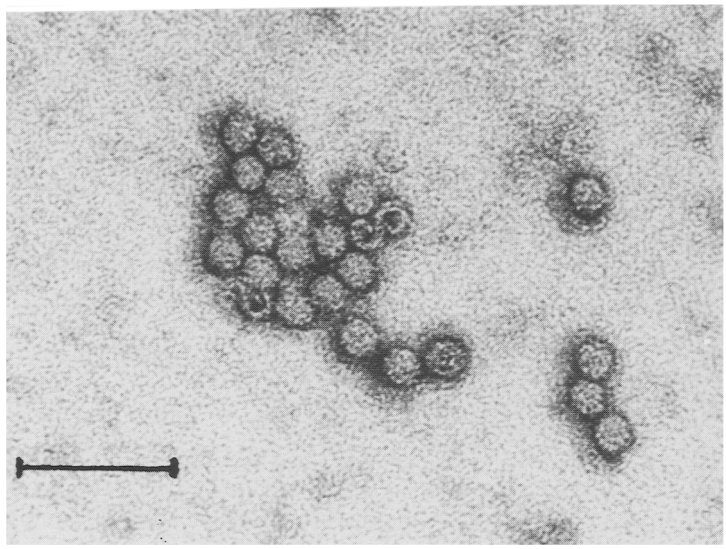

Fig. 1. Electron micrograph of chicken parvovirus-like particles purified in CsCl from the intestines of experimentally infected broiler chickens. Bar marker represents 100 nm.

infected cells (Berns & Hauswirth, 1978). Consequently, parvovirus ssDNA can be converted *in vitro* into a double-stranded form by incubation with a DNA polymerase and appropriate precursors (Bourguignon *et al.*, 1976). Thus, we first tested whether ABU virus is a parvovirus by determining the ability of virion nucleic acid to serve as a primer-template for *in vitro* polymerization of deoxyribonucleotides. This prediction was verified, as shown in Fig. 2.

Incubation of extracted viral nucleic acid with DNA polymerase I (Klenow fragment) from *Escherichia coli* and radioactive precursors resulted in the labelling of a unique class of molecules of about 5200 base pairs after agarose gel electrophoresis. This reaction was completely prevented by digesting ABU viral nucleic acid with DNase but not with RNase prior to polymerization (lanes 2 and 3). In full agreement with this result, the nucleic acid labelled *in vitro* was resistant to RNase treatment but was completely digested with DNase (lanes 4 and 5). To prove that a full-length incorporation of precursor nucleotides took place under the *in vitro* conditions, we stopped the reaction after various intervals. As can be seen on lanes 6 to 13, a continuous increase of the molecular weight occurred up to 160 min. After 1 or 2 min of incubation a faint band of about 5000 bases was detectable which became more intensely labelled as it increased in size, and resulted in a band of about 5200 base pairs at the end of the reaction. It should be noted that bands of lower electrophoretic mobility were also seen, the nature of which is unclear. Similar observations were reported with the genome of Kilham rat virus (Salzman *et al.*, 1978) and human parvovirus (Clewley, 1984). Most probably, an inappropriate proportion of precursors in the reaction is responsible for the aggregation of partially converted nucleic acid molecules. The efficiency of the conversion of extracted ABU DNA *in vitro* was not enhanced by prior denaturation treatment (data not shown). This result suggests that most virion DNA molecules have the same polarity and therefore are not susceptible to reannealing after extraction (Rose *et al.*, 1969). Selective encapsidation of one of the complementary strands is characteristic of most members of the Parvovirus genus, but there are exceptions (Muller & Siegl, 1983; Summers *et al.*, 1983).

In the next experiment we further tested the strandedness of ABU viral nucleic acid in comparison with MVM DNA. As illustrated in Fig. 3, treatment of unconverted viral nucleic acid with single strand-specific S1 nuclease (Ando, 1966) prevented the formation of double-stranded DNA molecules (lane 2), whereas the same enzyme had no effect on the double-stranded form of ABU viral DNA synthesized *in vitro* (lane 4). The same results were obtained with MVM DNA (lanes 5, 6 and 7) as we expected (Bourguignon *et al.*, 1976).

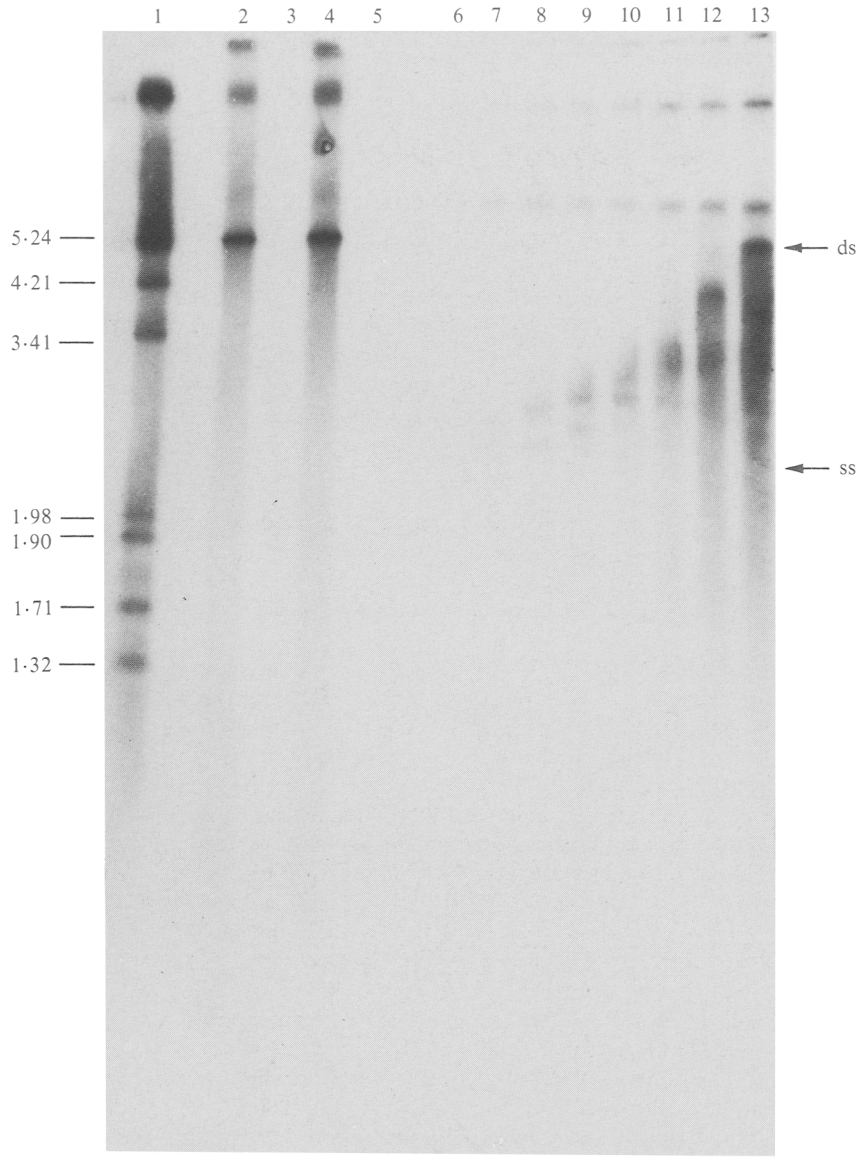

Fig. 2. Electrophoretic analysis of converted chicken parvovirus DNA in 1·4% non-denaturing agarose gel according to Sharp *et al.* (1973). Viral DNA was extracted from virions and treated with 20 μg/ml bovine pancreatic RNase (lane 2) or 20 μg/ml pancreatic DNase (lane 3) according to Clewley (1984) followed by precipitation with ethanol. The dissolved nucleic acid was incubated in 50 mM-Tris–HCl pH 7·5, 6 mM-$MgCl_2$ with 10 units DNA polymerase I (Klenow fragment) in the presence of 20 μM each of dGTP, dTTP, dCTP and 10 μCi [^{32}P]dATP (sp. act. 3000 Ci/mmol) for 40 min at 25 °C. After that, unlabelled dATP was added to a concentration of 20 μM followed by incubation at 25 °C for an additional 20 min. The reaction was terminated by adding EDTA to 10 mM. The same conditions were employed again, but the nucleic acid was converted prior to RNase (lane 4) or DNase (lane 5) treatment. In a parallel experiment viral nucleic acid was incubated for conversion into a double-stranded form as above but using four radioactive dNTPs (20 μCi of each, with specific activities of 3000 Ci/mmol) without adding unlabelled ones. Aliquots were taken after 1 (lane 6), 2 (lane 7), 5 (lane 8), 10 (lane 9), 20 (lane 10), 40 (lane 11), 80 (lane 12) and 160 (lane 13) min of incubation and the reaction was immediately stopped by adding EDTA to a final concentration of 15 mM. As markers, lambda phage DNA fragments generated by restriction enzymes *Eco*RI and *Hin*dIII and end-labelled according to Maniatis *et al.* (1982) were used (lane 1); their sizes are indicated in kilobase pairs. After electrophoresis the gel was dried and autoradiographed with an intensifying screen at −70 °C.

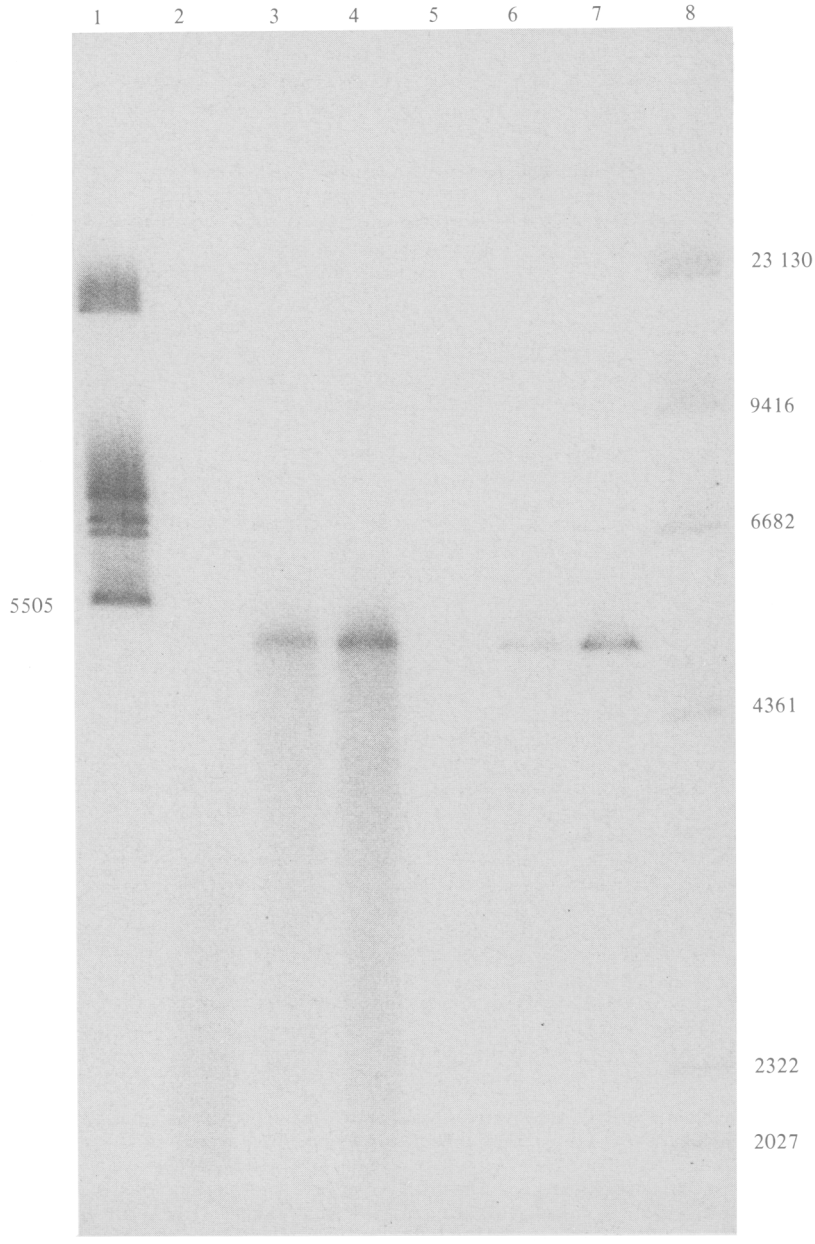

Fig. 3. S1 nuclease treatment of ABU and MVM viral DNAs performed according to Maniatis *et al.* (1982). The DNAs were converted as given in Fig. 2 for lanes 2 to 5. The samples were electrophoresed in a 1% non-denaturating agarose gel. Lane 2, ABU DNA converted after treatment with S1 nuclease; lane 3, ABU DNA converted without prior treatment with S1 nuclease; lane 4, ABU DNA treated with S1 nuclease after conversion; lanes 5, 6 and 7, MVM DNA processed as ABU DNA in lanes 2, 3 and 4. Lanes 1 and 8 show lambda phage DNA fragments generated by restriction enzymes *Bam*HI and *Hin*dIII, respectively, and used as size markers (base pairs).

We conclude from these data that the genome of chicken parvovirus-like particles is a predominantly single-stranded DNA molecule of about 5200 nucleotides and that a unique strand of DNA is preferentially packaged into the virions. Together with the presence of a covalently linked primer for the synthesis of a complementary strand, these properties are characteristic of the DNA of members of the *Parvovirus* genus of the Parvoviridae (Matthews, 1982). Therefore, the morphology, size and buoyant density of the virions as well as their genomic structure classify this newly recognized virus as fowl parvovirus type 1.

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