Cell

The Making of an mRNA

Brenton R. Graveley[1,*]
[1]Department of Genetics and Developmental Biology, University of Connecticut Stem Cell Institute, University of Connecticut Health Center, 400 Farmington Avenue, Farmington, CT 06030, USA
*Correspondence: graveley@neuron.uchc.edu

We are pleased to present a series of Annotated Classics celebrating 40 years of exciting biology in the pages of *Cell*. This installment revisits "An Amazing Sequence Arrangement at the 5′ Ends of Adenovirus 2 Messenger RNA" by Louise T. Chow, Richard E. Gelinas, Thomas R. Broker, and Richard J. Roberts. Here, Brenton Graveley comments on the work that first documented assembly of an mRNA sequence from discontinuous segments of DNA. The process came to be called pre-mRNA splicing, and Richard Roberts shared the 1993 Nobel Prize in Physiology or Medicine with Phillip Sharp for this discovery.

Each Annotated Classic offers a personal perspective on a groundbreaking *Cell* paper from a leader in the field, with notes on what stood out at the time of first reading and retrospective comments regarding the longer-term influence of the work.

To see Brenton Graveley's thoughts on different parts of the manuscript, just download the PDF and then hover over or double-click the highlighted text and comment boxes on the following pages. You can also view Graveley's annotation by opening the Comments navigation panel in Acrobat.

Cell, Vol. 12, 1–8, September 1977, Copyright © 1977 by M

An Amazing Sequence Arrangement at the 5′ Ends of Adenovirus 2 Messenger RNA

Louise T. Chow, Richard E. Gelinas, Thomas R. Broker and Richard J. Roberts
Cold Spring Harbor Laboratory
Cold Spring Harbor, New York 11724

Summary

The 5′ terminal sequences of several adenovirus 2 (Ad2) mRNAs, isolated late in infection, are complementary to sequences within the Ad2 genome which are remote from the DNA from which the main coding sequence of each mRNA is transcribed. This has been observed by forming RNA displacement loops (R loops) between Ad2 DNA and unfractionated polysomal RNA from infected cells. The 5′ terminal sequences of mRNAs in R loops, variously located between positions 36 and 92, form complex secondary hybrids with single-stranded DNA from restriction endonuclease fragments containing sequences to the left of position 36 on the Ad2 genome. The structures visualized in the electron microscope show that short sequences coded at map positions 16.6, 19.6 and 26.6 on the R strand are joined to form a leader sequence of 150–200 nucleotides at the 5′ end of many late mRNAs. A late mRNA which maps to the left of position 16.6 shows a different pattern of second site hybridization. It contains sequences from 4.9–6.0 linked directly to those from 9.6–10.9. These findings imply a new mechanism for the biosynthesis of Ad2 mRNA in mammalian cells.

Introduction

In contrast to the detailed knowledge of the mechanics of transcription in procaryotic cells (Losick and Chamberlin, 1976), little is known about this process in eucaryotic cells. Several possible schemes exist: one, analogous to the bacterial system, requires independent promoters for each mRNA; a second postulates the production of long primary transcripts in the nucleus which are subsequently cleaved to yield individual mRNAs (Darnell, Jelinek and Molloy, 1973); and a third invokes the use of RNA primers coded at one region on the genome but acting at some other region(s) and becoming elongated into mRNAs (Dickson and Robertson, 1976). Experiments to test these hypotheses directly have been hampered by the complexity of the eucaryotic genome. We have chosen to study these processes in a simpler system—lytic infection of human cells by adenovirus 2 (Ad2).

Ad2 DNA is transcribed by RNA polymerase II (Price and Penman, 1972; Wallace and Kates, 1972), and its transcription shows features characteristic of that of the host genome (Lewin, 1975a, 1975b). For example, long polyadenylated transcripts appear in the nucleus, but only a small percentage of this nuclear RNA appears as polyadenylated mRNA on cytoplasmic polysomes (Philipson et al., 1971). These mRNAs are "capped" at their 5′ ends (Moss and Koczot, 1976; Sommer et al., 1976). Gelinas and Roberts (1977) found that most Ad2 mRNAs isolated at late times during infection contain the same "capped" 11 nucleotide sequence at their 5′ ends. This sequence was sensitive to ribonuclease cleavage in mRNA:DNA hybrids (Gelinas and Roberts, 1977; Klessig, 1977) and led to the suggestion that this 5′ terminal sequence might not be coded immediately adjacent to the main body of the mRNA.

Thomas, White and Davis (1976) have shown that individual RNA molecules can be displayed as RNA displacement loops (R loops) in the electron microscope, and map coordinates have been obtained for many Ad2 mRNAs (Meyer et al., 1977; Chow et al., 1977). In the present studies, we have used mRNAs visualized in such R loops to examine more closely the sequences present at the 5′ end of late Ad2 mRNAs.

Results

R loops were formed between Ad2 DNA and polysomal RNA isolated 22 hr after Ad2 infection. The 5′ ends of the mRNA should form single-stranded projections if they are not coded immediately adjacent to the rest of the mRNA, and so might be visualized by hybridization to a single-stranded DNA fragment containing their complement. We therefore prepared a set of restriction endonuclease fragments of the Ad2 genome, separated their strands by agarose gel electrophoresis (Hayward, 1972; Sharp, Gallimore and Flint, 1974) and added each single strand in turn as a third hybridization component after the preparation of the R loops. Since R loops were formed from a mixed population of late mRNAs, many different species were examined simultaneously. By using a restriction endonuclease fragment as the single-stranded probe, complicated structures which might arise from hybridization of the probe to the single-stranded DNA segment of the R loop were limited to one region of the genome. Figure 1a shows the results of such an experiment using the slow strand of Hind III-B (map position 17.0–31.5) as the single-stranded DNA probe. The probe hybridized with the 5′ end of hexon mRNA in the R loop but not with the displaced DNA strand. It adopted a looped configuration, indicating that sequences from the 5′ end of the mRNA were complementary to two separate regions within the probe. The 5′ ends of other

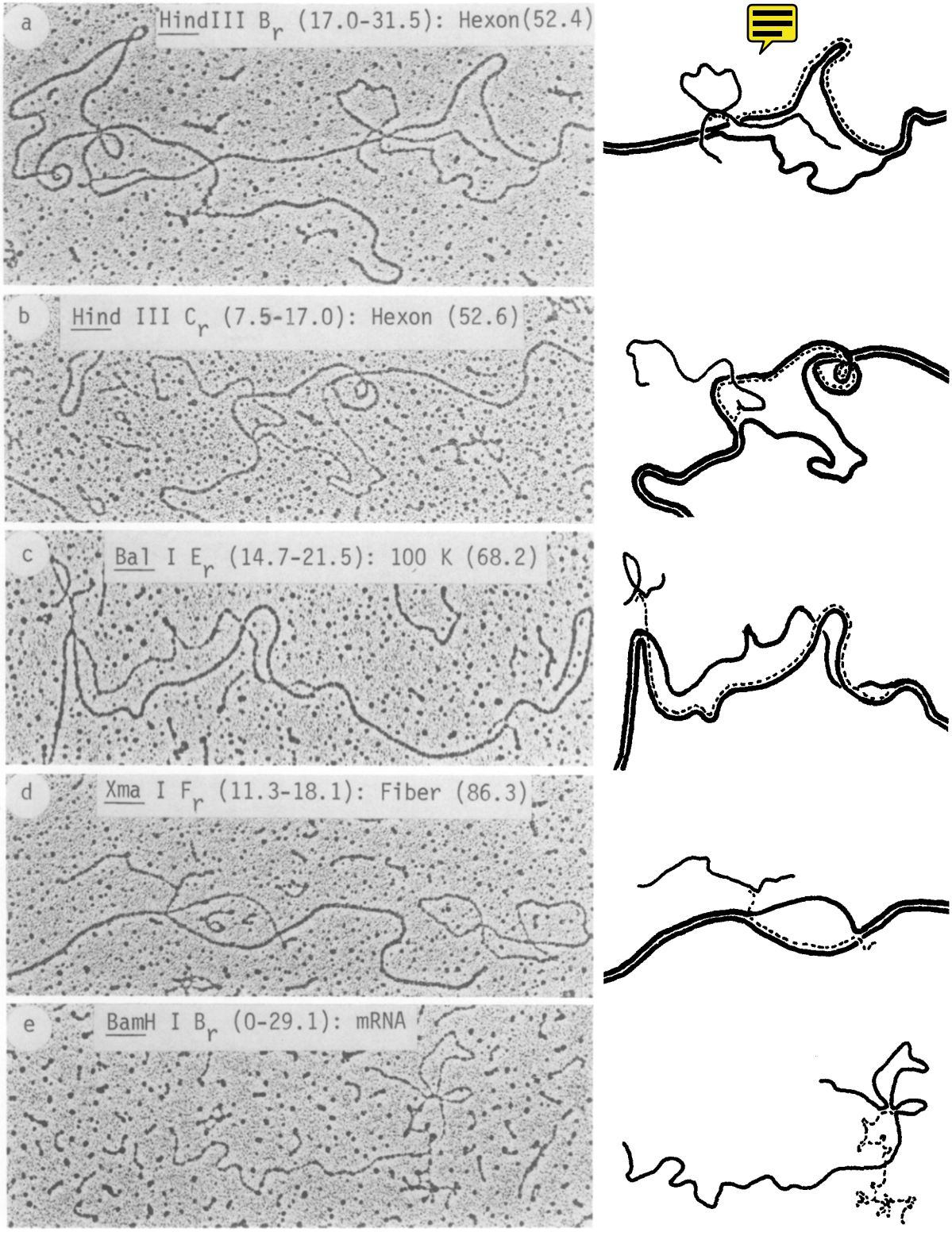
a
HindIII B_r (17.0-31.5): Hexon(52.4)
b
Hind III C_r (7.5-17.0): Hexon (52.6)
c
Bal I E_r (14.7-21.5): 100 K (68.2)
d
Xma I F_r (11.3-18.1): Fiber (86.3)
e
BamH I B_r (0-29.1): mRNA

Table 1. Map Coordinates of Labeled 5′ Termini of Late Ad2 mRNAs

mRNA Assignment	Previous Map Coordinates[a]	Map Coordinates of 5′ Label (Mean ± Standard Deviation)	Number R Loop Molecules Labeled with Restriction Endonuclease Fragments				
			Bam HI-B	Hind III-B	Bal I-E	Xma I-F	Total[b]
Core	–	36.6 ± 0.6	2	1	0	2	5
Penton	38.8	38.9 ± 1.0	1	3	3	0	7
Core	45.4	45.0 ± 1.0	1	6	1	1	9
pVI	49.9	49.8 ± 0.5	3	4	2	2	11
Hexon	51.9	52.2 ± 0.8	28	20	4	4	56
100K	67.9	67.9 ± 0.4	3	3	2	3	11
pVIII	74.6	74.1 ± 0.5	5	2	0	2	9
Fiber	86.3	86.4 ± 0.5	29	26	5	2	62
Totals			72	65	17	16	170

[a] Chow et al. (1977).
[b] Not included in the table are two molecules, each labeled at 66 and 71, that could be alternative 5′ ends for 100K and pVIII, respectively.

mRNAs also show identical two-site hybridization with the slow strand of Hind III-B and are compiled in Table 1. Length measurements place the contact points between the Hind III-B single strand and the mRNA at approximately 900 ± 60 nucleotides (42 measurements) from the end of the short arm and 1800 ± 120 nucleotides (42 measurements) from the end of the long arm. The distance between the two contact points on the DNA (the loop) was about 2400 ± 90 nucleotides (49 measurements). To orient these two arms, determine the strandedness and obtain accurate map positions for the points of hybridization, we used the separated strands of an overlapping fragment Bam HI-B (map position 0–29.1) in a similar experiment. The results are shown in Figure 2a, in which the slow strand of Bam HI-B is hybridized to the 5′ ends of both fiber and hexon mRNAs. In these cases and in others reported in Table 1, more complicated structures were observed. Three contact points between the single-stranded DNA probe and the 5′ end of the mRNA are now evident, and the Bam HI-B fragment is held into two loops. Length measurements give values of 5800 ± 180 nucleotides (39 measurements) for the long arm, 950 ± 100 nucleotides (58 measurements) for the short arm, 2400 ± 130 nucleotides (48 measurements) for the large loop and 1000 ± 100 nucleotides (47 measurements) for the small loop. Comparison with the hybridization sites on the Hind III-B strand suggests map positions of 16.6, 19.6 and 29.6 for the three segments of Ad2 DNA which hybridize to the 5′ ends of mRNA. Examination of these structures revealed that the contact point closest to the main portion of the mRNA was on the long arm of the Hind III-B fragment and on the short arm of the Bam HI-B fragment. Thus the 3′ end of the leader sequence is at 26.6 and the 5′ end is at 16.6. Because the mRNAs labeled by these probes are transcribed from the R strand from left to right, and because nucleic acids form anti-parallel base pairs, we conclude that these probes are from the R strand. Weingartner et al. (1976) have also shown that the slow strand of Bam HI-B is the R strand. All the mRNA species labeled are transcribed from the R strand (Sharp, Gallimore and Flint, 1974; Pettersson, Tibbetts and Philipson, 1976).

The 5′ terminal leader sequence of an mRNA in an R loop occasionally formed an intramolecular structure by hybridizing to its complementary DNA at coordinates 19.6 or 26.6 within the same DNA molecule. One example involving hexon mRNA is shown in Figure 2b. Such interaction constrains the intervening DNA, which often assumes a supercoiled configuration during spreading for electron microscopy.

To ensure that our interpretation of these structures was correct, we performed a number of control experiments. In separate hybridizations, single strands from restriction fragments encompassing

Figure 1. Hybridization of Rightward-Transcribed Strands (r) of Restriction Fragments to the Common 5′ Leader Sequences of Late Ad2 mRNA (e) or mRNAs in R Loops on Ad2 DNA (a–d)

(a) represents Hind III-B_r annealed to mRNA for hexon; (b) represents Hind III-C_r annealed to mRNA for hexon; (c) represents Bal I-E_r annealed to mRNA for the 100K protein; (d) represents Xma I-F_r annealed to mRNA for fiber; (e) represents Bam HI-B_r annealed to free mRNA. Map coordinates covered by each restriction fragment and locations of the hybridization are given in parentheses. Illustrative tracings are provided. (——) Ad2 DNA; (——) restriction fragments; (----) mRNA. In (c) and (d), most of the RNA "bridge" between the R loop and the restriction fragment is due to branch migration of the mRNA. The remaining portion is due to the unhybridized leader sequence.

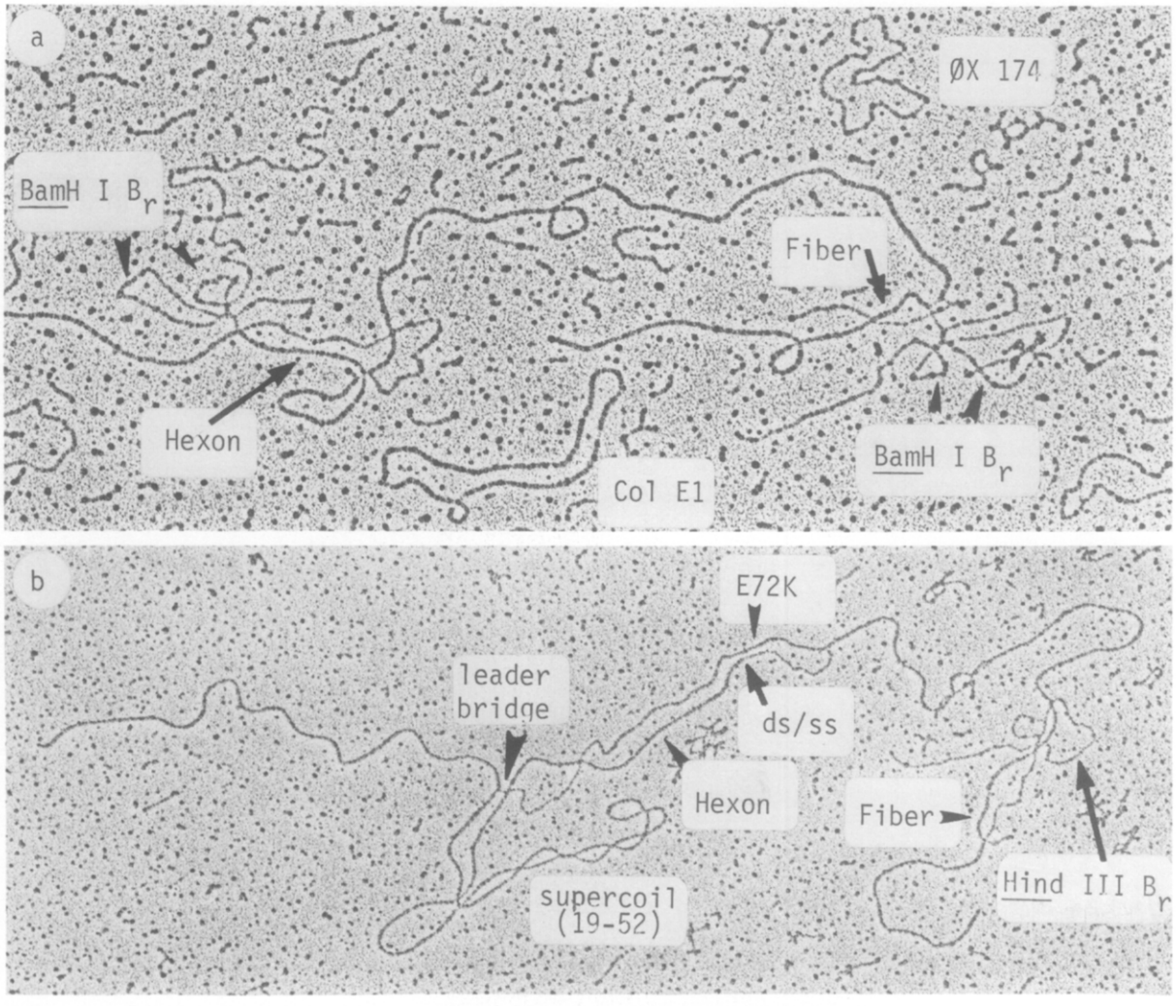

Figure 2. Multiple Site Hybridization of the 5′ Leader Sequences of the Hexon and Fiber mRNAs in R Loops

(a) Hybridization with the R strand (r) of the Bam HI-B restriction fragment. Arrows point to the DNA:RNA hybrids. Arrowheads point to the large and small loops formed in Bam HI-B_r DNA due to the hybridization. An additional 100 nucleotides at the 5′ ends of the hexon R loops have been displaced by branch migration. The spreading force during the preparation of grids has denatured about 200 nucleotides at the 5′ end of the fiber mRNA/DNA hybrid. (b) The leader of the fiber mRNA in an R loop was labeled by an added R strand of the Hind III-B_r fragment. The leader on the hexon mRNA was labeled by intramolecular hybridization to complementary DNA at coordinate 19 on the same molecule. The intervening DNA segment was constrained, and it formed tertiary superhelical twists when solvent conditions were changed during preparation of the sample for electron microscopy. The hexon RNA formed a convergent R loop with the mRNA for the E72K protein hybridized to the opposite (L) DNA strand.

the entire Ad2 genome were used as probes, and a summary of these data is shown in Figure 3. Only the slow strands of Hind III-B (Figure 1a, two contacts at 19.6 and 26.6), Hind III-C (Figure 1b, one contact at 16.6), Bam HI-B (Figure 2a, three contacts at 16.6, 19.6 and 26.6), Bal I-E (Figure 1c, two contacts at 16.6 and 19.6) and Xma I-F (Figure 1d, one contact at 16.6) showed consistent hybridization to RNA branches at the 5′ ends of R loops. In particular, it should be noted that the fast strands of these five fragments did not interact with any of the R loops. When the slow strands of Bam HI-B or Bal I-E were incubated alone and spread under identical conditions, no loops of the same size or with the same coordinates as those formed in the presence of mRNA were detected. If polysomal RNA was present during the incubation of the slow strand, but not the fast strand, of Bam HI-B, however, loops of the type shown in Figure 1e (identical to those seen at the 5′ ends of many late mRNAs in R loops) were frequently observed and were associated with collapsed RNA. The possibility that sequences at map positions 16.6, 19.6 and 26.6 were reiterated on the Ad2 genome was tested by isolating small fragments of the genome containing these sequences, labeling them to high specific

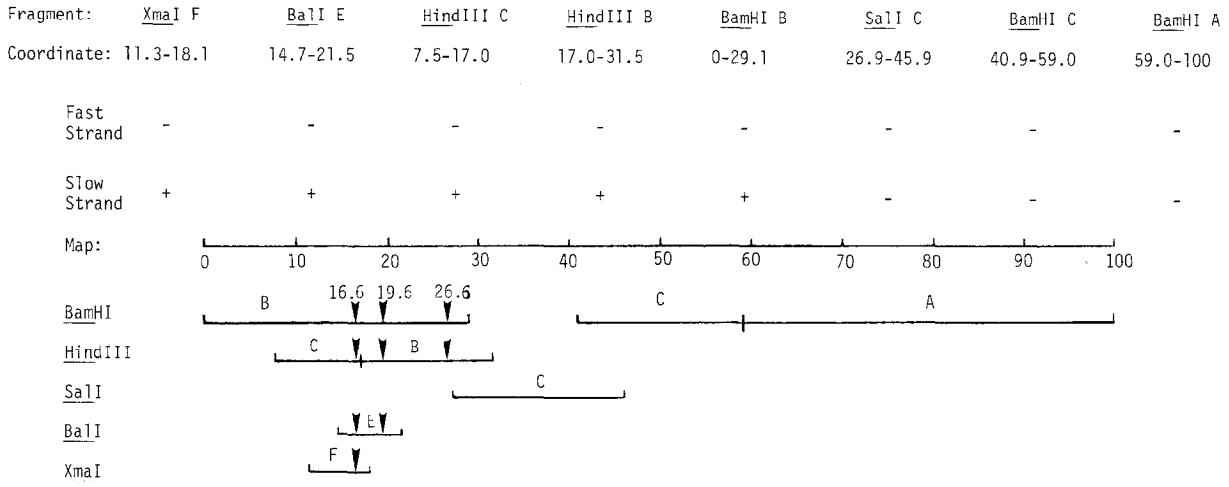

Figure 3. Hybridization of Separated Strands of Ad2 DNA Restriction Fragments to the 5′ Leader Sequence of Late Polysomal mRNAs.
Strands from restriction fragments spanning the entire Ad2 genome were used in separate experiments to label the R loops. All (+) slow strands come from the R strand, as discussed in Results. (+) indicates consistent hybridization of the strands to the leader sequences of the mRNAs in R loops; (−) indicates negative results. Arrowheads point to the locations of hybridization on the R strands of the fragments. The map coordinates of the restriction fragments are obtained from C. Mulder and R. Greene for Bam HI and Xma I (unpublished observations), from R. J. Roberts and J. Sambrook for Hind III; from J. R. Arrand and R. J. Roberts for Sal I (unpublished observations); and from R. E. Gelinas and R. J. Roberts for Bal I (unpublished observations).

activity in vitro by nick translation and using them as hybridization probes against fragments of the Ad2 genome immobilized on nitrocellulose filters (Southern, 1975). In each case, as shown in Figure 4, the fragments rehybridized only to that region of the genome from which they were derived and failed to hybridize to any other sequences on the Ad2 genome.

Hybrids between any one component of the leader sequences of the mRNA in an R loop and single-stranded DNA probes are stable in 70% formamide, 0.4 M NaCl, 0.1 M HEPES at 30°C, and yet there is only a hint of a duplex at positions 26.6 and 19.6 when Bam HI-B and Hind III-B are used, or at position 19.6 in the Bal I-E fragment. The duplex regions were measured to be 50–100 nucleotides at each of these two positions, and we believe it is improbable that more than a total of 200 nucleotides are involved at all three contact points.

The results described above refer to transcripts located to the right of position 36. Several other late mRNAs are known to map to the left of this coordinate. One of these, coding for polypeptide IVa_2 (map position 14.9–11.2), is transcribed from the L strand, and a second, coding for virion-associated component IX (map position 9.7–11.0), is transcribed from the R strand (Chow et al., 1977; U. Pettersson and M. B. Mathews, manuscript submitted). Both have been visualized in R loops, but neither showed secondary hybridization with any of the fragments used in this study. Some of the R loops formed by a polysomal RNA which contains sequences from coordinates 9.6 (± 0.2)–10.9 (± 0.2) (24 measurements each), however, have an unusual structure. Sequences from the 5′ end of this RNA form a second R loop with a noncontiguous region of the Ad2 genome located between coordinates 4.9 (± 0.3)–6.0 (± 0.2) (Figures 5a and 5b). As a result, the intervening double-stranded DNA was held into a third loop, and a short bridge of displaced RNA between the two R loops is clearly visible. This structure has frequently been observed in molecules containing a convergent R loop formed between IVa_2 mRNA and this new RNA (Figure 5c). Because a strand switch at 11.0 can be seen in this structure, as has been observed earlier (Chow et al., 1977), the new RNA species can be assigned to the R strand.

Discussion

The results presented in this paper show that sequences present at three separated sites (16.6, 19.6, 26.6) on the R strand of the Ad2 genome are complementary to a continuous sequence at the 5′ end of late Ad2 mRNAs that are transcribed from the R strand and map to the right of position 36. Since these sequences are available for hybridization when mRNA is displayed in R loops and are not reiterated elsewhere in the Ad2 genome, we conclude that they are not coded at a site immediately adjacent to the main portion of the mRNAs. Biochemical evidence by Gelinas and Roberts (1977) and Klessig (1977) has been presented to support this idea. Since it seems improbable to us that the sequence present at the 5′ end of many of these late Ad2 mRNAs is actually coded by the host genome and is only complementary to these three

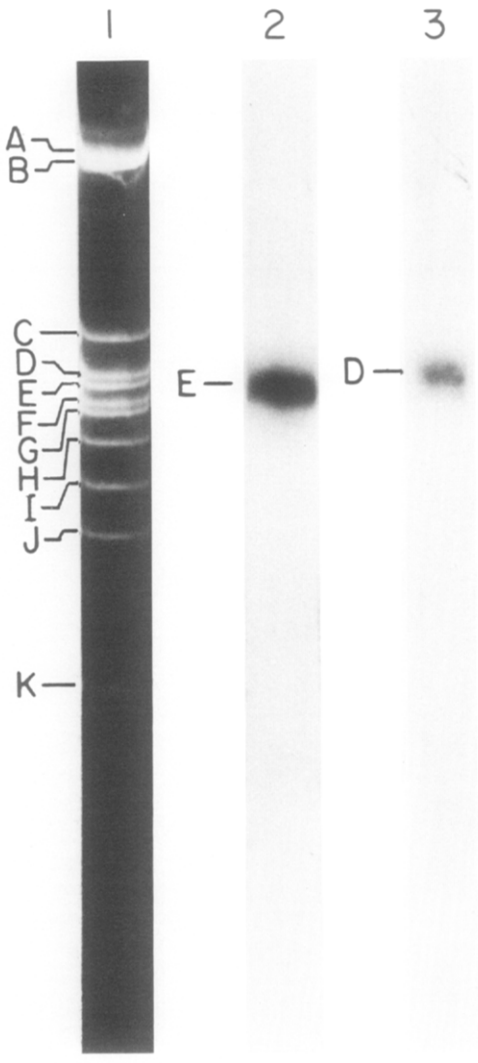

Figure 4. Hybridization of Bal I-E DNA (14.7–21.5) and Bal I-D DNA (21.5–28.5) to Bal I Fragments of Ad2 DNA

Bal I-E and -D fragments of Ad2 DNA were isolated after two cycles of purification by agarose gel electrophoresis, labeled with ^{32}P by nick translation and used as hybridization probes against all Bal I fragments of Ad2 DNA bound to nitrocellulose membranes. Slot 1 represents 2.0 μg of Bal I fragments of Ad2 DNA fractionated on a 1.4% agarose gel and stained with ethidium bromide. The same amount of DNA was present in slots 2 and 3. Slot 2 represents ^{32}P-Bal I-E DNA (10^6 dpm; about 20 μg) hybridized to Bal I fragments of Ad2 DNA. Slot 3 represents ^{32}P-Bal I-D DNA (10^6 dpm; about 20 μg) hybridized to Bal I fragments of Ad2 DNA. The minimal length of sequence homology which can be detected by this method has not been determined.

Ad2 sequences by chance, we believe that these sequences are probably transcribed from positions 16.6, 19.6 and 26.6 on the R strand of the Ad2 genome, and that their juxtaposition is an inherent feature of Ad2 mRNA biosynthesis.

Two mRNAs (for polypeptides IVa_2 and IX) mapping to the left of position 30 seem to have a different sequence arrangement at their 5′ ends. Particularly surprising is the finding that a polysomal RNA containing sequences from coordinate 9.6–10.9, the coding region for component IX, has an additional sequence at its 5′ end which is complementary to a noncontinguous segment from 4.9–6.0. This RNA may be related to early transcripts for E15K, which map between 5.0–11.0 or between 5.0–6.4 (Chow et al., 1977), and also to the component IX mRNA, which maps between 9.7–11.0 (Chow et al., 1977; U. Pettersson and M. B. Mathews, manuscript submitted). The absence of the tripartite leader and the occurrence of this new mRNA would account for the hybridizational and translational data reported for mRNAs originating from this region of the genome (Lewis, Anderson and Atkins, 1977).

These observations, together with the results presented in the accompanying papers on late Ad2 mRNA (Klessig, 1977; Lewis, Anderson and Atkins, 1977) and on Ad2-SV40 mRNA (Dunn and Hassell, 1977) are not directly consistent with any mechanism previously suggested for the biosynthesis of mRNA in eucaryotic cells. They imply that an alternate scheme must exist for Ad2 mRNAs, and perhaps for eucaryotic mRNA in general. One such mechanism is outlined in the accompanying paper by Klessig (1977). The experiments described herein provide a convenient method to map accurately the 5′ termini of Ad2 mRNAs, and have confirmed many of the previous assignments (Chow et al., 1977) and established new ones. We have recently learned of similar experiments by Berget, Moore and Sharp (1977) who used electron microscopy to examine hybrids between purified hexon mRNA and single strands of DNA. They observed that the 5′ terminal mRNA sequence appeared as a single-stranded tail, which was complementary to three noncontiguous regions of the Ad2 genome with map coordinates essentially identical to those reported here.

Experimental Procedures

Restriction Endonucleases

Bal I (Gelinas et al., 1977) and Xma I (Endow and Roberts, 1977) were purified as described. Bam HI, Sal I and Xma I were purified by unpublished procedures of P. A. Myers and R. J. Roberts. In all cases, DNA was digested at 37°C in 6 mM Tris–HCl (pH 7.9), 6 mM $MgCl_2$ and 6 mM 2-mercaptoethanol.

Isolation of Viral DNA and RNA

DNA was prepared from Ad2 virions grown on HeLa or KB cells in suspension cultures as described by Pettersson and Sambrook (1973) and Pettersson et al. (1973). Fragments of the Ad2 genome were produced by digestion with the restriction endonucleases Bal I, Bam H-I, Hind III, Sal I and Xma I. DNA fragments were fractionated by agarose slab gel electrophoresis (Sugden et al., 1975) and recovered from the agarose by chromatography on hydroxylapatite (Lewis et al., 1975), or by homogenization and diffusion followed by phenol extraction. Ad2 mRNA was a gift from Dr. J. B. Lewis. Polysomes were isolated 22 hr after Ad2 infection of KB cells by the method of Schreier and Staehelin (1973), and the RNA was recovered by the method of Anderson et al. (1974).

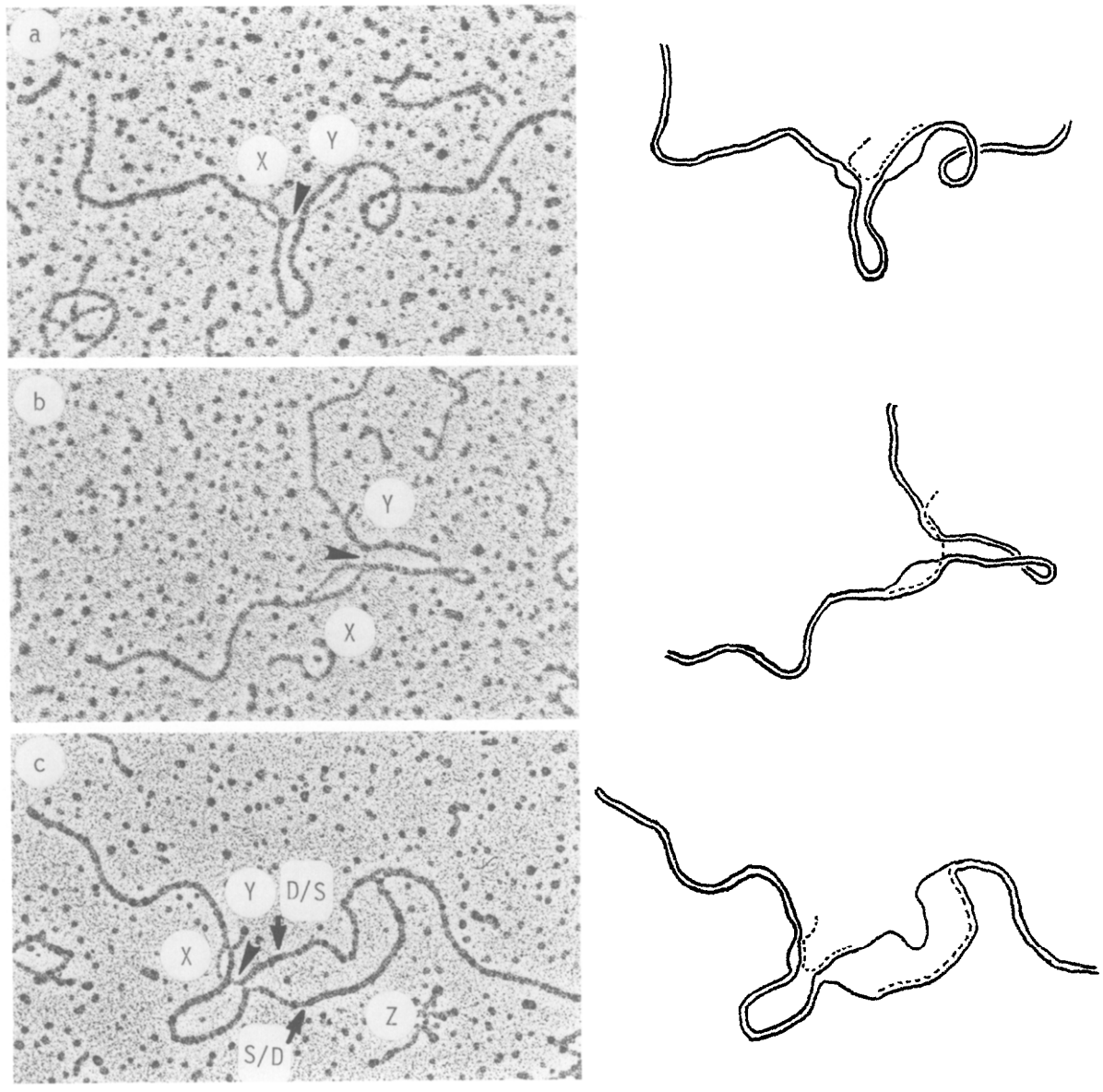

Figure 5. R Loops Formed between Ad2 DNA and a Polysomal RNA Containing Sequences from Map Coordinates 4.9–6.0 (X) and 9.6–10.9 (Y)

(a) Sequence Y, with the same coordinates as the mRNA for peptide IX, is totally contained in an R loop, whereas sequence X, possibly coding for the 15K protein, is only partially contained in an R loop with its 5′ end displaced as a tail. (b) Y is present in a collapsed and partially displaced R loop, whereas X is totally contained in an R loop. (c) X is in a partial R loop. Y is in a convergent R loop with the mRNA (Z), tentatively assigned to peptide IVa_2, on the opposite DNA strand. The RNA bridge (indicated by arrowheads) between X and Y is visible because of some RNA displacement by the intervening DNA segment. D/S and S/D indicate the double-strand/single-strand junctions in the convergent R loop.

Strand Separation of Endonuclease Fragments

Purified restriction fragments were denatured in 0.25 M NaOH and subjected to electrophoresis on 1.4% agarose slab gels containing the Tris-phosphate-EDTA buffer described by Hayward (1972), but at half the stated ionic strength. After electrophoresis, bands of single strands were located by staining with ethidium bromide. Single-stranded DNA was recovered by homogenizing the gel in several volumes of 0.01 M Tris-HCl (pH 7.9), 0.001 M EDTA and allowing the DNA to diffuse out for several hours. The aqueous supernatant was extracted first with phenol and then with chloroform. E. coli rRNA was added as carrier, and the single-stranded DNA was recovered by ethanol precipitation.

Filter Hybridizations

Bal I-E (14.7–21.5) and Bal I-D (21.5–28.5) DNAs were labeled in vitro by nick translation (Kelly et al., 1970) as described by Maniatis et al. (1975) and were used as probes to challenge Bal I fragments of Ad2 DNA adsorbed to nitrocellulose membranes by the method of Southern (1975).

Electron Microscopy

R loops were formed on intact Ad2 DNA at 51.5°C for 14–16 hr as described previously (Chow et al., 1977). Aliquots were diluted with an equal volume of the same buffer-formamide mixture containing purified, separated strands of Ad2 restriction fragments.

The concentration of the single strands was 5–10 μg/ml. The solution was returned to the water bath and cooled to 42 or 30°C over a period of 3–5 hr. Electron microscope grid preparation and data processing have been described by Chow et al. (1977). Single-stranded φX174 (5375 bases) and double-stranded φX174 RF or Col E1 DNA (6300 base pairs) were included as internal length standards.

Acknowledgments

We thank Dr. J. B. Lewis for a gift of late polysomal RNA; J. Bonventre, P. A. Myers and J. Scott for technical assistance; and M. Moschitta for secretarial help. This work was supported by a grant from the National Cancer Institute.

Received June 9, 1977; revised July 5, 1977.

Note Added in Proof

The mRNA for IVa_2 (14.9–11.2, L strand) also has a short single component leader present at its 5′ end. There is only a short deletion of the RNA sequences between the leader and the coding sequences, which is visible in Figure 5c as a small loop.

References

Anderson, C. W., Lewis, J. B., Atkins, J. F. and Gesteland, R. F. (1974). Proc. Nat. Acad. Sci. USA *71,* 2756–2760.

Berget, S. M., Moore, C. and Sharp, P. A. (1977). Proc. Nat. Acad. Sci. USA, in press.

Chow, L., Roberts, J. M., Lewis, J. B. and Broker, T. R. (1977). Cell *11,* 819–836.

Darnell, J. E., Jelinek, W. R. and Molloy, G. R. (1973). Science *181,* 1215–1221.

Dickson, E. and Robertson, H. D. (1976). Cancer Res. *36,* 3387–3393.

Dunn, A. R. and Hassell, J. A. (1977). Cell *12,* 23–36.

Endow, S. A. and Roberts, R. J. (1977). J. Mol. Biol. *112,* 521–529.

Gelinas, R. E. and Roberts, R. J. (1977). Cell *11,* 533–544.

Gelinas, R. E., Myers, P. A., Weiss, G. H., Murray, K. and Roberts, R. J. (1977). J. Mol. Biol., in press.

Hayward, G. S. (1972). Virology *49,* 342–344.

Kelly, R. B., Cozzarelli, N. R., Deutscher, M. P., Lehman, I. R. and Kornberg, A. (1970). J. Biol. Chem. *245,* 39–45.

Klessig, D. F. (1977). Cell *12,* 9–21.

Lewin, B. (1975a). Cell *4,* 11–20.

Lewin, B. (1975b). Cell *4,* 77–93.

Lewis, J. B., Anderson, C. W. and Atkins, J. F. (1977). Cell *12,* 37–44.

Lewis, J. B., Atkins, J., Anderson, C., Baum, P. and Gesteland, R. (1975). Proc. Nat. Acad. Sci. USA *72,* 1344–1348.

Losick, R. and Chamberlin, M. (1976). RNA Polymerase (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press), p. 899.

Maniatis, T., Jeffrey, A. and Kleid, D. G. (1975). Proc. Nat. Acad. Sci. USA *72,* 1184–1188.

Meyer, J., Neuwald, P. D., Lai, S. P., Maizel, J. V., Jr. and Westphal, H. (1977). J. Virol. *21,* 1010–1018.

Moss, B. and Koczot, F. (1976). J. Virol. *17,* 385–392.

Pettersson, U. and Sambrook, J. (1973). J. Mol. Biol. *73,* 125–130.

Pettersson, U., Tibbetts, C. and Philipson, L. (1976). J. Mol. Biol. *101,* 479–501.

Pettersson, U., Mulder, C., Delius, H. and Sharp, P. A. (1973). Proc. Nat. Acad. Sci. *70,* 200–204.

Philipson, L., Wall, R., Glickman, G. and Darnell, J. E. (1971). Proc. Nat. Acad. Sci. USA *68,* 2806–2809.

Price, R. and Penman, S. (1972). J. Virol. *9,* 621–626.

Schreier, M. H. and Staehelin, T. (1973). J. Mol. Biol. *73,* 329–349.

Sharp, P. A., Gallimore, P. H. and Flint, S. J. (1974). Cold Spring Harbor Symp. Quant. Biol. *39,* 457–474.

Sommer, S., Salditt-Georgieff, M., Bachenheimer, S., Darnell, J. E., Furuichi, Y., Morgan, M. and Shatkin, A. J. (1976). Nucl. Acids Res. *3,* 749–765.

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

Sugden, B., DeTroy, B., Roberts, R. J. and Sambrook, J. (1975). Anal. Biochem. *68,* 36–46.

Thomas, M., White, R. L. and Davis, R. W. (1976). Proc. Nat. Acad. Sci. USA *73,* 2294–2298.

Wallace, R. D. and Kates, J. (1972). J. Virol. *9,* 627–635.

Weingartner, B., Winnacker, E-L., Tolun, A. and Pettersson, U. (1976). Cell *9,* 259–268.