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Leader arrangement in the adenovirus fiber mRNA

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Four oligonucleotides, complementary to the adenovirus 2 (ad2) fiber mRNA were chemically synthesized and used as primers to study the 5′ end of that messenger. The oligonucleotides which were 8, 10, 12, and 14 nucleotides long had a common 3′-terminal sequence, TAC, complementary to the initiating codon of the fiber mRNA. The 5′-^{32}P-labeled oligonucleotides were hybridized to polyadenylated mRNA from ad2-infected cells and the primer was extended, using reverse transcriptase. When the resulting products were analyzed by polyacrylamide gel electrophoresis multiple distinct bands could be identified by autoradiography. The extension products were characterized by hybridization and sequence analysis. The most prominent band contained the well-known tripartite lead which is attached to many different late adenovirus mRNAs. In addition, four differently spliced leaders were characterized which, besides the three segments of the tripartite leader, contain one or two auxilliary segments. A kinetic analysis of the different leaders during lytic adenovirus infection shows no temporal variation.
Key words: synthetic oligonucleotides/reverse transcriptase/sequence analysis/adenovirus fiber mRNA

Introduction

The recent progress in organic synthesis of DNA now makes it possible to construct oligonucleotides with a defined sequence. The combination of organic synthesis of DNA and molecular cloning procedures is a very powerful tool in eukaryotic molecular biology. A recent spectacular achievement in this field is the synthesis of the complete interferon gene and its subsequent cloning in *Escherichia coli* (Edge *et al.*, 1981). Synthetic oligonucleotides can also be used for identification of specific genes in DNA libraries, since it is often possible to predict a fairly unambiguous DNA sequence from the amino-acid sequence of the protein. In this way, the mRNAs for the hormone gastrin (Mevarech *et al.*, 1979) as well as the mRNA for human transplantation antigens (Sood *et al.*, 1981) have been identified. Here we describe the use of synthetic oligonucleotides to study different splicing patterns of the mRNA for the adenovirus fiber protein, a capsid protein located at each vertex of the virion. The results show that at least five different leader sequences can be attached to the fiber mRNA.

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Results

Synthetic oligonucleotides as primers for cDNA extension

The adenovirus fiber mRNA seems to be present in several forms which differ in their untranslated 5′ ends (Dunn *et al.*, 1978; Chow and Broker, 1978; Zain *et al.*, 1979a). A correct splicing of the fiber mRNA may determine the host range of the virus since monkey cells which are semi-permissive for replication of human adenoviruses appear to splice the fiber mRNA in an aberrant fashion (Klessig and Chow, 1980). Chow and Broker (1978) have identified several different leader fragments linked to the fiber mRNA by heteroduplex analysis (Figure 1) and the sequences of two alternative 5′ ends of the fiber mRNA have already been established (Akusjärvi and Pettersson, 1979; Zain *et al.*, 1979b). To extend these studies and to examine the abundance of different forms of the fiber mRNA, four oligonucleotides were synthesized which all were complementary to the 5′ end of the coding sequence for the fiber (Zain *et al.*, 1979b). Thus, all the oligonucleotides have a common 3′ end, TAC, corresponding to the initiator AUG of the fiber but differ in size between 8 and 14 nucleotides (Figure 2). All four oligonucleotides were 5′-end labeled and mixed with poly(A)-containing mRNA isolated from HeLa cells either late (17 h) or early (7 h) after adenovirus 2 (ad2) infection. The mixture was heated at 80°C for 5 min, then rapidly cooled to 0°C. The primers were then extended with avian myeloblastosis virus (AMV) reverse transcriptase. The resulting cDNA was fractionated by electrophoresis through a 6% polyacrylamide gel, containing 7 M urea. Autoradiography showed that no specific cDNA bands were obtained when the octamer was used as a primer and that the different procedures (see Materials and methods) to hybridize the primer to the template did not yield cDNA transcripts of significantly different size or intensity (not shown). After extension of the three longest oligonucleotides five major bands, A–E, could be observed (Figure 3A). A dramatic increase in the intensity of the bands was observed going from the decamer to the dodecamer but no enhancement could be detected if two additional nucleotides were added to the primer (Figure 3A). The most abundant band "E" had the expected size of the primer plus the major late tripartite leader which consists of 203 nucleotides (Akusjärvi and Pettersson, 1979). To estimate the relative abundance of the five cDNA species the films were scanned with a densitometer (Figure 3B) and the area under each peak was determined. The results showed that the peaks were the same irrespective of whether the decamer or the tetradecamer was used as a primer. Excision and counting of each band separately also gave the same relative distribution of counts. Therefore, the relative abundance of these cDNA species is likely to reflect the concentration of the different forms of the mRNA. Table I demonstrates that the fiber mRNA species with the tripartite leader constitutes ~70% of the fiber mRNA whereas each of the other four species account for 5–10%. None of the five cDNA bands A–E could be detected when early adenovirus mRNA was used as a template (lane 4, Figure 3A). In contrast, a 72-nucleotide transcript was observed in early and late RNA,

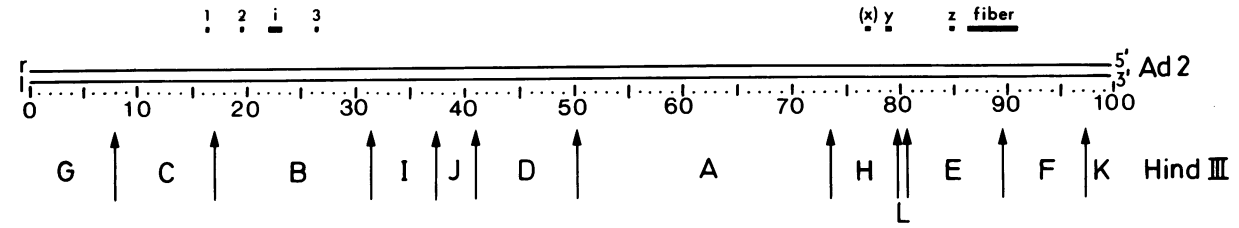

Fig. 1. A map of the exons which constitute the ad2 fiber mRNA. The approximate positions of the leaders and the body of the fiber mRNA are indicated (Akusjärvi and Pettersson, 1979; Zain *et al.*, 1979a). The *Hind*III restriction map for the ad2 DNA is also shown.

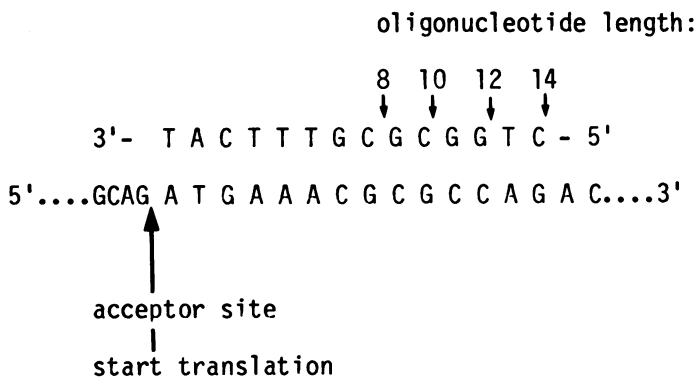

Fig. 2. The structure of three synthetic oligonucleotides which varied in length from 8–14 nucleotides. The genomic sequence of the 5′ end of the structural gene for the fiber is shown including the ATG start codon for the fiber polypeptide.

although only when the tetradecamer primer was used (band F, Figure 3A).

Kinetic analysis of the appearance of different forms of fiber mRNA

To establish whether the different forms of fiber mRNA are confined to a defined period of the lytic infection, cytoplasmic mRNA was prepared from uninfected cells and at regular intervals during the infection cycle with ad2; it was then reverse transcribed with the tetradecamer as a primer. Figure 4 shows that a band corresponding to the tripartite leader is observed already at 10 h post-infection and accumulates thereafter. The four additional bands A–D are mainly observed at 15 and 20 h after infection and their relative abundance appears to be somewhat less at the earliest time point. Since this experiment only identifies steady-state levels of the mRNA it cannot be established whether the minor species are splicing intermediates or authentic mRNAs. The 72-nucleotide transcript (band F, Figure 3A) appears to be present also in uninfected cells although the transcript is enhanced early after infection. This transcript is, therefore, probably derived from a cellular RNA which contains a sequence related to that of the primer. When the entire sequence of band F was determined it could not be identified in any known sequence from the ad2 genome (data not shown). This observation and the fact that only the tetradecamer primer gave rise to band F suggests that it is derived from a cellular RNA. This transcript was, therefore, not further considered in the present study.

Characterization of the cDNA species by hybridization

From the work of Chow and collaborators (Chow and Broker, 1978; Chow *et al.*, 1979) it was expected that the four largest cDNA bands (A–D) contain different combinations of the leader segments 1, 2, i, 3, x, y, and z as illustrated in Figure 1. To identify which leader segments are involved in

Table I. Leader arrangements for the different transcripts of the fiber mRNA

Band (see Figure 3A)	Leader fragments	Total size of leader sequence (in nucleotides)	Relative abundance (%)
A	1,2,i,3	643	5
B	1,2,3,y,z	532	7
C	1,2,3,y	387	10
D	1,2,3,z	348	9
E	1,2,3	203	69

the different cDNA species, hybridization studies were carried out. Purified ad2 DNA was cleaved by endonuclease *Hind*III and the cleavage products were separated by electrophoresis through a 1.4% agarose gel and transferred to nitrocellulose according to the method of Southern (1975). Strips from the nitrocellulose sheets were hybridized either to labeled cDNA or to the ^{32}P-labeled oligonucleotide primer. The hybridization with the primer was carried out at 22°C because of the small size of the oligonucleotide, whereas the extended cDNA species were hybridized at 65°C. The autoradiogram (Figure 5) shows that the tetradecamer primer exclusively hybridizes to the *Hind*III-E fragment (Figure 5, lane 6) as expected from the location of the fiber gene (Figure 1). The cDNA species which is present in band "E" (Figure 3A) hybridizes to the *Hind*III-B and C fragments (Figure 5, lane 5) which is also expected since this band is the same size as the tripartite leader plus the primer. The hybridization to the *Hind*III-E fragment, which contains the coding sequence for the fiber, was barely detectable, probably due to the small size of the complementary sequence (16 nucleotides). Bands A–D (see Figure 3A) all seem to contain the tripartite leader since they hybridize both to the *Hind*III-B and the *Hind*III-C fragments (Figure 5, lanes 1–4). Band "D" also hybridizes to the *Hind*III-E fragment (Figure 5, lane 2) which suggests that it contains the z-leader (see Figure 1). Band "C", in contrast, hybridizes to the *Hind*III-H fragment (Figure 5, lane 3) which suggests that it contains the x- or the y-leader (Figure 1). Band "B" hybridizes to both the *Hind*III-E and the *Hind*III-H fragments suggesting that it contains the x- or the y-leader as well as the z-leader. Band "A" shows strong hybridization to the *Hind*III-B fragment and weak hybridization to fragment *Hind*III-C. The length of this cDNA species indicates that it may contain the i-leader which is located in the *Hind*III-B fragment.

Sequence analysis of the different cDNA species

To determine the structures of the variant 5′ ends of the fiber mRNA, the cDNA species were eluted from the gels and sequenced according to the Maxam and Gilbert procedure (1977). The results which are summarized in Table I agree with the results obtained by the hybridization analysis (Figure

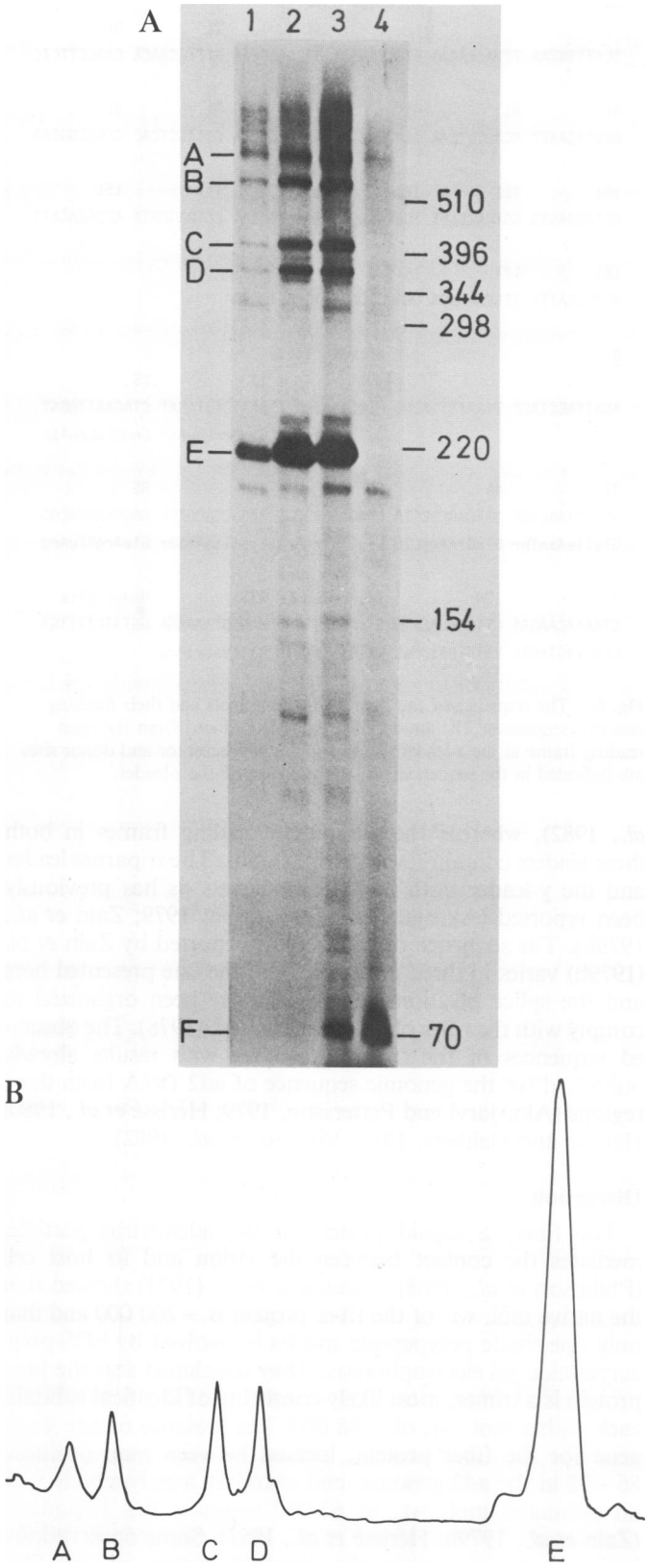

Fig. 3. **A**, Urea-polyacrylamide gels of the reverse transcripts of late and early adenovirus mRNA using synthetic oligonucleotides of different sizes. **Lane 1**: the decamer; **lane 2**: the dodecamer; **lane 3**: the tetradecamer; **lane 4**: the tetradecamer. **Lanes 1–3** represent reverse transcripts from late, and **lane 4** from early, adenovirus mRNA. **B**, A densitometer scan of **lane 2** in **A** corresponding to a region of the gel from ~700–200 nucleotides.

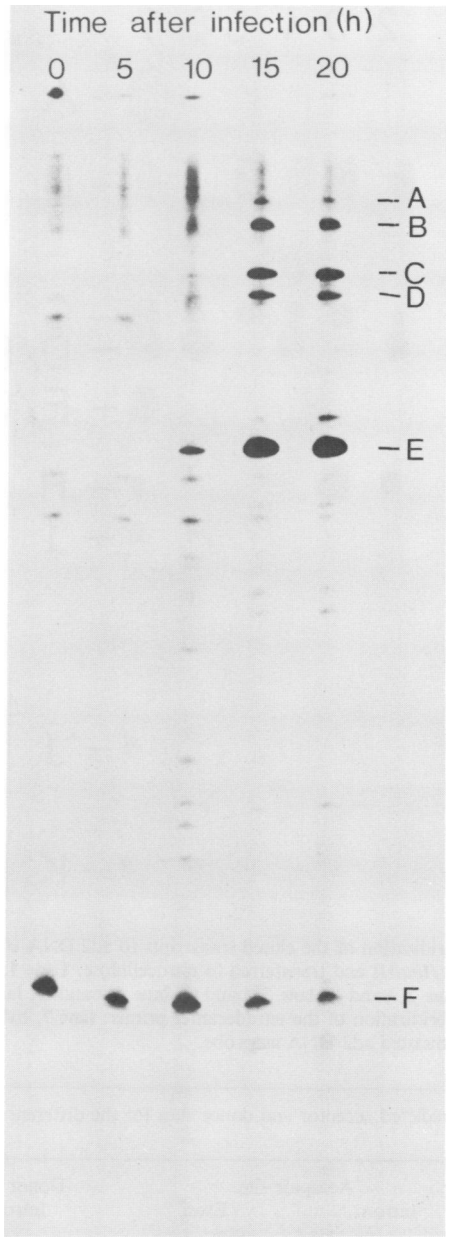

Fig. 4. Appearance of fiber mRNA species at different times during productive infection with ad2. Cytoplasmic RNA collected from uninfected cells and at 5-h intervals throughout infection with ad2 was transcribed with reverse transcriptase and analyzed on urea polyacrylamide gels. The tetradecamer primer was used in this experiment.

5) and also with the size estimates of the cDNA species as determined by gel analysis (Figure 3). The sequence analysis confirmed that species "E" corresponds to the previously published 203-nucleotide tripartite leader and that species "C" in addition contains the 184-nucleotide y-leader. The z-leader was found in species "D" and is 145 nucleotides long; its sequence is shown in Figure 6. None of the cDNA bands was found to contain the x-leader which is at variance with the results of Chow and Broker (1978). Sequence analysis of cDNA species "A" revealed the i-leader between the second and the third segment of the tripartite leader. Since the estimated size of the i-leader is >400 nucleotides only the 3′ end of this leader segment could be established by sequence analysis. However, the splice junction at the 5′ side was recently determined in our laboratory (Virtanen *et al.*,

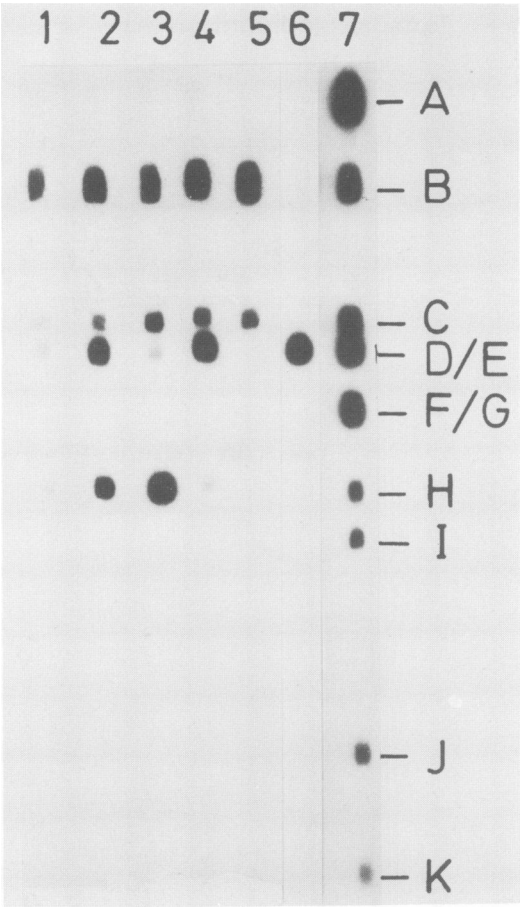

Fig. 5. Hybridization of the eluted transcripts to ad2 DNA cleaved with endonuclease *Hind*III and transferred to nitrocellulose. **Lane 1**, band A in Figure 3A; **lane 2**, band B; **lane 3**, band C; **lane 4**, band D; **lane 5**, band E; **lane 6**, hybridization of the tetradecamer primer; **lane 7**, hybridization using nick-translated ad2 DNA as probe.

Table II. Predicted acceptor and donor sites for the different forms of the fiber mRNA

Leader	Acceptor site			Donor site	Size in nucleotides
	Intron	Exon		Intron	
first	–	–	... GTTGGG	GTGAGT	41
second	CCACAG	CTCGCG	... CGAACG	GTAAGA	72
third	GTGTAG	GTACTC	... CGCAAG	GTAGGC	90
i	TCGTAG[a]	GTGACA	... CGACAG	GTCAGG	440
y	CAACAG	TTTCCA	... CAGGAG	GTGAGC	184
z	TGACAG	GTGGAG	... AAAGAG	GTATCT	145
main body	TTGCAG	ATGAAA	... –	–	–

[a]Data from Virtanen *et al.*, 1982.

1982) using a similar approach. Since the genomic sequence of these regions in the ad2 DNA are known (Akusjärvi and Pettersson, 1979; Hérissé *et al.*, 1980; Hérissé and Galibert, 1981; Virtanen *et al.*, 1982) the cDNA sequences could be matched and the splice junctions exactly localized. The sequences of the different leader segments that were identified in the present report and their flanking sequences are shown in Figure 6 and the exact positions of the splice sites is indicated in Table II. It is noteworthy that the z-leader contains an AUG triplet which is followed by an open translational reading frame as is also the case for the i-leader (Virtanen *et al.*, 1982), whereas the alternative reading frames in both these leaders contain several stop codons. The tripartite leader and the y-leader both lack AUG triplets as has previously been reported (Akusjärvi and Pettersson, 1979; Zain *et al.*, 1979b). The sequence of the y-leader reported by Zain *et al.* (1979b) varies in three positions from the one presented here and the splice positions (Table II) have been organized to comply with the rules of Breathnach *et al.* (1978). The observed sequences of the transcripts agree with results already published for the genomic sequence of ad2 DNA from these regions (Akusjärvi and Pettersson, 1979; Hérissé *et al.*, 1980; Hérissé and Galibert, 1981; Virtanen *et al.*, 1982).

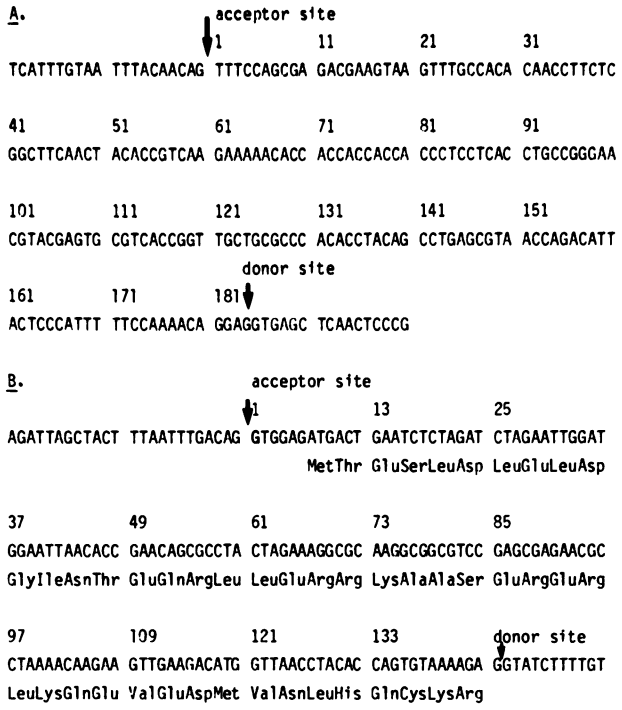

Fig. 6. The sequence of the fiber leader fragments and their flanking genomic sequences. The amino acid sequence deduced from the open reading frame in the z-leader is also shown. The acceptor and donor sites are indicated in the sequences. **A**: the y-leader. **B**: the z-leader.

Discussion

The fiber, a capsid protein in the adenovirus particle, mediates the contact between the virion and its host cell (Philipson *et al.*, 1968). Sundquist *et al.* (1973) showed that the native mol. wt. of the fiber protein is ~200 000 and that only one single polypeptide species is resolved by SDS-polyacrylamide gel electrophoresis. They concluded that the fiber protein is a trimer, most likely consisting of identical subunits each with a mol. wt. of ~68 000. The presence of one single gene for the fiber protein, located between map positions 86–92 in the ad2 genome, and encoding a polypeptide with an estimated mol. wt. of 62 000 supports this hypothesis (Zain *et al.*, 1979b; Hérissé *et al.*, 1981). Some observations, however, argue against the identical nature of the fiber subunits. Ishibashi and Maizel (1974) have reported that the native fiber contains only one N-acetylglucosamine residue on two of the three polypeptides and it appears to be possible to resolve the fiber subunits into separate bands in urea-containing polyacrylamide gels (Dorsett and Ginsberg, 1975). Moreover, the asymmetric morphology of the fiber protein speaks against a structure composed of identical subunits.

However, a comparison between the amino-acid composition of the fiber protein (Pettersson *et al.*, 1968) and that predicted from the DNA sequence of the fiber gene (Hérissé *et al.*, 1981) shows only minor differences. Thus, if different subunits are present in the fiber protein they must have closely related sequences. One remaining possibility, is however, that different subunits are created by attaching different 5′ ends to the fiber mRNA. Chow and Broker (1978) have shown that the 5′ end in the fiber mRNA can be spliced in several different ways. However, their data does not indicate whether the different splicing patterns of the 5′ end result in different coding properties. We have therefore examined the structure of the 5′ end of the fiber mRNA with a simple technique which can be used to characterize the 5′ end of any mRNA species. Using oligonucleotides having the same 3′ end and ranging in size between 8 and 14 nucleotides we found that dodecamers or longer oligonucleotides are required for efficient reverse transcription of mRNA populations. Although not tested in this study, it appears likely that oligonucleotides 12 or 14 units long represent a minimum size for the detection of clones in a cDNA library by colony hybridization. Our results show that the fiber mRNA is present as five major species representing 5–70% of the total fiber mRNA population and that the different species differ with regard to the structure of the 5′ end. In addition to the five major fiber mRNA species, several minor components were observed which have not been investigated further. The x-leader identified by Chow and Broker (1978) could not be found among the major transcripts, suggesting that it is only present in very low abundance. Many of the components which are longer than species "A" are likely to represent splicing intermediates formed during the splicing reaction. From hybridization studies and sequence analysis the structure of all five major classes of fiber mRNA were deduced, as summarized in Table I. The predominant species "E" contains the 203-nucleotide tripartite leader alone whereas the other four species contain the three segments of the tripartite leader in combination with other segments (Table I). The results pose the interesting question why the fiber mRNA is spliced in so many different ways. At least two possible explanations are conceivable: first the different 5′ ends of species A–E can give rise to different mRNA populations which may be translated with different efficiencies and the virus may in this way regulate the production of fiber protein. The fiber protein has additional functions besides being a structural protein in the adenovirus particle (Levine and Ginsberg, 1967) and its regulation, particularly at intermediate times after infection, could be very important for viral replication. The results of Klessig and Chow (1980) give additional evidence for the notion that the splicing of the fiber mRNA is an important event during adenovirus replication since monkey cells which fail to replicate human adenoviruses efficiently splice the fiber mRNA in an abnormal fashion. The kinetic study of the appearance of different leaders does not, however, lend support for their temporal regulation. An alternative explanation is that the different leaders at the 5′ end of the fiber mRNA alter the coding properties of the mRNA. A prerequisite for such a function would be that the leaders contain AUG triplets. It has previously been established that neither the tripartite leader itself nor the y-leader contain AUG triplets (Akusjärvi and Pettersson, 1979; Zain *et al.*, 1979b). Consequently, they cannot change the coding properties of the mRNA bodies to which they are attached. The i-leader contains an AUG triplet, followed by an open translational reading frame (Virtanen *et al.*, 1982) which would make species "A" a candidate for encoding an altered fiber polypeptide. It is, however, clear from the sequence of the i-leader (Virtanen *et al.* 1982) that the open translational reading frame will terminate in the third segment of the tripartite leader encoding a hypothetical 15.9 K polypeptide. Consequently, species "A" cannot be translated into an alternative form of the fiber polypeptide. Species "B" and "D" contain the z-leader and our analysis of the z-leader sequence shows that it also contains an AUG triplet followed by an open translational reading frame. From the splice junction between the fiber mRNA body and the z-leader it is possible to predict that the open translational reading frame which starts in the z-leader will terminate shortly after the junction to the fiber mRNA body and could at the most give rise to a 6 K polypeptide. Consequently, none of species A–E can give rise to alternative forms of the fiber polypeptide by changing the coding properties of the mRNA.

The hypothetical function of the different fiber mRNA species, if any, may perhaps be related to the efficiency by which the different species are translated. Another possibility is that additional fiber mRNA species exist in which the z-leader is spliced into the fiber body in a region downstream from the initiator AUG.

From purely structural considerations it is of interest that three of six leaders investigated in this report (i.e., the third tripartite component, the i- and the z-leaders) have sequences at the 5′ and 3′ ends corresponding to introns instead of exons (Table II). This finding may suggest that these leaders are intermediates in the splicing reaction.

Reverse transcription with defined oligonucleotide primers has already been used to make cDNA clones of several genes from mammalian cells including those for gastrin and transplantation antigens (Mevarech *et al.*, 1979; Sood *et al.*, 1981). It might also be used to establish the fine details of mRNA splicing patterns and, as our results show, to establish the relative abundance of scarce mRNA species. A calculation of the abundance of some of the aberrant forms of the fiber mRNAs, assuming $\sim 4 \times 10^6$ mRNA molecules/cell and 2000 copies of fiber RNA at 18 h after infection (Flint and Sharp, 1976), suggests that they are present at $\sim 0.01-0.002\%$ or less of the total mRNA in the cell. mRNA species this scarce can be difficult to identify by other methods, including *in vitro* translation and specific immunoprecipitation.

Materials and methods

Cells and viruses

Ad2 was propagated in suspension cultures of HeLa cells as described previously (Persson *et al.*, 1978). Cells, grown in the presence of 25 μg/ml cycloheximide from 2 h post-infection, were harvested after 7 h for early RNA whereas late RNA was prepared from untreated cells harvested 17 h post-infection. In one experiment RNA was collected at different times in the lytic cycle without any drug treatment. Cytoplasmic RNA was extracted by the procedure of Brawerman *et al.* (1972) and fractionated by chromatography on oligo(dT) cellulose (Collaborative Research).

Preparation of primers

The tetradecamer, $^{5'}$d(CTGGCGCGTTTCAT)$^{3'}$, was prepared by condensation of a 5′-O-(9-phenylxanthen-9-yl)-protected hexamer phosphodiester block with a 5′-hydroxy octamer block in 65.0% yield as previously reported in detail (Josephson *et al.*, 1981a).

The dodecamer, $^{5'}$d(GGCGCGTTTCAT)$^{3'}$ was prepared by condensation of a 5′-O-(9-phenylxanthen-9-yl)-dimer phosphodiester block with the appropriately protected 5′-hydroxy decamer block in 40.5% yield which was de-

protected using our standard procedure (Balgobin *et al.*, 1981).

The decamer, $^{5'}$d(CGCGTTTCAT)$^{3'}$, was prepared by a block condensation of 5'-O-(9-phenylxanthen-9-yl)(Px)-tetramer phosphodiester component with the appropriately protected 5'-hydroxy hexamer block in 41.2% yield using 1-mesitylenesulfonyl-3-nitro-1,2,4-triazole as a condensing agent (Balgobin *et al.*, 1981) and the octamer $^{5'}$d(CGTTTCAT)$^{3'}$ was similarly prepared in 72.5% yield by the condensation of a 5'-O-Px-tetramer-phosphodiester-5'-O-Px-d(CpGpTpTp-) with the 5'-hydroxy tetramer block (^{HO}TpCpApT-O-Bz).

The oligonucleotides were purified, after removal of the protecting groups, by h.p.l.c. on a permaphase AAX column (linear gradient: 0.01 M KH_2PO_4, 0.01 M KCl to 0.05 M KH_2PO_4 and 0.7 M KCl, pH 4.45 at 55°C) (Balgobin *et al.*, 1981; Josephson *et al.*, 1981b). The appropriate fractions were collected and dialyzed using per-acetylated dialysis tubing. They were then concentrated, γ^{32}P-labeled, and purified by electrophoresis on a 20% polyacrylamide gel. Their structures were confirmed by sequence analysis using the Maxam and Gilbert procedure (1977).

Radio-labeling of primers

The oligonucleotides were 5'-end labeled with [^{32}P]ATP (New England Nuclear, 2700 Ci/mmol) and T4 polynucleotide kinase (Boehringer Mannheim) as described previously (Houghton *et al.*, 1980; Maxam and Gilbert, 1980). 40 pmol of primer were incubated with 40 pmol of [^{32}P]ATP and the reaction was monitored by chromatography on PEI thin layer plates (Macherey-Nagel) in 0.75 M Na_2HPO_4 at pH 3.5. More than 90% of the primer molecules were usually recovered in the labeled fraction. The reaction was terminated by phenol extraction followed by repeated ether extractions. The oligonucleotides were precipitated with 2.5 volumes of ethanol after the addition of KAc to 0.3 M and $MgAc_2$ to 0.01 M.

cDNA synthesis

50 pmol of ^{32}P-labeled primer were mixed with 50 μg of poly(A)-containing mRNA in 250 μl of a buffer containing 5 mM Tris-HCl pH 7.5 and 0.25 mM EDTA. After heating at 80°C for 5 min, the mixture was chilled on ice and 250 μl of the reaction buffer was added giving the following final concentrations: 50 mM Tris-HCl pH 7.9, 10 mM $MgCl_2$, 20 mM dithiothreitol, and 0.7 mM each of the four deoxyribonucleoside triphosphates. The reaction mixture was incubated at 37°C for 2 h with 120 units of AMV reverse transcriptase (kindly supplied by J.W. Beard, Life Science Center, St. Petersburg, FL). The reaction was terminated by the addition of EDTA and SDS to final concentrations of 50 mM and 0.5%, respectively, followed by extraction with 500 μl phenol. The aqueous phase was then fractionated by chromatography on Sephadex G50 (Pharmacia Fine Chemicals, Uppsala). The percolate was precipitated with ethanol, and the cDNA was then dissolved in 4 μl of sample buffer (0.2 M NaOH with 10% Ficoll) and separated on a 6% polyacrylamide gel containing 7 M urea according to Maxam and Gilbert (1977). The gel was exposed to Kodak XRP film for 1 h at +8°C and the resulting autoradiograph was traced densitometrically with a laser densitometer (LKB 2202) equipped with an internal integrator. Different conditions for prehybridization of primers to mRNA were tested including pre-incubation at 41°C for 2 h (Sood *et al.*, 1981) or slow cooling of the sample from 90°C to 20°C over a 20-min period (Agarwal *et al.*, 1981).

DNA sequence analysis

The cDNA bands were excised from the polyacrylamide gels and eluted with 10 mM Tris-HCl pH 7.9, 1 mM EDTA, 0.2 M NaCl, at 37°C overnight. The crude eluates were passed over a Whatman glass fiber filter and adjusted to 20 mM Tris-HCl pH 7.9, 1 mM EDTA, 0.1% SDS, 0.3 M NaCl, and 10 mM $MgCl_2$. Ethanol was added to a final concentration of 50% and the mixture was then passed through a column of Whatman cellulose powder (CF-11) saturated with the precipitation buffer. After washing of the column, the cDNA was eluted in fractions of 100 μl with 10 mM Tris-HCl pH 7.9, 1 mM EDTA, 0.1 SDS prewarmed to 37°C. The cDNA-containing fractions were pooled and sequenced according to Maxam and Gilbert (1977) after several ethanol precipitations.

DNA-DNA hybridization

Ad2 DNA was cleaved with endonuclease *Hind*III and the fragments were separated by electrophoresis in a 1% agarose gel. DNA fragments were transferred to nitrocellulose filters according to the method of Southern (1975). The filters were baked for 2 h at 80°C and presoaked in 6 x SSC, 3 x Denhardt's solution, and 0.5% SDS at 65°C for 3 h. The radiolabeled probe consisting either of gel slices containing the cDNA, the kinased primer, or nick-translated ad2 DNA was added to the presoaked strips together with the hybridization solution. Hybridizations were carried out overnight at 65°C in 6 x SSC, 3 x Denhardt's solution, and 0.5% SDS or, when the primers were used as probes, at 22°C in 6 x SSC, 10 x Denhardt's solution, and 0.5% SDS. After hybridization the strips were washed four times in 2 x SSC, 0.5% SDS at the temperature used for hybridization. Autoradiography was performed at −70°C using intensifying screens.

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References

Agarwal,K.L., Brunstedt,I., and Noyes,B.E. (1981) *J. Biol. Chem.*, **256**, 1023-1028.

Akusjärvi,G., and Pettersson,U. (1979) *J. Mol. Biol.*, **134**, 143-158.

Balgobin,N., Josephson,S., and Chattopadhyaya,J.R. (1981) *Acta Chem. Scand.*, **B35**, 201-212.

Brawerman,G., Mendecki,J., and Lee,S.Y. (1972) *Biochemistry (Wash.)*, **11**, 637-641.

Breathnach,R., Benoist,C., O'Hare,K., Gannon,G., and Chambon,P. (1978) *Proc. Natl. Acad. Sci. USA*, **75**, 4853-4857.

Chow,L.T., and Broker,T.R. (1978) *Cell*, **15**, 497-510.

Chow,L.T., Broker,T., and Lewis,J.B. (1979) *J. Mol. Biol.*, **134**, 265-303.

Dorsett,P.H., and Ginsberg,H.S. (1975) *J. Virol.*, **15**, 208-216.

Dunn,A.R., Matthews,M.B., Chow,L.T., Sambrook,J., and Keller,W. (1978) *Cell*, **155**, 511-526.

Edge,M.D., Greene,A.R., Heathcliffe,G.R., Meacock,P.A., Schuch,W., Scanlon,D.B., Atkinson,T.C., Newton,C.R., and Markham,A.F. (1981) *Nature*, **292**, 756-761.

Flint,S.J., and Sharp,P.A. (1976) *J. Mol. Biol.*, **106**, 749-771.

Hérissé,J., and Galibert,F. (1981) *Nucleic Acids Res.*, **9**, 1229-1240.

Hérissé,J., Courtois,G., and Galibert,F. (1980) *Nucleic Acids Res.*, **8**, 2173-2192.

Houghton,M., Stewart,A.G., Doel,S.M., Emtage,J.S., Eaton,M.A.W., Smith,J.C., Patel,T.P., Lewis,H.M., Porter,A.G., Birch,J.R., Cartwright, T., and Carey,N.H. (1980) *Nucleic Acids Res.*, **8**, 1913-1931.

Ishibashi,M., and Maizel,J.V. (1974) *Virology*, **58**, 345-361.

Josephson,S., Balgobin,N., and Chattopadhyaya,J.B. (1981a) *Nucleic Acids. Res. Symp. Ser.*, **9**, 177-181.

Josephson,S., Balgobin,N., and Chattopadhyaya,J.R. (1981b) *Tetrahedron Lett.*, **22**, 4537-4540.

Klessig,D.F., and Chow,L.T. (1980) *J. Mol. Biol.*, **139**, 221-242.

Levine,A.J., and Ginsberg,H.S. (1967) *J. Virol.*, **1**, 747-757.

Maxam,A., and Gilbert,W. (1977) *Proc. Natl. Acad. Sci. USA*, **74**, 560-564.

Maxam,A.M., and Gilbert,W. (1980) in Grossman,L., and Moldave,K. (eds.), *Methods in Enzymology*, Vol. 65, Academic Press, NY, pp. 499-560.

Mevarech,M., Noyes,B., and Agarwal,K.L. (1979) *J. Biol. Chem.*, **254**, 7472-7475.

Persson,H., Pettersson,U., and Mathews,M.B. (1978) *Virology*, **70**, 67-90.

Pettersson,U., Philipson,L., and Höglund,S. (1968) *Virology*, **35**, 204-215.

Philipson,L., Lonberg-Holm,K., and Pettersson,U. (1968) *J. Virol.*, **2**, 1064-1075.

Sood,A.K., Pereiva,D., and Weissman,S.H. (1981) *Proc. Natl. Acad. Sci. USA*, **78**, 616-620.

Southern,E. (1975) *J. Mol. Biol.*, **98**, 503-517.

Sundquist,B., Pettersson,U., Thelander,L., and Philipson,L. (1973) *Virology*, **51**, 252-256.

Virtanen,A., Aleström,P., Persson,H., Katze,M.G., and Pettersson,U. (1982), submitted to *Nucleic Acids Res.*

Zain,A., Sambrook,J., Roberts,R.J., Keller,W., Fried,M., and Dunn,A.R. (1979a) *Cell*, **16**, 851-861.

Zain,S., Gingeras,T.R., Bullock,P., Wong,G., and Gelinas,R.E. (1979b) *J. Mol. Biol.*, **135**, 413-433.