VIROLOGY
MONOGRAPHS

14

L. PHILIPSON, U. PETTERSSON, AND U. LINDBERG

# MOLECULAR BIOLOGY OF ADENOVIRUSES

SPRINGER-VERLAG
WIEN NEW YORK

# MOLECULAR BIOLOGY OF ADENOVIRUSES

BY

L. PHILIPSON, U. PETTERSSON,
AND U. LINDBERG

1975
SPRINGER-VERLAG
WIEN NEW YORK

Printed in Austria by R. Spies & Co., A-1050 Wien

Library of Congress Cataloging in Publication Data. Philipson, Lennart, 1929–. Molecular biology of adenoviruses. (Virology monographs; 14) Bibliography: p. 1. Adenoviruses. 2. Molecular biology. I. Pettersson, Ulf, 1942–, joint author. II. Lindberg, Uno, 1939–, joint author. III. Title. IV. Series. [DNLM: 1. Adenovirus. 2. Adenovirus infections. 3. Molecular biology. W1 V183 v. 14 / QW160. A2 P555m] QR360. V52. no. 14 [QR396]. 576'. 64'08s [616. 01'94]. 75-6658

ISBN 3-211-81284-9 Springer-Verlag Wien-New York
ISBN 0-387-81284-9 Springer-Verlag New York-Wien

# Molecular Biology of Adenoviruses

By

**L. Philipson, U. Pettersson,** and **U. Lindberg**

Department of Microbiology, The Wallenberg Laboratory
Uppsala University, Uppsala, Sweden

With 20 Figures

## Table of Contents

# Molecular Biology of Adenoviruses

By

**L. Philipson, U. Pettersson,** and **U. Lindberg**

Department of Microbiology, The Wallenberg Laboratory
Uppsala University, Uppsala, Sweden

With 20 Figures

## Table of Contents

# I. Introduction

In his biography "Arrow in the Blue" the author Arthur Koestler suggests ironically that the fate of an individual may be predicted by examining the content of the newspapers at birth. Adenoviruses were discovered in 1953 (ROWE *et al.*, 1953; HILLEMAN and WERNER, 1954). At this time the Salk poliomyelitis vaccine was developed (SALK *et al.*, 1954) and in the same year the discovery of the double helical structure of DNA (WATSON and CRICK, 1953) and the plaque assay for one animal virus (DULBECCO and VOGT, 1953) was announced. Thus, this new group of viruses was born with great hopes for progress in molecular biology and for the control of animal virus infections. In the short interval between 1953 and 1956 the adenoviruses were discovered, methods for laboratory diagnosis and serotyping were established, the epidemiology was clarified and a highly effective vaccine was developed and approved (for a review see HILLEMAN, 1966). Succeeding years showed, however, that the vaccines were contaminated with the oncogenic SV40 virus and that the adenoviruses themselves were tumorigenic.

Since the discovery of adenoviruses animal virology was developed into a quantitative science offering explanation for viral functions at the molecular level. Precise biochemical tools to characterize the genome and its transcription products as well as the structural proteins of these viruses are now available. Many of the pathways involved in control of the host cell and the viral genome during lytic infection with adenoviruses as well as the properties of the structural and the non-structural polypeptides synthesized during early and late productive infection are currently investigated. The dissection of the virion itself and its component parts has been rewarding since most of the structural proteins are soluble under non-denaturing conditions and available in sufficient quantities for structural, immunological and functional studies.

The name adenovirus was coined in 1956 (ENDERS *et al.*, 1956) to designate this group of viruses isolated from the respiratory tracts of man and other animals. The adenoviruses are non-enveloped icosahedral viruses with genomes which are larger than those of papovaviruses, but less overwhelming than those of the T-even bacteriophages, the pox and the herpes viruses. The adenovirus chromosome has a molecular weight of about $23 \times 10^6$ and could thus code for 25—50 average sized polypeptides. Today more than 80 different adenovirus serotypes have been isolated from a variety of animal species (WILNER, 1969; WADELL, 1970) and all except the avian and some bovine adenoviruses seem to share one antigenic determinant. It was recognized early that the different adenovirus serotypes possess a high degree of individuality with regard to a number of attributes such as cytopathology, host range, hemagglutination properties, neutralization kinetics and oncogenicity. The human adenoviruses have been divided into subgroups on the basis of their ability to agglutinate rhesus monkey and rat erythrocytes (ROSEN, 1960) and on the basis of their oncogenicity (HUEBNER, 1967). Each subgroup contains several serotypes characterized by type specific antigens present in their capsids, as revealed by hemagglutination inhibition or neutralization tests.

In humans, adenoviruses cause primarily mild respiratory diseases, but conjunctivitis, myocarditis, enteritis, and lymph node involvement have also been reported (SOHIER *et al.*, 1965). The epidemic character of the respiratory illness caused by some serotypes among military recruits has emphasized the need for the production of multivalent vaccines (HILLEMAN, 1966).

One biological aspect of the adenoviruses, which has received much attention during the last ten years, is their oncogenicity. In 1962 TRENTIN *et al.* (1962), discovered that human adenovirus type 12 (ad12) induces tumors in newborn hamsters and HUEBNER *et al.* (1962), reported that ad18 also has this property. Subsequently, a series of reports have confirmed the oncogenicity of human ad 12 and also described the oncogenicity of several other human and non-human adenoviruses for hamsters and other rodents (HUEBNER *et al.*, 1963; RABSON *et al.*, 1964a; YABE *et al.*, 1964; PEREIRA *et al.*, 1965; HUEBNER *et al.*, 1965; GIRARDI *et al.*, 1964; HULL *et al.*, 1965; DARBYSHIRE, 1966; SARMA *et al.*, 1965). The tumors usually have the characteristics of undifferentiated sarcomas, although malignant lymphomas have occasionally been observed (LARSON *et al.*, 1965).

Transformation of *in vitro* cultured cells by an adenovirus was first demonstrated by MCBRIDE and WIENER (1964), who showed that the oncogenic human ad12 could transform newborn hamster kidney cells. Subsequently, the transformation of rat embryo fibroblasts by the same adenovirus type was reported by FREEMAN *et al.*, (1967a) Since then, several adenovirus types have been shown to cause *in vitro* transformation of rodent cells (FREEMAN *et al.*, 1967b and c; VAN DER NOORDA, 1968a and b; MCALLISTER *et al.*, 1969a and b; RIGGS and LENETTE, 1967; CASTO, 1969), and the tumorigenic properties of the *in vitro* transformed cells have been established. The biochemical studies of cells transformed by oncogenic viruses *in vitro* have progressed rapidly during recent years and much of our basic knowledge concerning growth regulation and tumorigenesis has been derived from studies on such *in vitro* systems (for a review see PONTÉN, 1971).

In addition to providing an insight about the changes involved in transformation of normally growing cells to tumor cells, the adenoviruses have become important in providing a convenient model system for studies of regulation of gene expression in eukaryotic cells. In the transformed cells, viral genes are apparently integrated in the host cell genome (GREEN, 1970), and results of studies on the expression of these viral genes would thus reflect the mechanisms used by the host cell in expressing its own genes. Recent developments in the analysis of adenoviruses reproduction indicate that *productively infected cells* also could serve as a convenient model system for studies on the synthesis of macromolecules in the eukaryotic cell. Several observations suggest that also under these conditions the adenoviruses mimic the host cell in its gene expression. After the infection the virus is rapidly established in the nucleus of the cell, where its DNA is transcribed and replicated (GREEN *et al.*, 1970). The viral DNA is transcribed into large RNA molecules (GREEN *et al.*, 1970; WALL *et al.*, 1972; MCGUIRE *et al.*, 1972), which by posttranscriptional events are cleaved into smaller mRNA molecules found associated with polyribosomes (GREEN *et al.*, 1970; PHILIPSON *et al.*, 1971; LINDBERG *et al.*, 1972). Adenovirus mRNA is polyadenylated at the 3′terminus in the same way as cell mRNA. Messenger ribonucleoprotein particles

(mRNP) isolated from polysomes during virus infection contain five major labeled polypeptides, four of which are identical in size to those found in the mRNP from uninfected cells (LINDBERG and SUNDQUIST, 1974).

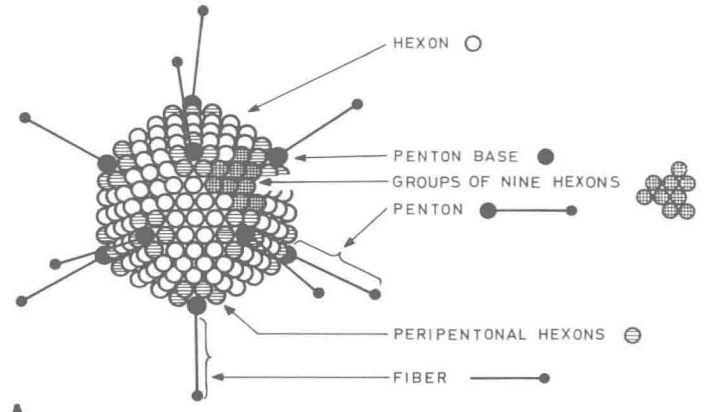
Conditional lethal mutants of adenoviruses have recently been isolated. A large number of temperature-sensitive mutants of serotype 5 and 12 and CELO virus have already been described (WILLIAMS *et al.*, 1971; LUNDHOLM and DOERFLER, 1971; ENSINGER and GINSBERG, 1971; SHIROKI *et al.*, 1972; ISHIBASHI, 1970, 1971). More than 50 ad5 ts-mutants distributed between at least 17 complementation groups have been isolated and are currently undergoing biochemical and genetic analysis (WILLIAMS and USTACELEBI, 1971; RUSSELL *et al.*, 1972a; WILKIE *et al.*, 1973; WILLIAMS, personal communication). A genetic map of the virus chromosome based on two-factor crosses has already appeared (WILLIAMS *et al.*, 1974).

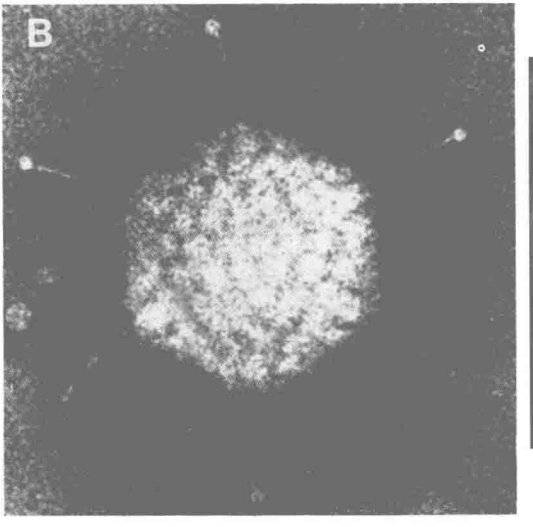
During recent years new physical-chemical techniques have been developed which allow mapping of loci for specific functions on viral chromosomes. Thus, studies of adenovirus DNA utilizing liquid phase hybridization, restriction enzyme fragments and electron microscopic mapping are in progress. Adenovirus DNA of several types has been cleaved by restriction endonucleases into unique sets of fragments, which can be separated (PETTERSSON *et al.*, 1973; MULDER *et al.*, 1974). The DNA strands of at least three adenovirus types have been separated (LANDGRAF-LEURS and GREEN, 1971; PATCH *et al.*, 1972), and in the case of ad 2 strand separation can be achieved also with isolated DNA fragments generated by restriction endonucleases (TIBBETTS and PETTERSSON, 1974; SHARP *et al.*, 1974a). Hopefully, with the aid of these techniques we will soon learn how the expression of the viral genome is controlled both in productive infection and also in cells transformed by adenoviruses.

This rapidly expanding field has been reviewed frequently during recent years with emphasis on different aspects of adenovirus research. Relevant reviews of a general character are those of GREEN (1966), SCHLESINGER (1969), GINSBERG (1969), GREEN (1970), TOOZE (1973) and PHILIPSON and LINDBERG (1974). The characteristics of the structural proteins (PHILIPSON and PETTERSSON, 1973) and the biological properties of the capsid components (NORRBY, 1968, 1971) have also been reviewed. Reviews on the oncogenic and transforming capacity of adenoviruses have also appeared (GREEN, 1970; HOMBURGER, 1973; TOOZE, 1973).

## II. The Architecture of the Virion

The adenoviruses are nonenveloped viruses with a diameter of 65—80 nm. The capsid is composed of 252 capsomeres arranged into an icosahedron with 20 triangular facets and 12 vertices as originally shown in the classical electron micrographs of HORNE *et al.* (1959). Figure 1A shows a schematic drawing of the virion with the major components of the capsid indicated. Figures 1B and C show electron micrographs which reveal the vertex region and the triangular facets of the virion, respectively. 240 out of the 252 capsomeres have six neighbours and are called *hexons* (GINSBERG *et al.*, 1966) whereas the 12 capsomeres at the vertices



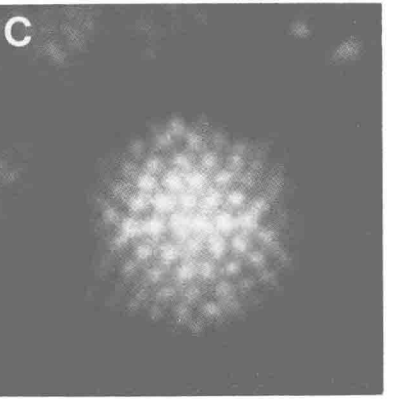




Fig. 1. Structure of the adenovirus capsid.
A. Schematic drawing showing the icosahedral outline of the adenovirus capsid and the location of various components. Reprinted from Philipson and Pettersson (1973)
B. Electronmicrograph showing the ad 5 virion contrasted with sodium silicotungstate (Valentine and Pereira, 1965). Note the antenna-like fiber protruding from each vertex. This figure was kindly provided by Dr. Pereira
C. Electron micrograph of ad 5 virus contrasted with sodium silicotungstate showing the regular icosahedral symmetry of adenoviruses (Horne *et al.*, 1959)

have five neighbours and are called *pentons* (GINSBERG *et al.*, 1966). Each penton unit consists of a penton base anchored in the capsid and a projection (VALENTINE and PEREIRA, 1965; NORRBY, 1966). The latter consists of a rod like portion with a knob attached at the distal end and is referred to as the *fiber*. Two types of hexons may be defined: (i) Those located in juxtaposition with the pentons, *peripentonal hexons*, which differ topologically from the remaining hexons since they have the penton base as one of their six neighbours; (ii) Those 180 which form the triangular facets and the edges of the icosahedron. The latter hexons may be released from the virions in aggregates of nine hexons (ninemers) which form a defined structure with 3-fold rotational symmetry as shown in Figure 1 A (SMITH *et al.*, 1965; RUSSELL *et al.*, 1967 b; MAIZEL *et al.*, 1968 b; PRAGE *et al.*, 1970; CROWTHER and FRANKLIN, 1972; ISHIBASHI and MAIZEL, 1974 a). Inside the capsid there is a core, which contains the DNA and additional proteins as originally revealed by electron microscopy of thin sections of particles stained with uranyl acetate. This core has a diameter of 40—45 nm and its structure is destroyed by both DNase and trypsin (EPSTEIN 1959; EPSTEIN *et al.*, 1960; BERNHARD *et al.*, 1961). Isolated core structures have also been studied in the electron microscope by negative staining after disintegration of the virus with heat (RUSSELL *et al.*, 1967 b) or formamide (STASNY *et al.*, 1968); a mesh-like unorganized morphology has usually been observed (RUSSELL *et al.*, 1967 b; STASNY *et al.*, 1968; LAVER *et al.*, 1968). The core contains all the DNA and about 20 per cent of the total protein of the virion. Acid extraction (PRAGE *et al.*, 1968, 1970; RUSSELL *et al.*, 1968), treatment with 6 M lithium iodide (NEURATH *et al.*, 1970 b) or SDS (MAIZEL *et al.*, 1968 a) releases all the core proteins from the DNA. ROBINSON *et al.* (1973) have reported that a circular protein-DNA complex is released after degradation of virus particles with guanidine-HCl. Subsequent phenol extraction linearized the DNA, which suggests that inside the virus particle the two termini of the adenovirus DNA are linked to each other with a protein.

## III. The Composition of the Virion

The ad 2 virion which has been studied in detail, has an estimated particle weight of $175 \times 10^6$ daltons (GREEN *et al.*, 1967 a). It contains 13 per cent DNA corresponding to $23 \times 10^6$ daltons of DNA (GREEN *et al.*, 1967 b; VAN DER EB *et al.*, 1969) and the adenovirion is composed only of DNA and protein. Unlike most other viruses the adenoviruses have capsid units which are soluble in non-denaturing solvents. This has greatly facilitated the purification and characterization of the virus components and made it possible to develop methods for sequential disintegration of the virion (MAIZEL *et al.*, 1968 b; LAVER *et al.*, 1969; PRAGE *et al.*, 1970; EVERITT *et al.*, 1973). The pentons alone or together with peripentonal hexons can be selectively removed by dialysis against distilled water (LAVER *et al.*, 1969) or Tris-maleate buffer pH 6.0—6.5 (PRAGE *et al.*, 1970). The release of pentons is accompanied by the release of additional antigens probably located in the peripentonal region. After treatment of the virus particle with SDS, urea or pyridine (SMITH *et al.*, 1965; MAIZEL *et al.*, 1968 b; PRAGE *et al.*, 1970) the capsid is disrupted and the hexons from the triangular facets are released as groups of nine hexons or ninemers (Fig. 1 A).

The polypeptide composition of adenoviruses has been studied by SDS polyacrylamide electrophoresis. MAIZEL *et al.* (1968a, b) showed that the virion of adenovirus types 2, 7 and 12 contains a minimum of 9 polypeptides which range

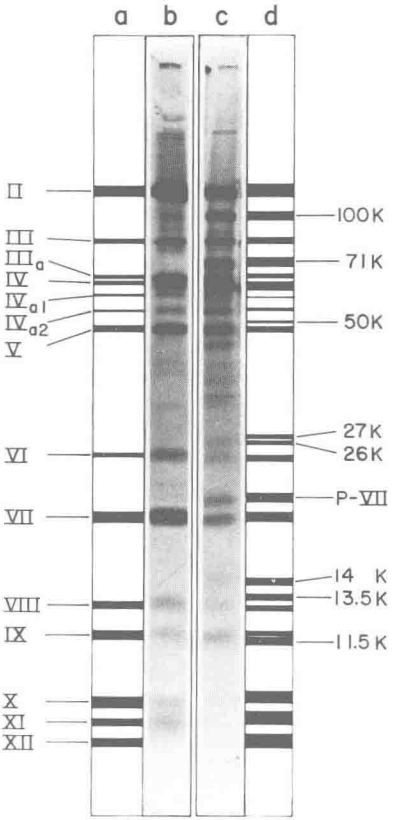



Fig. 2. Virion and virus-induced polypeptides in adenovirus infected cells. SDS-polyacrylamide gel autoradiograms of $^{35}$S-methionine-labeled purified virus (b) and an extract of infected cells (c). The gel contained 15 per cent acrylamide and 0.08 per cent bisacrylamide. The extract was obtained from cells which were labeled for 1 hour with $^{35}$S-methionine 18 hours after infection and then chased for 12 hours with 30 μg/ml of unlabeled methionine. Frames *a* and *d* are drawings which indicate all virion polypeptides (*a*) and all 22 virus-induced polypeptides (*d*). The nomenclature of EVERITT *et al.* (1973) and ANDERSON *et al.* (1973) is used to designate polypeptide bands. The non-structural polypeptides in the cell extract are designated by their molecular weight $\times 10^{-3}$ (K). P VII has been shown to be a precursor of virion polypeptide VII (ANDERSON *et al.*, 1973). The virus induced polypeptide 27 K appears to be the precursor to virion polypeptide VI (ANDERSON *et al.*, 1973; ÖBERG *et al.*, 1975), and polypeptide 26 K is probably the precursor to virion polypeptide VIII (ÖBERG *et al.*, 1975). Reprinted by permission from ANDERSON *et al.*, (1973)

in size from 7.500 to 120,000 daltons. With a high resolving SDS polyacrylamide gel technique as many as 15 polypeptides have been resolved from ad2 virions (MAIZEL, 1971; EVERITT *et al.*, 1973; ANDERSON *et al.*, 1973) as shown in Figure 2. Some polypeptides may be generated by proteolytic degradation (PEREIRA and

SKEHEL, 1971), and some may be precursor polypeptides (ISHIBASHI and MAIZEL, 1974a) but 8 of the polypeptides have so far been shown to be antigenically distinct and reside in different structures after sequential degradation of the virion (EVERITT *et al.*, 1973; EVERITT and PHILIPSON, 1974). Five of the polypeptides are integral parts of the capsomeres or the core. Thus, polypeptide II is the only

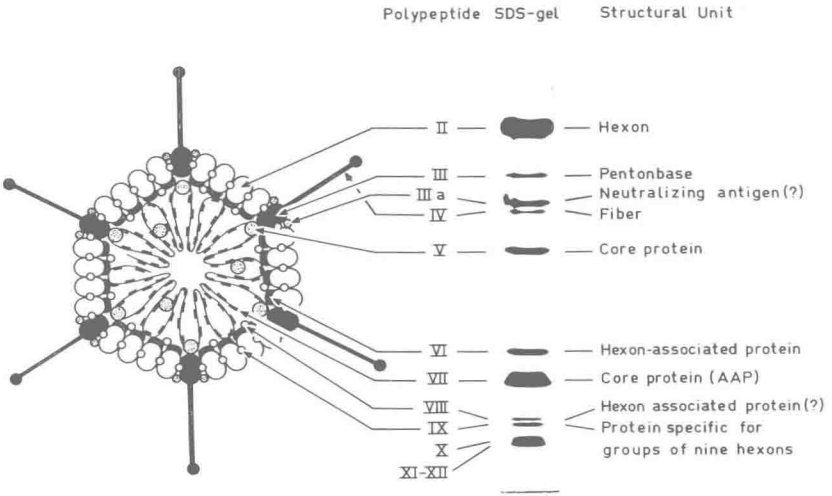



Fig. 3. A tentative model of the location of different proteins in the ad 2 virion. The core protein V may be located inside at the vertices since it is partially released with the peripentonal region (EVERITT *et al.*, 1973). It has been estimated that protein VII may neutralize about 50 per cent of all phosphate residues in the DNA (PRAGE and PETTERSSON, 1971). The molar ratio between polypeptide VI and the hexon polypeptide is about 2 and the native protein VI exists as a dimer (EVERITT and PHILIPSON, 1974). Protein VI is not iodinated by lactoperoxidase (EVERITT, personal communication), which suggests that protein VI is located at the inner surface of the hexons. Peripentonal hexons also possess this protein. Protein IX appears to be the cementing substance between hexons from the facets, since it is associated with groups of nine hexons (EVERITT *et al.*, 1973). Protein VIII is also associated with the hexons and may reside at the inner surface of the triangular facets since it is not enzymatically iodinated in intact virions (EVERITT, unpublished). Polypeptide IIIa has been proposed to be located at the peripentonal region. The localization of protein X is unknown. The polypeptide composition of the virion proteins as identified in a stained exponential (10—16 per cent) SDS-polyacrylamide gel are also shown. This figure was kindly provided by Dr. E. Everitt

polypeptide which is detected in purified hexons from infected cells. Polypeptide III resides in the penton base, IV in the fiber and polypeptides V and VII are associated with the core. The remaining polypeptides have been tentatively located in the virion as indicated in Figure 3. Polypeptide VI can be demonstrated in all fractions from disintegrated virus which contain hexons and sediments with hexons in sucrose gradients at low salt concentration irrespective of whether the hexons are obtained after disintegration of virions by freezing and thawing or pyridine treatment (EVERITT *et al.*, 1973). Polypeptide VI is therefore probably

associated with the hexons in the virion. Polypeptide VI cannot be iodinated by lactoperoxidase when intact virions are labeled (unpublished), which may suggest that it is located internal in the capsid as indicated in Figure 3. There appear to be about two molecules of polypeptide VI per molecule of hexon and this polypeptide seems to occur as a dimer in the native state (EVERITT and PHILIPSON, 1974). Polypeptide VIII is recovered in association with hexons after freezing and thawing but not after degradation with pyridine. Thus, it is possible that this polypeptide is associated with hexons in the capsid but that pyridine dissociates the complex (MAIZEL *et al.*, 1968b; EVERITT *et al.*, 1973). Polypeptide IX is present in ninemers of hexons (MAIZEL *et al.*, 1968b; EVERITT *et al.*, 1973) but is not recovered in association with hexons when the capsid is disintegrated into single capsomeres by freezing and thawing. Polypeptide IIIa is released from the virion together with the peripentonal hexons after dialysis against Tris-maleate buffer, pH 6.3 or after pyridine treatment, and may therefore be located in the peripentonal region (EVERITT *et al.*, 1973). The origin of the smallest polypeptide X is not yet established and this band has been resolved into 3 components — X, XI and XII (Fig. 2) (ANDERSON *et al.*, 1973). In addition to the polypeptides discussed above several minor components (polypeptides $IVa_1$, $IVa_2$, Va, Vb, VIa, VIb and VIIIa), which each constitute less than 0.1 per cent of the total mass of protein in the virion, can be observed in preparations of purified virions (EVERITT *et al.*, 1973; ANDERSON *et al.*, 1973; ISHIBASHI and MAIZEL, 1974a; see also Fig. 2). They are present in amounts which would correspond to only a few copies per virus particle and may not represent polypeptides in the mature virion (EVERITT *et al.*, 1973). ISHIBASHI and MAIZEL (1974a) have proposed that purified preparations of adenovirus contain two populations of particles with the same buoyant density. One consists of mature virions and the other of so called young virions. "Young virions" contain five polypeptides (Va, Vb, VIa, VIb, VIIIa), which are absent in mature particles. During virus maturation these five polypeptides are presumed to be cleaved into stable polypeptides. It has been possible to demonstrate polypeptide cleavage *in vitro* after incubation of pulse-labeled virions with extracts from infected cells (ISHIBASHI and MAIZEL, 1974a). Finally, it should be pointed out that we so far lack positive evidence that all polypeptides of mature virions have unique primary structures.

Several reports describe virus-specific antigens of unknown origin from disrupted virions or in extracts from adenovirus infected cells. BERMAN and ROWE (1965) described a "D" antigen in cells infected with ad12. It is possible that this antigen is identical to the free penton bases which later have been shown to be present in cells infected with ad12 (NORRBY and ANKERST, 1969). PEDERSEN and GINSBERG (1967) identified by immunoelectrophoresis an antigen from disintegrated ad5 virions which was shown to be distinct from the capsid antigens. RUSSELL *et al.* (1967a) reported on the presence of another antigen, the P-antigen. This antigen was detected by antisera prepared against extracts of infected cells in which DNA synthesis was inhibited with cytosine arabinoside. It is produced both early and late after infection with ad5 and RUSSELL and KNIGHT (1967) showed that disrupted virions in contrast to intact virus particles reacted with antisera against the P-antigen. This suggests that one of the P-antigen

components is an internal protein of the virion. Since the P-antigen is heat labile and accumulates in the nucleus without requirement for DNA synthesis, it has also been claimed to be related to the adenovirus T-antigen (see section V:G:1; RUSSELL and KNIGHT, 1967; HAYASHI and RUSSELL, 1968). Thus, it seems likely that P-antisera react with several antigens including T-antigen and an internal component of the virion.

# IV. The Adenovirus Genome

## A. Physical-Chemical Properties

The adenovirion contains one linear molecule of duplex DNA which has no single strand breaks. The molecular weight of DNA from different human serotypes has been determined to be 20 to $23 \times 10^6$ (GREEN *et al.*, 1967b; VAN DER EB *et al.*, 1969). The partial denaturation maps of DNA from ad2, ad5 and ad12 are unique (DOERFLER and KLEINSCHMIDT, 1970; DOERFLER *et al.*, 1972; ELLENS *et al.*, 1974) but the maps of the former two viruses are similar. In addition it has been shown that terminal fragments of adenovirus DNA are unique (MURRAY and GREEN, 1973) which together with the partial denaturation patterns show that the adenovirus DNA is not circularly permuted. Digestion of the adenovirus DNA with exonuclease III does not generate circular molecules (GREEN *et al.*, 1967b) indicating that adenovirus DNA lacks terminal redundancies. Instead, the adenovirus DNA has a novel property in that denaturation and renaturation of intact DNA molecules at low concentrations generates single stranded circles (GARON *et al.*, 1972; WOLFSON and DRESSLER, 1972). Since the majority of unit length single strands can be recovered as rings, both strands must be able to form circles. The circles are opened by digestion with exonuclease III which shows that they are maintained by hydrogen bonds. The most likely interpretation of these findings is that each strand of the adenovirus DNA contains inverted terminal repetitions, which form a circle which is held together by a panhandle-like structure. A terminal inverted repetition has so far only been observed in double stranded adenovirus DNA and in the single stranded DNA from adenovirus associated virus (AAV) — a member of the parvovirus group (KOCZOT *et al.*, 1973). The function of this inverted terminal repetition is unclear. Since a protein seems to circularize double stranded adenovirus DNA (ROBINSON *et al.*, 1973) it is conceivable that the identical sequences at the two ends of the viral DNA are recognition sites for this protein.

Usually single stranded DNA is not retained by hydroxylapatite in 0.12 M phosphate at 65° C. However, denatured adenovirus DNA is retained by hydroxylapatite under the same conditions (TIBBETTS *et al.*, 1973). The retention is dependent on the extent of fragmentation of the adenovirus DNA and the fraction retained decreases slowly until the length is reduced to about 10 per cent of the intact DNA. With more extensive degradation the fraction retained decreases drastically. The formation of single strand circles may contribute to the retention of intact single stranded adenovirus DNA by hydroxylapatite, but the described behaviour of the fragmented DNA points at an additional feature of adenovirus DNA. Presumably several short complementary or partially complementary se-

quences are dispersed throughout the single strands of the adenovirus DNA. Figure 4 shows schematically these structures in adenovirus DNA.

The complementary strands of adenovirus DNA have been separated by density gradient centrifugation in the presence of ribocopolymers such as poly (I, C) and poly (U, G) (KUBINSKI and ROSE, 1967; LANDGRAF-LEURS and GREEN, 1971; PATCH *et al.*, 1972), but the separation has proved difficult in practice. Not only do the conditions for the copolymer binding seem to be important but also the quality of the copolymer used. It is the experience of several investigators that many commercially available preparations of polymers are inefficient in

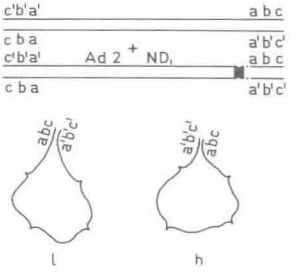



Fig. 4. Schematic drawing of some characteristic structures of ad 2 DNA. The adenovirus DNA contains an inverted terminal repetition (top figure, WOLFSON and DRESSLER, 1972; GARON *et al.*, 1972). The genome of the non-defective adeno-SV 40 hybrid virus, ad $2^{+}ND_1$, which has been used to map several restriction endonuclease fragments, is also shown. About 240,000 daltons of SV 40 DNA is inserted near the right hand end of the hybrid DNA where about $1.3 \times 10^6$ daltons of ad 2 DNA has been deleted (KELLY and LEWIS, 1973, see Table 6 and Fig. 17). The circles of the single stranded adenovirus DNA which are formed because of the inverted terminal repetition are illustrated for both the l- and h-strand at the bottom of the figure. The single strands appear to contain regions with intramolecular complementarity and there appear to be 4—8 such regions per genome (TIBBETTS *et al.*, 1973)

separating the adenovirus strands, but that occasional batches can give reproducible and near complete separation (TIBBETTS *et al.*, 1974). The reason for this variability is not known. Separated adenovirus strands have been used for the characterization of RNA synthesis and processing in productive infection as will be discussed in a separate section V: D.

After controlled shearing of ad2 DNA, double stranded half molecules can be separated by mercury-CsCl gradient centrifugation (KIMES and GREEN, 1970; DOERFLER and KLEINSCHMIDT, 1970). Endonuclease EcoRI (a restriction enzyme from *E. coli* carrying a drug resistant transfer factor RTF-I), cleaves ad2 DNA into six unique double stranded fragments which can be separated by gel electrophoresis (PETTERSSON *et al.*, 1973). They have been designated A-F and their order has been found to be A B F D E C. The ordering of the fragments was achieved by electron microscopic analyses of partially denatured fragments (DELIUS, personal communication) and of heteroduplex molecules formed between EcoRI fragment strands and strands from other adenoviruses (SHARP *et al.*, 1974b). The A fragment which accounts for 59 per cent of the genome