The Structural Proteins of Chick Embryo Lethal Orphan Virus (Fowl Adenovirus Type 1)

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SUMMARY

Chick embryo lethal orphan (CELO) virus (fowl adenovirus type 1) contains at least 14 structural proteins with polypeptide molecular weights ranging from 100K to about 6K. A nomenclature of the CELO virion polypeptides is presented and the molar proportion of each polypeptide has been estimated. The CELO virus pentons were specifically released from the virion by dialysis against borate-based calcium-magnesium saline. The penton base (polypeptide III, mol. wt. 92K) and two fibres were separated, characterized and their polypeptides were correlated with their morphological positions in the virion. Peptide mapping suggested that the long fibre (polypeptide IV, mol. wt. 65K), and the short fibre (polypeptide VII, mol. wt. 44.5K) were not related in their primary sequences and are therefore probably encoded by separate genes. The time course of synthesis of the CELO virion polypeptides indicated that, like their mammalian adenovirus counterparts, they are synthesized late (after viral DNA replication).

INTRODUCTION

Avian adenoviruses are only distantly related to human adenoviruses. Chick embryo lethal orphan (CELO) virus (fowl adenovirus type 1) has received more detailed study than other avian adenoviruses, being the type species. Although the DNA structure and DNA replication of CELO virus have been shown to share basic similarities with those of human adenoviruses (Younghusband & Bellett, 1971; Bellett & Younghusband, 1972), there is little information available concerning CELO virus-specific proteins. Avian adenovirus structural proteins do not cross-react immunologically with any human adenovirus protein, so that avian adenoviruses are classified as a separate genus, Aviadenovirus (Norrby et al., 1976).

Laver et al. (1971) identified the CELO virus hexon and at least four other virion proteins. Yasue & Ishibashi (1977) detected 11 polypeptides from virions, 14 polypeptides from virion-like light particles and 23 CELO virus-induced polypeptides from infected chicken kidney cells (CKC). In this paper, the CELO virus structural polypeptides from the virions, and from CELO virus-infected chicken embryo kidney (CEK) cells are further investigated by SDS-PAGE and by immunoprecipitation. A nomenclature is introduced for the structural proteins of CELO virus. The pentons of CELO virus are released by dialysis against borate-based calcium-magnesium saline, a method not described previously. The penton base and fibres are further dissociated, separated, and characterized by sucrose gradient sedimentation, SDS-PAGE, electron microscopy and peptide mapping. Experimental data presented in this paper and studies on CELO virus DNA structure (Younghusband & Bellett, 1971; Aleström et al., 1982a, b), CELO virus hexon and terminal protein (Laver et al., 1971; Li et al., 1983), CELO virus core proteins and CELO virus single-stranded DNA-binding proteins (Li et al., 1984) are discussed in comparison with those of human adenovirus in terms of protein structure and function and adenovirus evolution.

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METHODS

Viruses. The Phelps strain of CELO virus and human adenovirus type 5 (Ad5) were grown and purified as described previously (Laver et al., 1971; Younghusband & Bellett, 1971).

Immunoprecipitation of cell extracts was done as described previously (Braithwaite et al., 1983).

Gel electrophoresis. SDS–PAGE of viral proteins (Laemmli, 1970) was done on 10 to 20% gradient gels. Gels were stained with Coomassie Brilliant Blue using the formaldehyde fixation–staining method of Steck et al. (1980).

Electron microscopy. Grids were covered with parlodion and then carbon. Samples were applied directly to the grids, and excess was removed with filter paper. Samples were negatively stained with 2% silicotungstate, pH 7.0, and examined in a Philips 301S electron microscope.

Peptide mapping. $^{125}$I–labelled polypeptides were digested with chymotrypsin (50 µg/ml in 50 mM-ammonium bicarbonate, 16 h at 37°C), dried, dissolved in 10 µl 50 mM-ammonium bicarbonate, and the peptides mapped by electrophoresis followed by chromatography on thin-layer cellulose plates as described previously (Elder et al., 1977; Li et al., 1983).

RESULTS

Molecular weights and nomenclature of the CELO virion polypeptides

The molecular weights of the CELO virion polypeptides were estimated by SDS–PAGE. Ad5 virion polypeptides were used as molecular weight markers. The most recent estimates for these are: Ad5 hexon (polypeptide II), 103K (Bahr-Lindstrom et al., 1982); penton base (III), 85K; IIIa, 66K; fibre (IV), 64K; minor core protein (V), 48-5K; VI, 24K; major core protein (VII), 18-5K (Wadell et al., 1980); VIII, 13K; IX, 12K (Everitt et al., 1973; values for Ad2).

CELO virus particles freshly grown in embryonated eggs were purified and analysed on a linear 10 to 20% acrylamide gradient gel, which was used to cover a mol. wt. range from > 100K to < 10K. The polypeptides were visualized by Coomassie Brilliant Blue staining (Fig. 1a). $^{[35]}$S–Methionine–labelled CELO virus particles freshly grown from CEK cells were also purified and analysed on a 10% gel to show the larger polypeptides more clearly (Fig. 1b). The two gels agree very well and the results revealed that the polypeptide electrophoresis pattern of CELO virus is distinct from that of human adenoviruses. CELO virus particles contained at least 14 polypeptides (including the terminal protein; Li et al., 1983) with molecular weights ranging from 100K to about 6K. A nomenclature is introduced here for CELO virus structural proteins. The hexon polypeptide (Laver et al., 1971) is represented by roman numeral II in accordance with the convention used for human adenoviruses. Other polypeptides are not directly comparable to those of human adenoviruses. Table 1 summarizes the molecular weights and nomenclature of CELO virus structural polypeptides. The estimated numbers of molecules of each polypeptide per virion and the suggested morphological positions for these polypeptides are also included (see below).

There are some differences in the total number and the molecular weights of CELO virion polypeptides described in this paper and those reported by Yasue & Ishibashi (1977). The discrepancy in the total number of CELO virion polypeptides lies mainly in the low molecular weight range. Yasue & Ishibashi reported three virion polypeptides with molecular weights under 20K, whereas we detected six virion polypeptides in this range. This can be attributed partly to loss of small basic polypeptides in the conventional methanol and acetic acid destaining solution they used. The differences in the molecular weights of some of the CELO virion polypeptides seem to have arisen from the values of some of the molecular weight markers used by Yasue & Ishibashi. The commonly accepted molecular weight for Ad2 polypeptide III is 85K (Anderson et al., 1973; Tooze, 1980). The accurate molecular weight of β-galactosidase polypeptide is 116K (Fowler & Zabin, 1978), and that of Ad2 hexon polypeptide, 108K (Jornvall et al., 1981). The values Yasue & Ishibashi (1977) used for these two marker polypeptides were 135K and 120K respectively, as the more accurate sequence-based values were published later. For these reasons, we believe that the total number and the molecular weights of the CELO virion polypeptides, as well as the estimated numbers for each polypeptide in the virion (see below) listed in Table 1 are probably more accurate.
Estimation of the molar proportions of polypeptides in the CELO virion

The molar proportions of polypeptides in the CELO virion were first estimated by Yasue & Ishibashi (1977). But the following considerations justify a re-examination of the estimate by these authors. (i) We detected more protein species, and high relative concentrations of the virion proteins in the low mol. wt. range (6K to 20K). This is probably because the formaldehyde fixation–staining method we used retained small and basic protein molecules better (Steck et al., 1980). In addition, freshly grown virus was used in our experiments, so that storage reduction of
Table 1. *Polypeptide composition of CELO virus*

<table>
<thead>
<tr>
<th>Polypeptide</th>
<th>Molecular weight</th>
<th>Estimated number per virion*</th>
<th>Suggested morphological position†</th>
</tr>
</thead>
<tbody>
<tr>
<td>II</td>
<td>100K</td>
<td>720 (assumed)</td>
<td>Hexon</td>
</tr>
<tr>
<td>III</td>
<td>92K</td>
<td>36 (39)</td>
<td>Penton base</td>
</tr>
<tr>
<td>IV</td>
<td>65K</td>
<td>96 (100)</td>
<td>Long fibre</td>
</tr>
<tr>
<td>V</td>
<td>62K</td>
<td>66 (66)</td>
<td>-</td>
</tr>
<tr>
<td>VI</td>
<td>55K</td>
<td>96 (100)</td>
<td>-</td>
</tr>
<tr>
<td>VII</td>
<td>44.5K</td>
<td>96 (100)</td>
<td>Short fibre</td>
</tr>
<tr>
<td>VIII‡</td>
<td>30K</td>
<td>720 (723)</td>
<td>Core protein 1</td>
</tr>
<tr>
<td>IX</td>
<td>20K</td>
<td>740 (743)</td>
<td>Core protein 2</td>
</tr>
<tr>
<td>XI</td>
<td>12K</td>
<td>1330 (1326)</td>
<td>Core protein 3</td>
</tr>
<tr>
<td>XII</td>
<td>9.5K†</td>
<td>910 (905)</td>
<td>Covalently linked to DNA</td>
</tr>
<tr>
<td>XIII§</td>
<td>9K†</td>
<td>1910 (1908)</td>
<td>-</td>
</tr>
<tr>
<td>XIV</td>
<td>6K†</td>
<td>960 (960)</td>
<td>-</td>
</tr>
</tbody>
</table>

* Those in parentheses are calculated numbers.
† Hexon was identified by Laver *et al.* (1971). The identification of the three core proteins will be reported elsewhere (P. Li, A. J. D. Bellett & C. R. Parish, J. Virol., in press).
‡ Detected as faint, but distinct and well-defined bands. Estimation of numbers of those two polypeptides per virion was not done.
§ XII and XIII form one broad band on the gel. These are recognized as two separated bands, as XII is clearly associated with the core, while XIII is not (P. Li, A. J. D. Bellett & C. R. Parish, J. Virol., in press).
† Estimates based on extrapolation.
¶ Terminal protein, see Li *et al.* (1983).

The most distinct feature of the CELO virus polypeptide composition is that the virion contains more core polypeptides than do the human adenoviruses. The reason for this may be that the core proteins are needed to neutralize the negative charge of the CELO virus DNA, which is 30% longer than human adenovirus DNA (Younghusband & Bellett, 1972).

**Identification of the pentons of CELO virus**

In the icosahedral architecture of adenoviruses, pentons hold a morphologically prominent position as the vertex capsomeres. The penton of human adenoviruses is composed of a penton base and a fibre. In fowl adenoviruses, each penton base carries two fibres, one of which appears longer than the other in the electron microscope (Laver *et al.*, 1971; Gelderblom & Maichle-Lauppe, 1982).

As surface components of the virus, the pentons of intact virus particles can theoretically be probed by enzymic radioiodination (Marchalonis, 1969). This surface labelling approach was unsatisfactory when dealing with purified CELO virus. Most of the virion proteins were labelled, to different extents, even when solid-state lactoperoxidase–glucose oxidase beads
Fig. 2. Release of pentons from CELO virus. [35S]Methionine-labelled CELO virus particles were dialysed against two changes of calcium–magnesium saline at 4 °C for 16 h. Precipitation was observed within the first 4 h. The supernatant (a) and the precipitate (b) were prepared by low-speed centrifugation and analysed by electrophoresis on a 10 to 20% SDS-polyacrylamide gel. Protein bands were detected by fluorography. (c) A mixture of (a) and (b). Polypeptides III, IV and VII were specifically released, and subsequently shown to be the penton components.

(Enzymobead, Bio-Rad Laboratories) were used as the iodination agent (results not shown). CELO virus particles, at least after purification in CsCl, could not be specifically labelled only in the surface components.

An alternative approach is to release specifically the pentons. The reported methods of dialysis against water (Laver et al., 1971) or maleate buffer pH 6·0 to 6·8 (Prage et al., 1970) used with human and avian adenoviruses, did not produce satisfactory results. Among other methods we have tried, dialysis against calcium–magnesium saline (0·145 M-NaCl, 19 mM-H3BO3, 0·14 mM-Na2B4O7, 0·83 mM-MgCl2, 0·25 mM-CaCl2, pH 7·3) gave the clearest results.

[35S]Methionine-labelled CELO virus particles in about 0·5 ml CsCl were dialysed against two changes of 200 ml of calcium–magnesium saline at 4 °C for 16 h. Precipitation was observed. The supernatant (it will be referred to as Ca2+/Mg2+ supernatant in the rest of this paper) and the precipitate were separated by centrifugation at 1000 r.p.m. for 10 min. The supernatant, the precipitate and a mixture of the two were analysed by electrophoresis on a 10 to 20% SDS–polyacrylamide gel, and protein bands were detected by fluorography. As shown in Fig. 2, the Ca2+/Mg2+ supernatant contained four well-defined bands that co-migrated on the gel with CELO virion polypeptides II, III, IV and VII. The precipitate contained greatly reduced amounts of polypeptides III, IV and VII. A Coomassie Brilliant Blue-stained gel of unlabelled CELO virus treated in the same way gave the same results (not shown).

Polypeptide II of CELO virus was previously identified as hexon protein by gel electrophoresis and by electron microscopy (Laver et al., 1971). From the morphology of adenovirus and the relative quantities of the four polypeptides in the Ca2+/Mg2+ supernatant and in the precipitate, it is reasonable to consider that a small number of the total hexon capsomeres were released together with the vertex penton capsomeres, which are composed of...
polypeptides III, IV and VII. Confirmation of this conclusion by electron microscopic examination and further analysis of the \( \text{Ca}^{2+}/\text{Mg}^{2+} \) supernatant by sucrose gradient sedimentation under dissociating conditions are described in the following sections.

**Separation of the penton components**

The \( \text{Ca}^{2+}/\text{Mg}^{2+} \) supernatant of unlabelled CELO virus was iodinated with \(^{125}\text{I}\) using the Iodogen method (Fraker & Speck, 1978). The iodinated \( \text{Ca}^{2+}/\text{Mg}^{2+} \) supernatant was first analysed by electrophoresis on a 10\% SDS–polyacrylamide gel and protein bands were detected by autoradiography. As shown in Fig. 3(b), polypeptides II, III, IV and VII were labelled. A

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**Fig. 3. Separation of penton components.** (a) \(^{125}\text{I}\)-labelled CELO virus \( \text{Ca}^{2+}/\text{Mg}^{2+} \) supernatant was incubated with 10\% pyridine, 1\% Sarkosyl and 0.5 M-2-mercaptoethanol and subsequently analysed by sucrose gradient sedimentation as described in the text. Six protein peaks were designated A to F. (b) \(^{125}\text{I}\)-labelled CELO virus \( \text{Ca}^{2+}/\text{Mg}^{2+} \) supernatant analysed by electrophoresis on a 10\% polyacrylamide gel followed by autoradiography. Three tracks of the same supernatant were included in order to get enough material for peptide mapping. (c) A portion of each of the peaks marked in (a) was analysed by electrophoresis on a 10\% SDS–polyacrylamide gel, followed by autoradiography. Lanes A to F correspond to peaks A to F in (a).
very fast-moving band, and a faint and less-defined band migrating slightly faster than polypeptide V, were also detected on the gel. The low mol. wt. material (bottom band, Fig. 3b), which was not detected in a \[^{35}S\]methionine-labelled Ca\(^{2+}/\text{Mg}^{2+}\) supernatant, will be addressed later in this section. Apart from this, the results of \(^{125}\text{I}\) labelling in vitro agreed with those from \[^{35}S\]methionine labelling in vivo.

The \(^{125}\text{I}\)-labelled Ca\(^{2+}/\text{Mg}^{2+}\) supernatant was next analysed by sucrose gradient sedimentation. It is known that pyridine disrupts the adenovirus capsid without denaturing capsomeres to polypeptides (Prage et al., 1970). Pyridine was therefore used, together with a mild detergent and 2-mercaptoethanol, to dissociate the pentons further. The \(^{125}\text{I}\)-labelled Ca\(^{2+}/\text{Mg}^{2+}\) supernatant was adjusted to 10% pyridine, 1% Sarkosyl and 0.5 M 2-mercaptoethanol. The mixture, in a volume of 150 \(\mu\)l, was incubated at 37 °C for 1 h and then loaded onto a pre-formed 4-7 ml 10 to 40% linear sucrose gradient cushioned by 0.2 ml of 60% sucrose and containing 8% pyridine, 0.5% Sarkosyl, 0.05 M 2-mercaptoethanol, 20 mM-Tris-HCl pH 7.5, and 1 mM-EDTA. Centrifugation was at 64000 r.p.m. for 16 h at 5 °C using a Beckman SW65 rotor, after which fractions of 0.1 ml were collected from the bottom. The \(^{125}\text{I}\) radioactivity of each fraction was determined using a Beckman Series 9000 automatic gamma counter.

The sedimentation profile is shown in Fig. 3(a). There were six recognizable peaks, designated A to F. A portion of each peak fraction (A to F) was then analysed by electrophoresis on a 10% SDS–polyacrylamide gel and protein bands were detected by autoradiography (Fig. 3c). The results showed that peak A in Fig. 3(a) contained polypeptides III and VII, free of polypeptides II and IV. Peaks B and C contained mainly polypeptide II (hexon) as well as a considerable amount of polypeptides III and VII. Peak C contained less of polypeptides III and VII than peak B. Peaks D and E were composed of polypeptide IV slightly contaminated with an undefined protein. Peak F represented the low mol. wt. band detected in Fig. 3(b). This band, because of its low mol. wt., could not be adequately characterized in the 10% gel. Material from peak F was therefore analysed further on a 10 to 20% gradient SDS–polyacrylamide gel with stained CELO virion polypeptides as markers. It was found that the material from peak F migrated on the gel slightly faster than CELO virion polypeptide XIV, but slower than the bromophenol blue dye. Thus, it could be a virion protein of very low mol. wt. (less than 6K) and rich in tyrosine, or an iodinated peptide resulting from degradation of virion proteins. In any case, its very small size suggests that it cannot be the penton base or one of the fibres. This is confirmed by electron microscopy in the following section.

**Electron microscopic examination of the penton components**

Material from peaks A, C, E and F shown in Fig. 3(a), as well as the whole Ca\(^{2+}/\text{Mg}^{2+}\) supernatant of CELO virus, were further characterized by electron microscopy. Fractions 10 to 12 (peak A), 20 to 22 (peak C), 38 to 40 (peak E) and 46 to 48 (peak F) were separately pooled and dialysed against 5 mM-Tris-HCl pH 7.5, overnight. Samples were negatively stained and examined in the electron microscope. Fig. 4(a, b) shows that the supernatant contains mainly the released penton capsomeres. Arrows in (b) indicate the two-fibre structure of CELO virus pentons (Laver et al., 1971). Fig. 4(c) and (d) are electron micrographs of the material from peak C shown in Fig. 3(a). As expected, the hexon capsomere is the main structure (arrows), but some penton base can also be seen. Fig. 4(e, f) shows that penton base–short fibre complexes and free penton base are the main structures in peak A (arrows). Fibres were seen in peak E (not shown). No defined structure was seen in peak F.

It is clear that the penton of CELO virus is composed of polypeptides III, IV and VII. Polypeptides III and VII are probably the penton base and short fibre respectively. The possibility that polypeptide VII is the penton base and polypeptide III the short fibre is unlikely, because the densitometer tracing of CELO virion polypeptides showed that polypeptide VII is present in a smaller amount than polypeptide III in the virion, and the volume of the short fibre is smaller than that of penton base as shown in the electron micrographs in Fig. 4 and those published previously (Laver et al., 1971). By inference, it can be concluded that the long fibre is composed of polypeptide IV, although this needs further, direct confirmation by electron microscopy.
Fig. 4. Electron microscopy of pentons and penton components. (a, b) Whole Ca\(^{2+}\)/Mg\(^{2+}\) supernatant of CELO virus; intact penton is the main structure. (c, d) Material from peak C shown in Fig. 3(a); hexon is the main structure. (e, f) Material from peak A shown in Fig. 3(a); penton base and base–short fibre complex are the main structures. Bar markers represent 100 nm.
As the penton components must be present in multiples of 12, as required by the virion morphology, the estimated number of each of the three penton polypeptides listed in Table 1 suggests that the penton base is probably a trimer of polypeptide III, the short fibre is a trimer of polypeptide VII, and the long fibre is an octamer of polypeptide IV. These tentative conclusions, however, need to be confirmed by accurate measurement of the molecular weights of undenatured penton base and the two fibres.

Peptide analysis of the penton components

One interesting question concerning the molecular biology of fowl adenovirus is whether the two fibres are encoded by two separate genes. This ultimately requires physical mapping of the gene(s). A preliminary approach is to compare the peptide maps of the two fibres.

Gel slices containing 125I-labelled polypeptides III (the penton base), IV (the long fibre) and VII (the short fibre) were cut from the gel shown in Fig. 3(b) according to a duplicate autoradiogram. Proteins in these gel slices were digested with chymotrypsin, and 125I-labelled peptides of the three penton components were mapped as described in Methods. Fig. 5 shows the results. As expected, the peptide map of the penton base (Fig. 5a) exhibited no similarity to maps of either fibre (Fig. 5b, c). Interestingly, there was no detectable similarity between the maps of the long fibre (Fig. 5b) and the short fibre (Fig. 5c).

The two fibres of CELO virus showed a number of differences. Firstly, they differ in length. This has been previously documented (Laver et al., 1971; Gelderblom & Maichle-Lauppe, 1982) and can be readily recognized in the electron micrographs shown in Fig. 4. Secondly, the two fibres showed different strengths of binding to the penton base. In the presence of pyridine, mild detergent and 2-mercaptoethanol, the long fibre is dissociated from the penton base, whereas the short fibre is still partly associated. The broad distribution of the penton base and the short fibre in the sucrose gradient shown in Fig. 3 indicated that there was only partial dissociation under these experimental conditions. Thirdly, the two fibres are composed of different numbers of polypeptides with different molecular weights. Finally, 125I-labelled peptide mapping revealed that the two fibres are not related in their primary sequences. This indicates that the two fibres are probably encoded by different genes. These differences are interesting, because the two fibres are similar in shape and in morphological position in the virion. Presumably, the two fibres are also similar in their functional interaction with cells.

Time course of the synthesis of CELO virus structural proteins in CEK cells

Bellett & Younghusband (1972) reported that in CELO virus-infected CEK cells viral DNA synthesis was detectable at 16 h post-infection and infectious progeny virus increased rapidly from 20 h post-infection. The time of synthesis of the structural proteins was determined relative to these events.

Confluent monolayers of CEK cells were mock-infected, or infected by CELO virus at a multiplicity of 25 i.u./cell. At intervals, medium was removed from one dish, the cells were washed with phosphate-buffered saline and then incubated with 1 ml serum-free, methionine-free Eagle's medium containing 50 μCi [35S]methionine for 1 h. Cells were then harvested and cell extracts were prepared and immunoprecipitated using antiserum against CELO virus particles, followed by SDS–PAGE on a 10% acrylamide gel. CELO virus structural proteins were first detectable between 16 h and 20 h (Fig. 6). This agrees well with the results of replication studies (Bellett & Younghusband, 1972) and implies that the structural proteins of CELO virus, like human adenoviruses, are synthesized during the late phase in gene expression. Two polypeptides with molecular weights of 58K and 26.5K, which do not co-migrate with any virion polypeptides, were also precipitated by virion antiserum at late times (solid triangles in Fig. 6). These are most likely intracellular precursors to virion polypeptides.

DISCUSSION

Data presented in this paper demonstrate that CELO virus contains at least 14 structural proteins with polypeptide molecular weights ranging from 100K to about 6K. The polypeptide of the CELO virus hexon was previously identified (Laver et al., 1971). The polypeptides
Fig. 5. $^{125}$I-labelled peptide maps of CELO virus penton components. Gel slices containing $^{125}$I-labelled polypeptides III, IV and VII were cut from the gel shown in Fig. 3(b). The proteins were digested with chymotrypsin and mapped as described in Methods. (a) Penton base (polypeptide III); (b) long fibre (polypeptide IV); (c) short fibre (polypeptide VII).
CELO virus structural proteins

Fig. 6. CELO virus-induced structural polypeptides in CEK cells. Cells were infected at 25 i.u./cell and labelled with $[^{35}S]$methionine for 1 h at the time indicated by the arabic numerals (hours after infection, indicating the mid-point of 1 h labelling). Cell extracts were prepared and analysed by immunoprecipitation using CELO virion antiserum followed by electrophoresis on a 10% SDS-polyacrylamide gel. Protein bands were detected by fluorography. C, $[^{35}S]$methionine-labelled CELO virion polypeptide markers; M, mock-infected cell extract. Triangles indicate the possible precursors to some virion polypeptides.

M 3 6 9 12 16 20 24 C

II

III

IV/V

VI

VII

IX

The main structural differences between avian and human adenoviruses are that the penton base of avian adenovirus carries two fibres (Laver et al., 1971) which are probably encoded by corresponding to the CELO virus penton base and the two fibres are identified and characterized in this paper. The CELO virus genome-linked terminal protein (Robinson et al., 1973; Robinson & Bellett, 1974; Li et al., 1983), core proteins, and single-stranded DNA-binding proteins have also been characterized (Li et al., 1984).

Avian adenoviruses share with human adenoviruses basic structural similarities at the following levels: (i) the virion of both avian and human adenoviruses is an icosahedron of about 70 nm diam. and probably consists of the same numbers of morphologically similar hexon and penton base capsomeres (Table 1); (ii) inside the icosahedral capsid, both avian and human adenoviruses contain a roughly spherical core (P. Li, A. J. D. Bellett & C. R. Parish, J. Virol., in press) which is composed of viral DNA and virus-coded core proteins (Table 1); (iii) the DNA of both avian and human adenoviruses is covalently linked to a terminal protein at the 5' end of each strand (Robinson et al., 1973; Robinson & Bellett, 1974; Li et al., 1983); (iv) the DNA genome of both avian and human adenoviruses is a linear molecule with inverted terminal repetitions; a sequence within the repetition is conserved between avian and human adenoviruses (Aleström et al., 1982b) (v) there are regions of DNA sequence homology between avian and human adenoviruses (Aleström et al., 1982a), and possibly conformational similarities between the terminal proteins (Li et al., 1983).

Apart from these structural similarities, avian and human adenoviruses also share similar basic DNA replication mechanisms (Bellett & Younghusband, 1972), similar early and late phases of gene expression and at least one similar non-structural protein, the single-stranded DNA-binding protein (Li et al., 1984).
two different genes (Fig. 5); details of the capsid assembly of avian adenovirus may be different as revealed by the lack of 'group of nine' hexons (P. Li, A. J. D. Bellett & C. R. Parish, J. Virol., in press); the avian adenovirus core contains three prominent core proteins (Table 1), and is possibly a slightly more compact structure; avian adenovirus structural and non-structural proteins are not related to their human counterparts serologically.

Apart from these differences, the lytic cycle of avian adenovirus is considerably longer as revealed by studies on DNA replication (Bellett & Younghusband, 1972), and the time courses of synthesis of structural proteins (Fig. 6) and of the single-stranded DNA-binding protein (Li et al., 1984).

The overall similarities between avian and human adenoviruses are important. They imply that equivalent viral proteins and equivalent sub-virion structures play similar roles in the structure and lytic cycle of adenoviruses as a family, although no serological relatedness or significant sequence homology can be detected for most of the polypeptides. Sequence homology is a very stringent criterion in evolutionary comparisons of viruses. Subgroup D human adenoviruses, for example, have a mere 4% DNA sequence homology with subgroup A human adenoviruses (Green et al., 1979). Thus, the regions of DNA sequence homology between avian and human adenoviruses (Aleström et al., 1982a) and the possible conformational similarities in the terminal proteins of avian and human adenoviruses (Li et al., 1983) suggest that avian and human adenoviruses may have evolved from a common ancestor. From sequence analysis of the genomes of human adenoviruses, it was suggested that the regions of DNA sequence least affected by evolution were those involved in recognition by proteins (Sambrook et al., 1980), which are usually short sequences. The hexanucleotide AAATAA, a probable protein recognition sequence involved in the initiation of adenovirus DNA replication (Stillman & Tamanoi, 1982; van Bergen et al., 1983) is perfectly conserved in the inverted terminal repetition of all adenoviruses, including avian adenovirus (Aleström et al., 1982b).

The most prominent structural difference between avian and human adenoviruses is the two-fibre nature of the penton of most avian adenoviruses (Laver et al., 1971; Gelderblom & Maichle-Lauppe, 1982; Fig. 4) which apparently involves two separate fibre genes (Fig. 5). In both human and avian adenoviruses, the fibre is probably the most variable viral component in size and antigenicity (Wadell et al., 1980; Gelderblom & Maichle-Lauppe, 1982). At present, we do not know the location of the avian adenovirus fibre gene(s). But in human adenovirus the fibre gene is the 3'-terminal member of the major late transcriptional unit and therefore in a position to be affected by changes in the long interval between its promoter and the coding sequence. The different splicing combinations between the tripartite leader sequences, the i, x, y, z leader sequences and the coding sequence, may serve as a reservoir of genetic information that might be tapped during evolution (Sambrook et al., 1980).

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REFERENCES


CELO virus structural proteins


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