JOURNAL OF VIROLOGY, Mar. 1978, p. 897-906
0022-538X/78/0025-0897$02.00/0
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Vol. 25, No. 3
Printed in U.S.A.

Adenovirus Type 2 Late mRNA's: Structural Evidence for 3'-Coterminal Species

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Received for publication 27 September 1977

Adenovirus type 2-infected HeLa cells were labeled with $^{32}PO_4$ during the period 14 to 17 h postinfection. Viral mRNA's with polyadenylic acid were isolated by polyuridylic acid Sepharose chromatography and fractionated according to size by electrophoresis through an acrylamide-agarose slab gel. Messenger bands were eluted and partially degraded with alkali. RNA fragments from each band that contain polyadenylic acid were isolated by polyuridylic acid Sepharose chromatography and fingerprinted two-dimensionally after T1 RNase digestion. Three bands, with mobilities of ~26S, 21S, and 18S, shared two large characteristic T1 oligonucleotides in common in the fingerprints of their 3'-terminal sequences. These oligonucleotides were mapped with a *Hpa* II restriction fragment of adenovirus type 2 DNA with coordinates 49-50.2. We conclude that the three mRNA's are coterminal in sequence at their 3' ends and overlap at internal positions. Implications for the protein-coding potential of these mRNA's and the mechanisms of adenovirus type 2 late RNA processing are discussed.

Adenovirus type 2 (Ad2) expresses its genome through an intricate series of transcriptional events (11, 32). During infection of human cells, transcription of the viral DNA, a linear duplex of 35 kilobase pairs (18), takes place in two stages. Early mRNA is transcribed from four separated regions of the genome and is complementary to both strands of the DNA (16, 30, 33, 39). With the onset of the late stage, the majority of transcripts are initiated at a newly functional site located near coordinate 16 on the Ad2 physical map (10). Late transcripts initiated near 16 proceed rightward along the viral DNA and can extend virtually to the far end at coordinate 100 (3, 4). The primary product of transcription, a large RNA molecule with a sedimentation coefficient greater than 45S, has been proposed as precursor to late mRNA (3, 15, 40). Late transcripts are processed selectively, with certain portions transported to the cytoplasm as mRNA and others confined to the nucleus (12, 30, 33). The 5' ends of the late messengers are capped (25, 37) and contain a common 5'-terminal undecanucleotide released by T1 RNase (14). The common undecanucleotide is part of a leader RNA ~150 to 200 nucleotides long that is discontinuous in structure with the template for the main body of the mRNA (4, 7, 20). The leader is itself composed of three RNA segments that are linked to one another and the mRNA body by a mechanism that potentially utilizes RNA splicing or ligation (4, 7, 20). The 3' termini of the messengers are polyadenylated (22). Six different polyadenyated RNA sequences have been identified for Ad2 late mRNA's (N. Fraser and E. Ziff, submitted for publication). Five of these map in the region of the 16-100 large transcript.

In vitro translation studies have disclosed an additional important feature of Ad2 late mRNA structure. Within an 11-kilobase region of the genome, which lies between coordinates 31 and 62.2 and is expressed only late in infection, Lewis et al. (21) have mapped the following proteins: IIIa, peripentonal polypeptide, 66 kilodaltons (kd); III, penton, 70 kd; V, minor core, 48.5 kd; and pVII, major core precursor, 20 kd; followed by pVI, hexon-associated precursor, 27 kd; and II, hexon, 120 kd (see reference 11 for a review). These proteins total 367 kd and, with the assumption that their genes do not overlap, account for approximately 90% of the 400-kd coding potential of the 31-62.2 DNA region. Anderson et al. (2) have shown, however, that several of these mRNA's have the potential to encode protein in excess of their in vitro translation products. Protein III (70 kd) was from an mRNA with 145-kd coding potential, pVII (20 kd) was from an mRNA of 103-kd coding potential, V (48.5 kd) was from an mRNA with a 62-kd coding potential, and pVI (27 kd) was from a 165-kd-potential mRNA. To account for these excesses, Anderson et al. (2) suggested that the mRNA's were polycistronic.

In this report we present structural evidence that at least three Ad2 rightward-reading late

mRNA's of different size are coterminal in sequence at their 3′ ends. The position of the common 3′ terminus of these mRNA's is located near coordinate 50. We suggest that these species are overlapping in structure and that the larger of the coterminal mRNA's correspond to messengers with excess protein-coding capacity comparable to those identified by Anderson et al. (2). Implications for the arrangement of structural genes and processing sites within Ad2 transcripts are discussed.

MATERIALS AND METHODS

Cell culture and virus preparation. HeLa cells were maintained in exponential growth in Spinner culture with Joklik-modified minimal essential medium (F-13, Grand Island Biological Co.) supplemented with 5% fetal calf serum. Infection with Ad2 and virus isolation were essentially by the procedure of Green and Piña (17) as modified by Philipson et al. (31). Viral DNA was extracted from CsCl-banded virus by the Pronase-sodium dodecyl sulfate method of Doerfler (9) modified by Pettersson and Sambrook (29).

Preparation of viral DNA restriction fragments. Ad2 DNA was restricted with *Eco*RI endonuclease (Miles Laboratories, Inc.) to yield six fragment products (28) that were fractionated on 1.4% agarose slab gels. The *Sma* I-D restriction fragment was isolated from an *Sma* I digest of the *Eco*RI-A fragment by using similar slab gels. (*Sma* I enzyme preparation and digestion pattern were personal communications of C. Mulder.) DNA was recovered from gels by $NaClO_4$ solubilization and hydroxylapatite chromatography by the method of Lewis et al. (21). Restriction of *Sma* I-D or its subfragments was with *Kpn* I (35), *Hpa* II (34) (both from New England Biolabs), or *Hin*dIII (36) (gift of J. Ford). Bands were visualized by ethidium bromide staining (34). Fragment sizes were determined by the rate of their migration relative to polyoma A-2 strain DNA (gift of A. Cowie) *Hpa* II digestion fragments (19). Polyoma *Hpa* II fragments 1 through 8, calibrated by E. Johnson versus λdv fragments of known size, were taken to be 1,480, 1,240, 925, 740, 430, 390, 270, and 125 base pairs, respectively. In one experiment, *Sma* I-D termini were kinased by the method of Maxam and Gilbert (24).

Viral mRNA. Late viral mRNA was prepared from the cytoplasmic fraction of Ad2-infected HeLa cells labeled with $^{32}PO_4$ during the period 14 to 17 h postinfection by polyuridylic acid [poly(U)] Sepharose chromatography as previously described (Fraser and Ziff, submitted for publication). Polyadenylic acid [poly(A)]-containing RNA was denatured in 50% formamide at 100°C for 2 min and fractionated by electrophoresis on a 2.2% acrylamide–0.8% agarose slab gel (27; Fraser and Ziff, submitted for publication). RNA bands, visualized by autoradiography, were eluted electrophoretically (13; Fraser and Ziff, submitted for publication). mRNA in 0.01 M Tris-hydrochloride–0.01 M EDTA–0.2% sodium dodecyl sulfate was partially degraded by mixing with 0.25 volume of 1.0 M NaOH at 0°C, incubation for 30 min, and neutralization with 0.5 volume of 1 M *N*-2-hydroxyethyl piperazine-*N*′-2-ethane sulfonic acid (untitrated). Poly(U) Sepharose chromatography to isolate 3′-terminal regions was as previously described (Fraser and Ziff, submitted for publication).

RNA structure analysis. T1 RNase digests were fractionated two-dimensionally by pH 3.5 cellulose acetate electrophoresis and homochromatography as described by Brownlee and Sanger (6). Analysis of T1 oligonucleotides by RNase A digestion and pH 3.5 high-voltage DEAE paper electrophoresis was by the methods of Sanger and co-workers as described by Brownlee (5). In vitro transcription of Ad2 DNA or fragments with *Escherichia coli* RNA polymerase was as previously described (Fraser and Ziff, submitted for publication). Blots from DNA gels to nitrocellulose filters were by the method of Southern (38).

RESULTS

Gel electrophoretic fractionation of Ad2 late mRNA. Highly radioactive Ad2 late mRNA suitable for RNA fingerprint analysis was prepared by incubating Ad2-infected HeLa cells with $^{32}PO_4$ from 14 to 17 h postinfection and fractionating the cytoplasmic RNA by poly(U) Sepharose chromatography. The labeled mRNA with poly(A) tails that is isolated by this procedure has previously been shown to be almost completely viral in origin (22; Fraser and Ziff, submitted for publication). Purified viral messenger was fractionated by size by electrophoresis through a composite agarose-acrylamide slab gel. To ensure that the mRNA species migrated as individual molecules rather than as aggregates, the sample was denatured at 100°C in 50% formamide before gel loading. An autoradiogram of the gel (Fig. 1) reveals a series of intense RNA bands of discrete size that range in mobility from ~28S to ~10S. Sections of the gel with peaks of radioactivity were excised, and the RNA was recovered by electrophoretic elution.

Comparison of 3′-terminal regions by T1 RNase fingerprinting. The RNA structures near poly(A) of the gel-fractionated mRNA's were prepared by subjecting the mRNA to partial alkaline degradation and reselecting the fragments that contained poly(A) by poly(U) Sepharose column chromatography. Hydrolysis conditions were chosen that cleaved that mRNA into pieces approximately 100 to 200 residues long. The fragments that bound to the column contained poly(A) plus a short region of RNA located near the 3′ terminus. After isolation, the 3′-terminal fragments derived from the different size classes of mRNA were digested with T1 RNase, and the resultant oligonucleotides were fractionated two-dimensionally by standard methods of cellulose acetate electrophoresis and homochromatography (6).

Figure 2 shows the fingerprint patterns of the

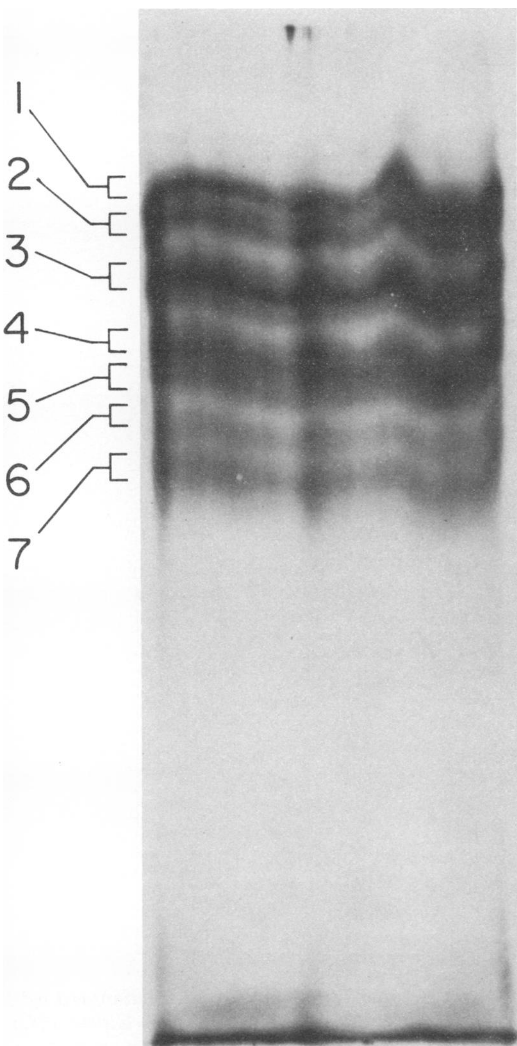

FIG. 1. *Autoradiogram of a 2.2% acrylamide-0.8% agarose slab gel fractionation of* ^{32}*P-labeled Ad2 late mRNA. Poly(A)-containing RNA labeled during the period 14 to 17 h postinfection was denatured by heating in 50% formamide at 100°C for 2 min before gel loading. S values of bands were determined from a second analytical gel that included HeLa rRNA markers. Band 1 ran in the position of 28S rRNA, and band 5 ran with 18S rRNA. Gel slices were excised with an X-ray film autoradiogram template that followed the contour of the bands. Bands 1 through 6, which were further analyzed in Fig. 2, had approximate mobilities of 28S, 26S, 23S, 21S, 18S, and 17S, respectively.*

3'-terminal regions of six consecutive RNA bands. In a previous study of messenger 3'-terminal regions (Fraser and Ziff, submitted for publication), each of these patterns was obtained from mRNA fractionated by hybridization to particular restriction fragments of Ad2 DNA. Specific T1 oligonucleotides near poly(A) were thus assigned to different mRNA 3'-terminal regions derived from defined positions within the Ad2 physical map. Comparison of the oligonucleotides of Fig. 2 with the earlier patterns obtained from mRNA hybridized to DNA restriction fragments (Fraser and Ziff, submitted for publication) identified panel a as a 3' terminus encoded in *Eco*RI-B (coordinates 58.5-70.7), panel c as a terminus in *Eco*RI-C (coordinates 89.7-100) and panel f as a terminus in *Eco*RI-D (coordinates 75.9-83.4). As previously discussed (Fraser and Ziff, submitted for publication), these patterns are distinct from one another and do not contain large T1 oligonucleotides in common. Oligonucleotides characteristic of the different mRNA 3'-terminal regions are noted in these fingerprints. In contrast to panels a, c, and f, comparison of panels b, d, and e of Fig. 2 identified two large oligonucleotides that have essentially identical mobilities in each of the three latter fingerprints. These oligonucleotides, designated spots A and B, were eluted, and their RNase A digestion products were compared by high-voltage electrophoresis on DEAE paper at pH 3.5 (Fig. 3). Spot A from each of these three panels yielded a distinctive group of three large RNase A products, consisting of a slowly migrating doublet and a third large product that migrates just ahead. None of these products had a mobility greater than an A_3G standard included in the electropherogram. A similar analysis was performed on the oligonucleotides labeled spot B in Fig. 2. In each case, one large RNase A digestion product was released together with 1 mol of A_2G and 1 to 2 mol of AU (Fig. 3b). For both spot A and spot B, the mononucleotides G_p, C_p, and U_p were not evident (region of electropherogram not shown), although a low yield of these mononucleotides per mole of oligonucleotide could not be excluded in this experiment. Because each of the spots labeled A and B have equivalent mobilities in the three fingerprints (Fig. 2b, d, and e) and yield equivalent RNase A digestion products (Fig. 3), we conclude that the three spots labeled A are the same oligonucleotide, and likewise for the three spots labeled B. The oligonucleotides that are not common to panels b, d, and e of Fig. 2 are derived from contaminating mRNA's that partially overlap the gel bands containing the common products.

Our earlier study (Fraser and Ziff, submitted for publication) found that spots A and B were obtained in hybrid of the 3'-terminal regions of unfractionated Ad2 late mRNA to the *Sma* I-D fragment of Ad2 DNA (coordinates 40.5-52.6; Fig. 6). The two spots could be isolated in greater

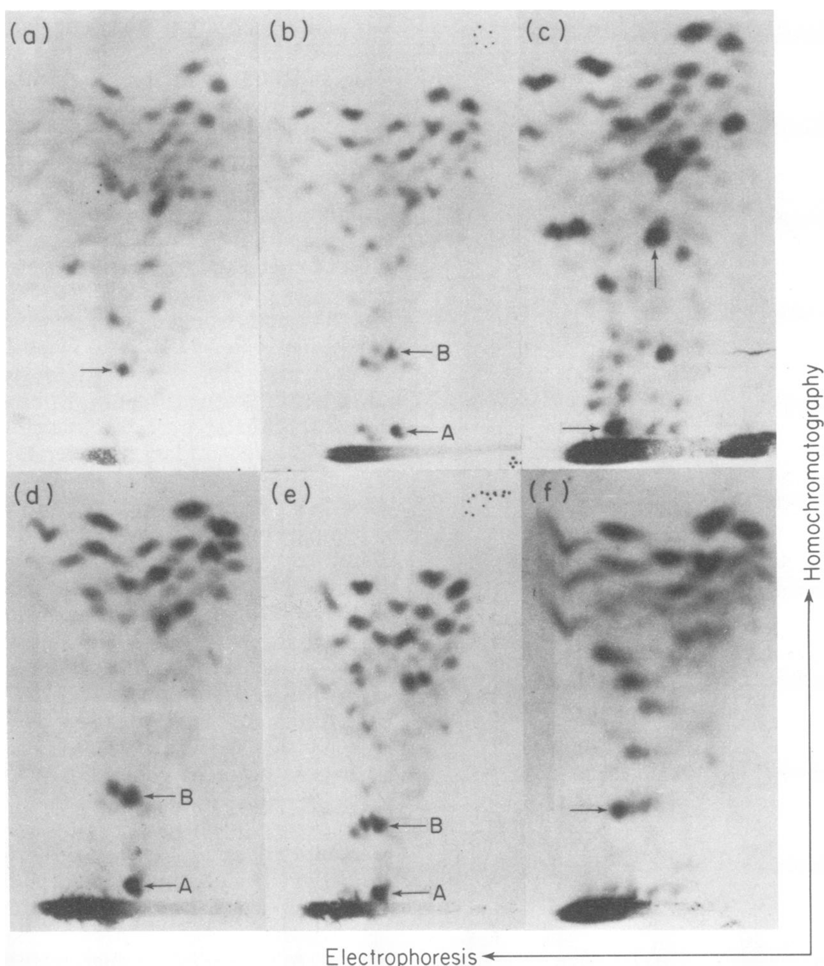

FIG. 2. *T1 RNase fingerprints of 100- to 200-nucleotide-long, 3'-terminal fragments of gel-fractionated Ad2 late mRNA's. Panels (a) through (d) are the analyses of bands 1 to 6, respectively, from Fig. 1. Eluted RNA was partially degraded with alkali to fragments ~100 to 200 residues long (Fraser and Ziff, submitted for publication). Fragments with poly(A) were reisolated by poly(U) Sepharose chromatography and then digested with T1 RNase. The resulting oligonucleotides were fractionated by standard methods of pH 3.5 cellulose acetate electrophoresis and homochromatography (6). Oligonucleotides A and B from (b), (d), and (e) were compared by RNase A redigestion as shown in Fig. 3 and described in the text. Oligonucleotides designated by unmarked arrows were shown by RNase A digestion to be distinct from spots A and B and map for (a) in EcoRI-B for (c) in EcoRI-C, and for (f) in EcoRI-D as described in the text. The RNase A products of these oligonucleotides and the deduction of their map positions are given by Fraser and Ziff (submitted for publication).*

radioactive yield when 3' ends of unfractionated Ad2 late mRNA were fingerprinted directly. When so obtained, the previous study found that spot A with RNase A gave $A_{5-6}U$, $A_{4-5}G$ (products with migration as the doublet of spot A, Fig. 3), and A_4U and C. Spot B gave A_5C (migrating as the slowest product of spot B, Fig. 3), A_2G, AU, 1-2C and 1-2U. The position of the template for this messenger 3' end (i.e., template for spots A and B) was further localized by identifying spot A in the fingerprint of an in vitro transcript of the *Kpn*I-D/*Sma*I-D subfragment of *Sma*I-D (coordinates 47.4-52.6) (Fraser and Ziff, submitted for publication).

Fine mapping of the common 3' terminus. To locate the template for spots A and B more precisely, rerestriction products of *Kpn*I-D/*Sma*I-D were analyzed. *Hpa* II restriction endonuclease digests of *Kpn* I-D/*Sma* I-D and of *Sma* I-D were subjected to electrophoresis side-by-side on an agarose-ethidium bromide slab gel (Fig. 4a). *Kpn* I-D/*Sma* I-D yielded two

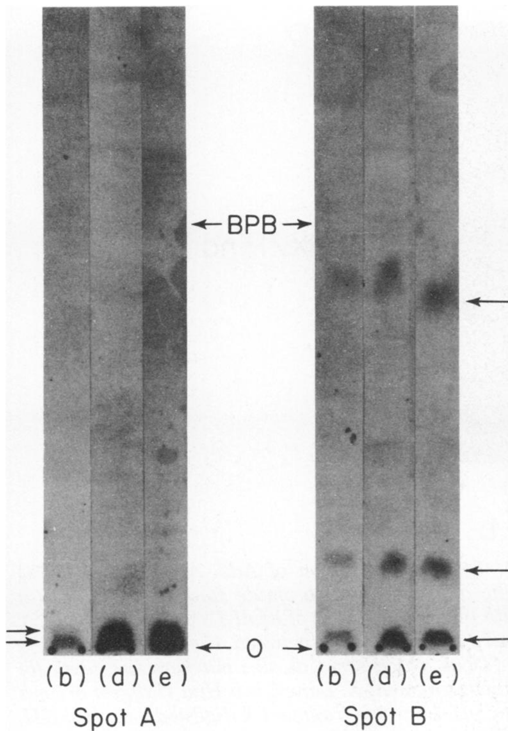

FIG. 3. *Comparison of RNase A digestion products of T1 oligonucleotide spots A and B isolated from (b), (d), and (e) of Fig. 2. Fractionation of digests was by high-voltage electrophoresis on DEAE paper at pH 3.5 (5). Spot A yielded an unresolved doublet (lower arrow) plus a third slowly migrating product (upper arrow). The compositions of these RNase A oligonucleotides as well as the products released from spot B are discussed in the text. The positions of the origin and bromophenol blue dye marker are noted. Mononucleotides (G_p, C_p, U_p) were not evident, and the position of their migration (upper section of electropherogram) is not shown. Analyses of mononucleotides in other experiments with greater quantities of radioactive sample are described in the text.*

major fragments that comigrated with the third- and fourth-largest *Hpa* II digestion products of whole *Sma* I-D. These two fragments are denoted X and Y, respectively. When *Sma* I-D terminally labeled with ^{32}P at its 5' ends with polynucleotide kinase was restricted with *Hpa* II, radioactivity migrated with the first- and fourth-largest bands of Fig. 4a, lane 1 (results not shown). Because the fourth band, Y, was from *Kpn* I-D/*Sma* I-D, it must include the right-hand terminus of *Sma* I-D. Thus, the third fragment, X, also from *Kpn* I-D/*Sma* I-D, was expected to be derived from an internal position of *Sma* I-D. Figure 6 shows that *Kpn* I-D/*Sma* I-D overlaps the position of the *Hin*dIII restriction endonuclease site at coordinate 50.1. Lane 7 of Fig. 4b shows the result of incubation of fragment X with *Hin*dIII. Restriction endonuclease treatment yielded a slightly shorter DNA fragment, with an increase of electrophoretic mobility corresponding to a cleavage of ~30 residues from one end of X. Fragment X, therefore, overlapped the *Hin*dIII site, but with two possible configurations. The bulk of the fragment (~430 residues) could lie to the right of coordinate 50.1 in *Hin*dIII-A or to the left in *Hin*dIII-D. To distinguish between these two orientations, the intact *Hpa* II fragment X was transcribed in vitro with *E. coli* RNA polymerase and [α-^{32}P]ATP. The radioactive transcript was hybridized to a nitrocellulose strip to which a gel-fractionated *Hin*dIII digest of whole Ad2 DNA had been transferred by the Southern blotting method (38). The transcript hybridized strongly to *Hin*dIII-D and weakly to *Hin*dIII-A (Fig. 5). Therefore, fragment X lies predominantly to the left of coordinate 50.1 in *Hin*dIII-D with the map position indicated in Fig. 6.

Figure 7 presents a T1 RNase fingerprint of an in vitro transcript of *Hpa* II fragment X. Labeling was with [α-^{32}P]ATP to ensure that the larger A-rich RNase A products released from T1 oligonucleotides could be detected. Because this fingerprint contains T1 oligonucleotides from transcripts of both strands of fragment X, spots not found in vivo are expected. Two oligonucleotides are noted with mobilities of spots A and B of Fig. 2. When redigested with RNase A, the oligonucleotide corresponding to spot A yielded three large products with mobilities equivalent to those seen for spot A in Fig. 3, plus C_p. The oligonucleotide corresponding to spot B yielded a product migrating with A_5C plus A_2G, C_p, and U_p. (The AU of spot B is evidently not adjacent to an A residue on its 3' side and, thus, was not detected in vitro in [α-^{32}P]ATP-labeled transcripts.) We conclude that these two in vitro oligonucleotides correspond to in vivo spots A and B and that the template for in vivo synthesis of the common 3' terminus of bands 2, 4, and 5 of Fig. 2 lies within *Hpa* II fragment X.

DISCUSSION

This report identifies three Ad2 late mRNA's with mobilities of ~26S, ~21S, and ~18S that share common oligonucleotide sequences at their 3' termini, adjacent to poly(A). A short *Hpa* II restriction fragment of Ad2 DNA that encodes these common oligonucleotides in in vitro transcription reactions has been isolated and mapped between coordinates 49 and 50.2 on the Ad2 genome. These Ad2 mRNA's are transcribed from a region of the viral genome that

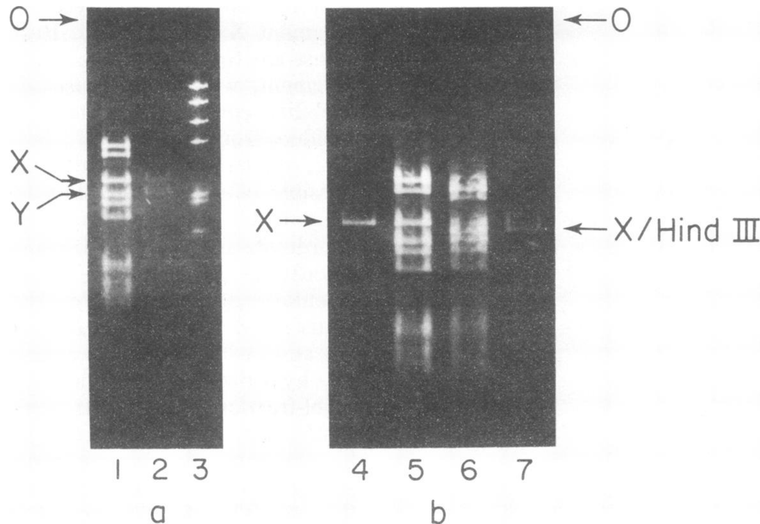

FIG. 4. *Mapping of Hpa II restriction fragments from the 47.4-52.6 region of Ad2. Agarose gel (2.5%) fractionations of DNA restriction fragments were visualized by ethidium bromide fluorescence (34). (a) compares the Hpa II digest of Sma I-D (40.5-52.6, lane 1) with the Hpa II digest of KpnI-D/Sma I-D (47.4-52.6, lane 2). Fragments X and Y are present in both lanes 1 and 2 and, thus, map between 47.4 and 52.6. Lane 3 contains Hpa II digestion products of polyoma A-2 strain DNA which serve as size markers (see text). (b) demonstrates a HindIII site within fragment X. Lane 4 is uncut fragment X. Lane 5 is a Hpa II digest of Sma I-D. Lane 3 is a combined Hpa II plus HindIII digest of Sma I-D. Lane 7 is fragment X digested with HindIII. Because fragment X increases mobility with HindIII digestion (lanes 6 and 7), it overlaps the HindIII site at 50.1 (Fig. 6).*

yields rightward-reading mRNA's (30, 33; Fig. 6), and thus the 5'-terminal portions of these mRNA's must lie to the left of coordinates 49-50.2. We conclude that the three mRNA's are coterminal in sequence at their 3' ends, overlapping, and staggered in length at their 5' ends. The deduced map position of the common 3' terminus and a proposed relationship of the mRNA's to one another, to the Ad2 physical map, and to the Ad2 mRNA map are given in Fig. 6.

Our conclusion requires that bands 2, 4, and 5 (Fig. 1) are distinct and separate mRNA species rather than degradation products or aggregates. Because the gel resolved sharp RNA bands with very little low-molecular-weight RNA, we regard random degradation as unlikely. Figure 2 shows that the mRNA bands 1, 3, and 6, which migrated alongside the three related mRNA's, yielded 3'-terminal regions with distinctive fingerprints. Thus, this gel was capable of resolving different RNA species as narrow bands. The fingerprint of the 3' end of the largest of the coterminal messengers (Fig. 2b) contains extra oligonucleotides and is probably only 50 to 70% pure. The shortest band (Fig. 2d) also contains a second minor component.

Our conclusion that three different mRNA's contain *Sma* I-D sequences is in accord with the work of McGrogan and Raskas (23). Their studies identified three mRNA's of 27S, 22S, and 18S mobility fractionated on formamide denaturing gels, which hybridize to *Sma* I-D, comparable to the coterminal mRNA's found in the present studies. Nevins and Darnell (26) also identified three gel-fractionated, *Sma* I-D-specific mRNA's and concluded from the pattern of their hybridization to rerestriction fragments of *Sma* I-D that the three are 3' coterminal.

Our conclusions are also based on the premise that the T1 oligonucleotides, spots A and B, which are present near poly(A) of the three coterminal mRNA's, are unique to the 49-50.2 region of Ad2 and not encoded at other positions on the genome. In a previous study (Fraser and Ziff, submitted for publication), we have identified the large T1 oligonucleotides of late mRNA 3' termini mapping in restriction fragments *Sma* I-M, *Sma* I-I, *Sma* I-D, *Eco*RI-B, *Eco*RI-D, and *Eco*RI-C. For each mRNA 3'-terminal region analyzed, the large T1 oligonucleotides were distinctive and not shared between two such regions. Spots A and B were found only in hybrid to *Sma* I-D.

Two other examples of overlapping and 3'-coterminal Ad2 late mRNA's have been deduced by Chow et al. (8) through R-loop mapping. They found that the protein pVI and II mRNA's

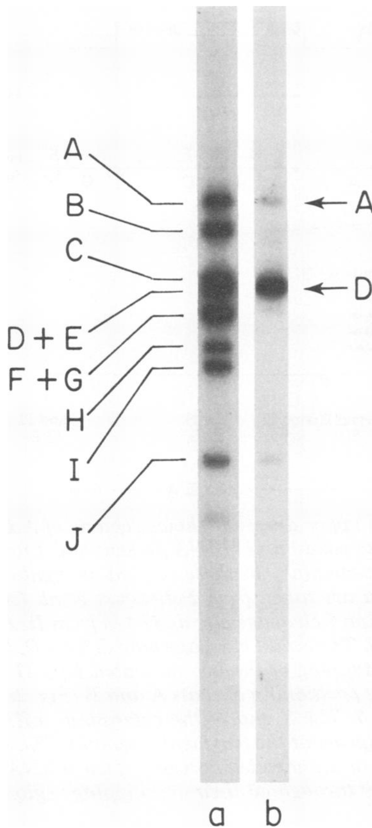

FIG. 5. *Mapping Hpa II fragment X (Fig. 4) with respect to the Ad2 HindIII cleavage map. A HindIII digest of Ad2 DNA was electrophoresed on a 1.4% agarose gel, and the DNA fragments were transferred to a nitrocellulose sheet by the Southern blotting method (38). (a) shows the hybrid of an in vitro [α-^{32}P]-ATP-labeled transcript of whole Ad2 DNA to one strip from this blot. (b) shows the hybrid of an [α-^{32}P]-ATP in vitro transcript of fragment X (same DNA as Fig. 4, lane 4) to a second strip. Hybridization of the transcript of X is much greater to HindIII-D than HindIII-A.*

are 3′ coterminal at coordinate 62.2, whereas the 100K protein and pVIII mRNA's are 3′ coterminal at 78.6. The studies of Nevins and Darnell (26) concluded that between coordinates 29.1 and 100 five banks of Ad2 late mRNA's exist. Each bank contains up to five 3′-coterminal mRNA species. In the case of simian virus 40 and polyoma virus, overlapping and 3′-coterminal 19S and 16S late mRNA's have also been reported (for a review, see reference 1). Thus, the synthesis of 3′-coterminal viral mRNA species appears to be a common phenomenon.

The proteins encoded by the coterminal mRNA's of this report may be tentatively identified by considering the in vitro translation studies of Anderson et al. (2) and Lewis et al. (21). Anderson and co-workers identified the in vitro translation products of Ad2 late mRNA's fractionated by size in formamide-sucrose gradients. They noted cases of mRNA's with excess protein-coding capacity, which suggested that some Ad2 messengers are polycistronic. Two proteins translated from mRNA's with clear excess coding capacity were viral protein III, the penton base, and pVII, the major core protein precursor. These two proteins, plus protein V, a core protein, have been mapped in the region of *Sma* I-D by Lewis et al. (21). By translating mRNA hybridized to the *Eco*RI fragments of Ad2, Lewis et al. concluded that the III, V, and pVII mRNA's mapped within the *Eco*RI-A fragment (coordinates 0-58.7), to the left of the positions of the protein II (hexon) and pVI (hexon-associated precursor) mRNA's. Using the R-loop technique, Chow et al. (8) have mapped the left-hand boundary of the pVI and II mRNA's at coordinates 49.9 and 51.6, respectively. Thus, the 3′-coterminal mRNA's identified in the present work (with 3′ end at coordinates 49-50.2) map to the left of pVI and II in the region reported for III, V, and pVII. The mobilities of the 3′-coterminal mRNA's of this report, ~26S, ~21S, and ~18S, correspond (within experimental accuracy) to the S values reported for the III, pVII, and V mRNA's, 25S, 21S, and 16S, respectively (2).

If initiation of translation of these mRNA's occurs near the 5′ end, excess coding sequences would presumably correspond to the portion of the mRNA on the 3′ side of the translated segment. Sequences untranslated in the larger mRNA's could be translated from the shorter of the 3′-coterminal species. In this instance the shorter messengers would yield the rightward-mapping proteins. RNA splicing events could, however, cause subtle alterations in the translational capacities of overlapping messengers. Because the exact order and map positions of the III, pVII, and V structural genes have not been established, the assignment of the coterminal mRNA's to these proteins must be regarded as tentative.

It is of interest to ask whether the region of Ad2 between coordinates ~38 and ~50 yields only mRNA's that are coterminal at coordinate ~50 or whether other mRNA species exist with polyadenylated 3′ ends mapping at intermediate positions. We have previously fingerprinted the hybrid of the 3′-terminal regions of unfractionated late mRNA to *Sma* I-D (submitted for publication). This fingerprint, which is expected to contain all oligonucleotides near poly(A) transcribed from *Sma* I-D, was virtually identical to the fingerprints obtained from the gel-fraction-

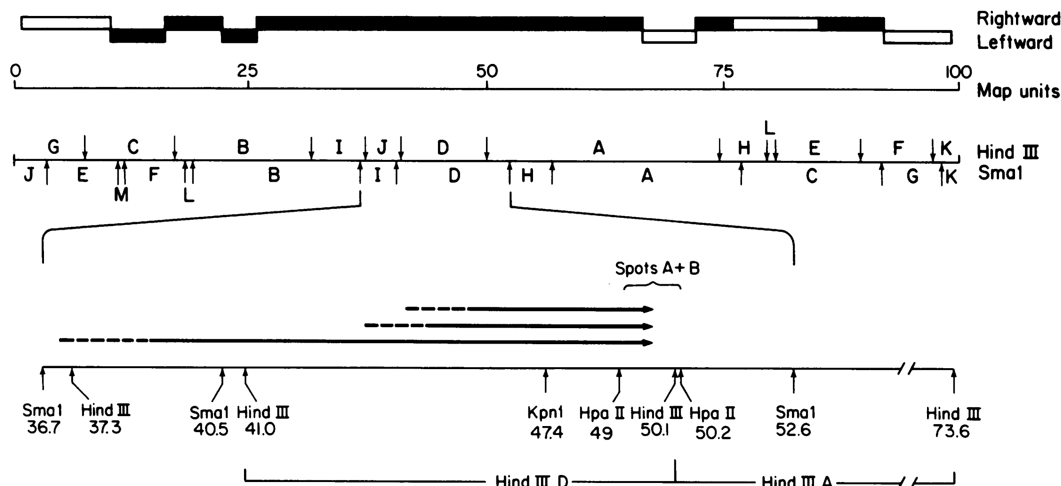

FIG. 6. *Deduced map positions of 3'-coterminal mRNA's. The upper diagram shows regions of Ad2 DNA that are complementary to mRNA present early in infection (white boxes) and mRNA present only late (black boxes). The 5' to 3' direction of transcription of mRNA's complementary to these regions is rightward or leftward with respect to this diagram, as indicated. These data are taken from Pettersson et al. (30). The HindIII restriction cleavage map of Ad2 and the location of a Kpn I cleavage site at 47.4 is from R. Roberts and co-workers (unpublished data). Other Kpn I sites are omitted. The Sma I cleavage map is from C. Mulder (unpublished data). The Sma I-I-D region is expanded below. Mapping of the two indicated Hpa II sites is from the data of Fig. 4 and 5. Other Hpa II sites are omitted. The positioning of spots A and B (Fig. 2) within the Hpa II 49-50.2 fragment is based on the fingerprint of Fig. 7. The 3' end of the coterminal mRNA's is encoded within this fragment and is arbitrarily placed in this diagram at the fragment midpoint. The dashed lines designate uncertainty in the map positions of the 5' termini of the encoded portions of the mRNA's. The diagram assumes that the mRNA's are colinear with one another throughout their overlapping regions.*

ated species of the present study (for example, the fingerprint from band 4, the purest of the three 3'-coterminal mRNA's, Fig. 2d). We suggest that no second major poly(A) site exists within the region of the largest *Sma* I-D mRNA (Fig. 1, band 2). Messengers without poly(A) would not be detected in these experiments.

The presence of 3'-coterminal mRNA's in *Sma* I-D allows several observations regarding the arrangement of sequences that participate in Ad2 late RNA processing. The 3'-coterminal *Sma* I-D mRNA's are derived from a larger region of Ad2 DNA (Fig. 6), which gives rise to an apparently contiguous series of rightward-reading mRNA's (8, 26, 30, 33). Our previous studies (submitted for publication) identified a rightward mRNA 3' terminus [or poly(A) site] located in the restriction fragment *Sma* I-I/*Hin*dIII-J (coordinates 37.3-40.5). This poly(A) site maps in the vicinity of the predicted map position of the 5' end of the encoded sequences of the largest of the three coterminal mRNA's analyzed in the present report (Fig. 6). A similar close positioning of the poly(A) site and encoded 5' end occurs near coordinate 50. The 5' end of the main "body" sequences for the pVI mRNA, mapped at coordinate 49.9 by Chow et al. (8), lies close to the poly(A) site of the *Sma* I-D mRNA's (coordinates 49-50.2). Because late viral mRNA's have ligated or spliced 5' leader sequences (4, 7, 20), in these two cases leader attachment sites are juxtaposed to poly(A) sites.

The coterminal *Sma* I-D mRNA's, therefore, form a related group of messengers that are flanked on either side by rightward-transcribed sequences that may also appear in the cytoplasm as mRNA. The mechanism of forming the mRNA's within this group is not known. Each could be formed directly from an RNA primary transcript. In this case, three leader attachment sites (one for each 3'-coterminal mRNA) would be expected between ~38 and 50. Polyadenylation at 50 would be coupled with three separate choices for splicing the leader. Alternatively, the larger coterminal mRNA's may be obligatory intermediates to the formation of the shorter. Splicing of the leader to ~38 would occur whenever polyadenylation was at 50. Subsequent processing events would generate the shorter mRNA's. The complexity of Ad2 late processing is underscored by the fact that at least five poly(A) sites exist within the 16-100 region (Fraser and Ziff, submitted for publication). If the large primary transcript described by Bach-

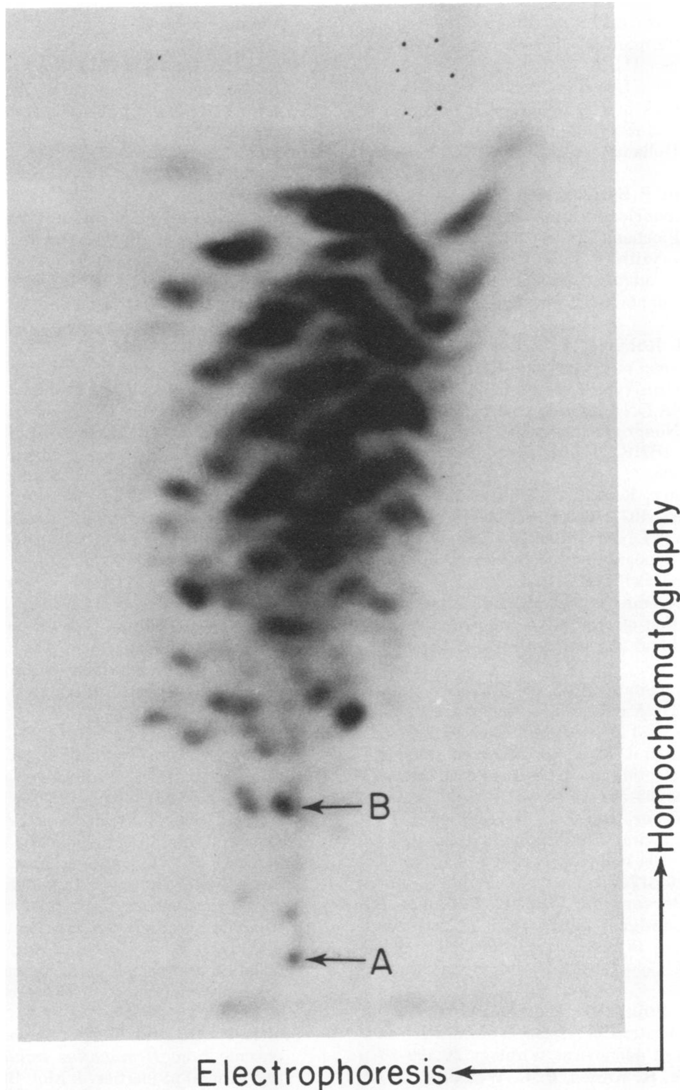

FIG. 7. *T1 RNase fingerprint of an in vitro [α-^{32}P]ATP-labeled transcript of Hpa II fragment X (same DNA as lane 4, Fig. 4B; coordinates 49-50.2, Fig. 6). Spots A and B from this fingerprint were characterized by RNase A digestion as described in the text.*

enheimer and Darnell (3) yields mRNA by an intramolecular mechanism, as suggested by Klessig (20), many different processing pathways may exist. To deduce these pathways, it will be of interest to determine the temporal order of leader splicing and polyadenylation and to analyze the structure of nuclear RNA intermediates to mature messenger.

ACKNOWLEDGMENTS

We acknowledge the technical assistance of D. Rosenweig-Baronofsky and J. Bailey-Miller. We thank J. Darnell and S. Bachenheimer for helpful discussions and A. English and L. Cousseau for aid in preparing the manuscript.

N.F. is the recipient of a Fulbright-Hayes Travel Fellowship. This work was supported by Public Health Service grants GM 21779 from the National Institute of General Medical Sciences to E.Z. and CA 16006-04 from the National Cancer Institute and by American Cancer Society grant NP-213G.

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