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In vivo transcription from the adenovirus E2 early promoter by RNA polymerase III

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ABSTRACT **We have previously reported that the subgroup C adenovirus E2 early (E2E) RNA polymerase II promoter can specify efficient *in vitro* transcription by RNA polymerase III. We now show that promoter proximal sequences of the E2E transcription unit are also transcribed by RNA polymerase III in nuclei isolated from adenovirus-infected cells. Small E2E RNA species that possessed the same properties as *in vitro* synthesized RNA polymerase III E2E transcripts were detected in cytoplasmic RNA populations from infected cells by using blotting, primer extension, and RNase protection assays. The 3′ termini of these RNAs were mapped to thymidine-rich sequences typical of RNA polymerase III termination sites. These results demonstrate that a single gene can be transcribed by both RNA polymerase II and RNA polymerase III *in vivo*.**

The ubiquitous function of the TATA-binding protein (TBP; see ref. 1) in initiation of eukaryotic transcription (refs. 2 and 3; see also refs. 4 and 5) suggests that the three eukaryotic RNA polymerases employ fundamentally similar mechanisms of initiation (4, 5). Certain RNA polymerase (pol) III promoters, moreover, closely resemble typical pol II promoters (6–8): for example, vertebrate U6 promoters comprise a set of upstream elements, including a TATA sequence essential for recognition by pol III (6–8). These properties suggest that some promoters might be transcribed by both enzymes. Although pol II transcription from the *Xenopus* U6 promoter and pol III transcription from several pol II promoters (9–12, 27) have been observed in experimental situations, such dual transcription has not been demonstrated *in vivo*. A pol III promoter which shares upstream elements with the well-characterized adenovirus type 2 (Ad2) E2E pol II promoter, is active *in vitro* (11). The comparable *in vitro* activities of the pol II and pol III E2E promoters prompted us to search adenovirus-infected cells for analogues of products of *in vitro* pol III transcription (Fig. 1).

MATERIALS AND METHODS

Cells and Virus. HeLa cells were maintained in suspension culture and infected with Ad2 (20–25 plaque-forming units per cell) as described (13).

Synthesis and Labeling of Complementary E2E RNAs and Oligonucleotides. Antisense RNAs complementary to positions −17 to +120 or +3 to +199 of the Ad2 E2E gene were synthesized with SP6 RNA polymerase. The latter RNA was synthesized in reaction mixtures containing [^{3}H]UTP (40 Ci/mmol, New England Nuclear/DuPont; 1 Ci = 37 GBq), and 1–2 pmol of the product was then 3′ end-labeled with [^{32}P]pCp (3000 Ci/mmol, Amersham) and T4 RNA ligase (14). The full-length RNA was electrophoretically purified. Oligonucleotides complementary to E2E positions −15 to +45 (exon 1) and +68 to +89 (intron 1) and to positions 10–36 of the coding, third exon (see Fig. 1*A*) were synthesized, purified, and 5′ end-labeled as described (11).

Run-On Transcription Assays. RNA labeled in run-on transcription mixtures containing 10^8 nuclei from Ad2-infected cells was purified and hybridized (15) to dot blots carrying the E2E −17 to +120 antisense RNA (2 μg per dot) or linearized plasmids containing either the Ad2 major late (ML) L2 plus tripartite leader sequences (2.5 μg per dot) or the viral VA-RNA$_I$ gene (2.5 μg per dot). Membranes were exposed for autoradiography, or the hybridized RNA was eluted by heating at 95°C for 10 min in 5 mM EDTA prior to electrophoresis in 6% polyacrylamide sequencing gels (16).

***In Vitro* Transcription.** pol III transcripts of the Ad2 E2E promoter were synthesized in the presence of α-amanitin (2 μg/ml) (17).

Preparation and Analysis of RNA from Ad2-Infected Cells. Cytoplasmic RNA was purified from uninfected or Ad2-infected HeLa cells (18) and separated into poly(A)-containing and poly(A)-lacking populations (17), or into populations precipitable or soluble in 2.5 M LiCl. Small RNAs were separated by electrophoresis in 10% polyacrylamide gels containing 7 M urea and were electrophoretically transferred to nylon membranes. mRNA and pre-mRNA were electrophoresed in 1.2% agarose gels containing 0.5 M formaldehyde and transferred to 0.4-μm nitrocellulose membranes. Wet membranes were UV irradiated for 90 sec and baked at 80°C for 1 hr in a vacuum oven. Prehybridization and hybridization to 5–10 fmol of 5′-end-labeled oligonucleotides, at appropriate temperatures, were as described by Church and Gilbert (19). In protection assays, RNA samples were coprecipitated with 5–10 fmol of the E2E antisense RNA 3′ end-labeled at position +2, prior to hybridization and digestion with RNase T1 (Calbiochem, 15–30 units) as described (11). Primer extension using 10 fmol of 5′-end-labeled intron 1 oligonucleotide per reaction was performed as described (20).

RESULTS

Transcription of the Adenoviral E2E Gene by pol III in Isolated Nuclei. To determine whether the adenovirus E2E pol III promoter identified *in vitro* (see Introduction) operated in infected cells, we examined the sensitivity of E2E transcription in isolated nuclei to increasing concentrations of α-amanitin. The RNA labeled during run-on transcription in nuclei isolated 18 hr after Ad2 infection was hybridized to an E2E antisense RNA (+120 to −17), as well as to adenoviral ML and VA-RNA$_I$ DNAs, which are transcribed by pol II and pol III, respectively (21). Transcription of VA-RNA$_I$ and E2E RNAs was refractory to α-amanitin at 1 μg/ml but sensitive to 150 μg/ml (Fig. 2*A*), suggesting that the E2E gene can be transcribed by pol III in isolated nuclei. To compare the putative E2E pol III transcripts synthesized in infected

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Abbreviations: Ad2, adenovirus type 2; ML, major late; pol, RNA polymerase.

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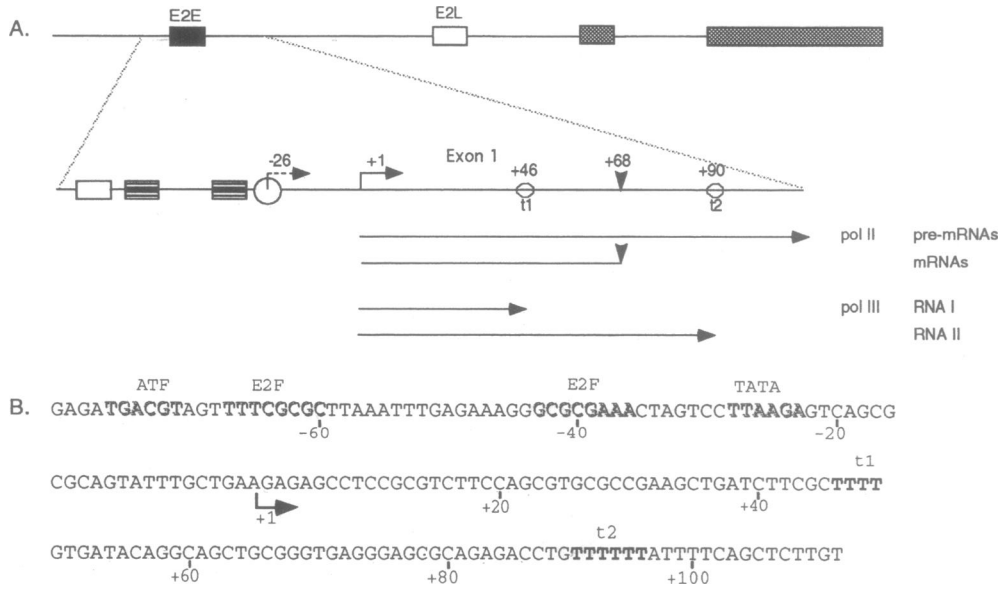

FIG. 1. E2 transcription units of subgroup C adenovirus. (*A*) Exons are represented by boxes and introns by lines. All E2E mRNAs include exons 2 and 3 shown in gray, but those expressed from the early (E2E) and late (E2L) promoters carry unique 5′-terminal exons, shown in black and white, respectively. The 5′ end of the viral genome is represented below by the solid line, and the major (+1) and minor (−26) sites of pol II initiation by the solid and dashed arrows, respectively. The vertical arrowhead at position +68 represents the 5′ splice site between exon 1 and intron 1 of E2E pre-mRNA. ATF and E2F binding sites and the TATA-like sequence are shown by the open and striped boxes and the circle, respectively, and termination sites for pol III transcription *in vitro* (11) by octagons. The pre-mRNA and mRNA products of pol II transcription and small RNA species transcribed by pol III *in vitro* are drawn in the direction of transcription. (*B*) The sequence of this segment of the Ad2 E2E transcription unit, with upstream promoter elements indicated in open-face type, and the *in vitro* sites of pol III termination in bold type.

cell nuclei with those made by this enzyme *in vitro*, labeled RNA was selected by hybridization to E2E antisense RNA and analyzed by electrophoresis. Viral ML and VA-RNA$_I$ transcripts were examined in the same way. No ML RNA was detected among products of run-on transcription reactions containing α-amanitin at 1 or 150 μg/ml (Fig. 2*B*, lanes 8 and 9), but VA-RNA$_I$, 157–160 nt long, (21), was efficiently synthesized in reaction mixtures containing low, but not high, concentrations of the drug (Fig. 2*B*, lanes 6 and 7). Thus, synthesis of pol II (ML) and pol III (VA-RNA$_I$) transcripts in adenovirus-infected cell nuclei exhibited the expected responses to increasing concentrations of α-amanitin. Three E2E RNA species, which comigrated with the products of *in vitro* pol III transcription, were labeled in isolated nuclei in the presence of α-amanitin at 1 μg/ml (Fig. 2*B*, lanes 3 and 4), but their synthesis was inhibited by α-amanitin at 150 μg/ml (Fig. 2*B*, lanes 3 and 5). As transcription of the small E2E RNA species exhibited the identical response to increasing α-amanitin concentration as synthesis of the well-characterized pol III product VA-RNA$_I$ (Fig. 2*B*), we conclude that they were transcribed by pol III.

The pol III-transcribed E2E RNA species of some 49 and 90/91 nt (Fig. 2*B*), subsequently designated E2E RNA I and RNA II, respectively, exhibited the lengths predicted for transcripts initiating at the +1 E2E initiation site and terminating at the t1 and t2 sites, respectively (Fig. 1); these termination sites are used by pol III *in vitro* (11). The largest E2E product of both run-on and *in vitro* transcription reactions (Fig. 2*B*, lanes 3 and 4), could be made by termination at the t2 site, but with initiation at the −26 site (Fig. 1) (11).

Small E2E Transcripts Are Present in Ad2-Infected Cells. The results of run-on transcription assays established that, in Ad2-infected cell nuclei, pol III can transcribe the 5′ segment of the E2E gene into RNA species indistinguishable from the E2E RNAs made by this enzyme *in vitro*. The failure of pol III to transcribe promoter-proximal sequences of the ML transcription unit (Fig. 2) indicated that this enzyme was not randomly initiating transcription from pol II promoters. Nevertheless, such experiments do not demonstrate that the E2E promoter is transcribed by pol III in intact cells. We therefore sought RNA species corresponding to the *in vitro* products of pol III E2E transcription in RNA populations of Ad2-infected cells, initially using blotting assays.

Based on the results of preliminary RNase protection assays, cytoplasmic RNA was prepared from Ad2-infected cells harvested during the late phase of infection, for hybridization to a series of E2E oligonucleotides designed to permit positive identification of pol III transcripts corresponding to those transcribed *in vitro*. Representative results of such experiments, in which a single set of infected-cell RNA samples was analyzed, are shown in Fig. 3. An intron 1 oligonucleotide hybridized to infected-cell nuclear RNAs of ≥5 kb, but no cytoplasmic E2E mRNA was detected (Fig. 3*A*, lanes 3 and 2, respectively), even when autoradiograms were overexposed. As expected (Fig. 1*A*), this oligonucleotide also detected the 91-nt RNA transcribed by pol III *in vitro* from a wild-type E2E template, but not the RNA synthesized from a template lacking the t2 termination site (Fig. 3*D*, lanes 3 and 4). A comigrating RNA species was present in cytoplasmic RNA of Ad2-infected, but not uninfected, HeLa cells (Fig. 3*D*, lanes 1 and 2). By contrast, an exon 3 oligonucleotide labeled to comparable specific activity detected abundant E2A mRNA (Fig. 3*B*, lane 2) but no small RNA species among infected-cell cytoplasmic RNA or *in vitro* synthesized pol III E2E RNAs (Fig. 3*E*). The results shown in Fig. 3 *A–C* indicate that neither intron 1-containing pol II transcripts (i.e., pre-mRNA or alternatively spliced mRNA) nor products of E2E mRNA degradation were present in the cytoplasm at greater than about 1% of the E2E 2-kb mRNA concentration. Thus, putative products of degradation of intron 1-containing pol II transcripts recovered in cytoplasmic fractions would be present at a concentration well below the detection limit of this assay.

If the 91-nt E2E RNA species were a product of specific pol III transcription *in vivo*, it should also contain exon 1 sequences (Fig. 1*A*). Two small RNA species, with apparent lengths of 45 and 91 nt, were readily detected among the products of *in vitro* pol III transcription from the E2E

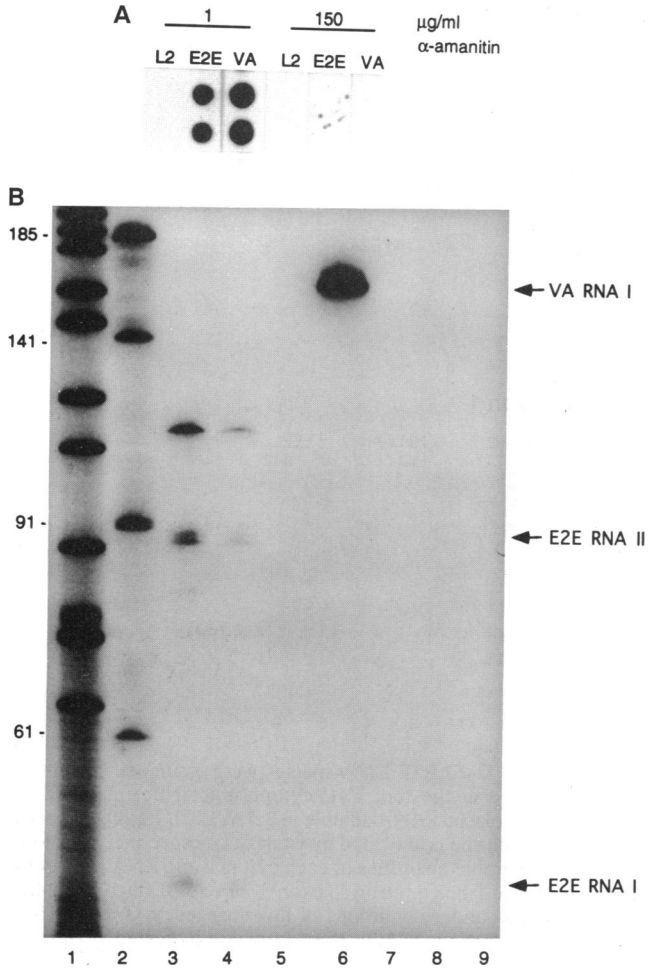

FIG. 2. E2E transcription by pol III in nuclei from adenovirus-infected cells. (*A*) RNA synthesized in the presence of 1 or 150 μg/ml α-amanitin was purified and hybridized to the probes indicated. (*B*) RNA transcribed in the presence of α-amanitin at 1 μg/ml (lanes 3, 6, and 8) or 150 μg/ml (lanes 5, 7, and 9) was selected by hybridization to E2E (lanes 3–5), VA-RNA$_I$ (lanes 6 and 7), or ML (lanes 8 and 9) probes and analyzed by electrophoresis. Hybridization-selected E2E RNA synthesized by pol III *in vitro* was examined in parallel (lane 4). End-labeled *Hpa* II fragments of pBR322 DNA and a set of SP6 run-off transcription products, whose lengths in nucleotides are indicated at left, were run in lanes 1 and 2, respectively.

promoter (Fig. 3*F*, lane 5). As predicted, only the smaller RNA was synthesized *in vitro* when the t2 termination site was removed (Fig. 3*F*, lane 6). RNA preparations from infected, but not uninfected, HeLa cells contained an RNA species comigrating with pol III E2E RNA II synthesized *in vitro* (Fig. 3*F*, lanes 4 and 3). An infected-cell RNA slightly shorter than *in vitro* synthesized E2E RNA I could just be discerned under these conditions (Fig. 3*F*, lane 4). When membranes were washed at lower stringency, this RNA species (≈42 nt) was detected more readily, as was a small quantity of an RNA species comigrating with E2E RNA I made *in vitro* (Fig. 3*F*, compare lanes 2 and 5).

The specificity and efficiency with which the small E2E RNA species present in infected-cell cytoplasmic RNA preparations hybridized to the exon 1, intron 1, and exon 3 oligonucleotides (Fig. 3 *D–F*) indicated that they did not represent products of degradation of abundant E2E mRNA species or E2E pre-mRNA. The recovery of the small E2E RNA species in the infected-cell RNA population soluble in 2.5 M LiCl (Fig. 3*D*, lane 2; Fig. 3*F*, lanes 2 and 4) and in the poly(A)-lacking fraction (data not shown)—populations that contained little or no E2E mRNA (Fig. 3*C*, lanes 3 and 4)—provided further support for this conclusion. These blotting experiments therefore established that cytoplasmic RNA preparations from Ad2-infected HeLa cells contained a 91-nt RNA with the properties predicted for an E2E transcript extending from position +1 to the t2 termination site (see Fig. 1): this RNA comigrated with such E2E transcripts synthesized by pol III *in vitro* (Fig. 3 *D* and *F*) and contained sequences from the −15 to +45 and +68 to +89 segments, but not from exon 3, of the pol II E2E transcription unit. Such infected-cell E2E RNA was further characterized by mapping its 5′ and 3′ termini.

When infected-cell cytoplasmic RNA was examined by primer extension using the intron 1 oligonucleotide, an infected cell-specific cDNA estimated to be 89 nt in length was observed (Fig. 4*A*, lanes 1–3). A comigrating cDNA, as well as smaller quantities of a 91-nt cDNA, were generated by pol III E2E transcripts synthesized *in vitro* from a wild-type template, but not from a template lacking the t2 termination site (Fig. 4*A*, lanes 4 and 5). The enrichment of the E2E RNA yielding the 89-nt cDNA in cytoplasmic RNA populations soluble in LiCl (data not shown) and the specificity of the intron 1 oligonucleotide (Fig. 3*A*) indicate that this cDNA cannot be generated from cytoplasmic pol II transcripts. Its length places the 5′ ends of the major *in vitro* pol III transcript detected in this assay and the small, intron 1-containing infected-cell RNA at position +1 of the pol II E2E transcription unit.

The 3′ ends of small E2E RNA species detected *in vivo* were mapped by using an E2E antisense RNA labeled at position +2 in RNase T1 protection assays. *In vitro* pol III E2E transcripts yielded major protection products of apparent length 55 and 99 nt (Fig. 4*B*, lane 7), as predicted (see Fig. 1). These can be unambiguously assigned to pol III transcripts terminating at the t1 and t2 sites, for mutations that disrupted these sites eliminated the 55- and 99-nt products, respectively (Fig. 4*B*, compare lanes 6 and 8 with lane 7). In addition to the 78-nt protection product generated by E2E mRNA exon 1, protection products comigrating with those generated by *in vitro* synthesized E2E pol III transcripts were generated by cytoplasmic RNA from Ad2-infected cells, but not by RNA from uninfected cells (Fig. 4*B*, lanes 1 and 2). These species were enriched, relative to E2E mRNA, in cytoplasmic RNA soluble in 2.5 M LiCl (Fig. 4*B*, lanes 2 and 3) and were recovered in the poly(A)-lacking fraction (Fig. 4*B*, lanes 4 and 5). These results indicate that infected cells must contain E2E RNA species with 3′ ends within the regions +45 to +55 and +90 to +100, which contain the T-rich t1 and t2 sequences characteristic of pol III termination sites (22) (Fig. 1*B*). Indeed, the length of the intron 1 sequence-containing, infected-cell E2E RNA species, 91 nt (Fig. 3*D*), and the location of its 5′ end at position +1 (Fig. 4*A*) place its 3′ terminus at position +91, within the t2 termination site used by pol III *in vitro*.

Small E2E RNAs Are Present in Ad2-Infected Cells at Low Concentrations. Estimates of the concentrations of small E2E RNA species in infected cells by RNase T1 protection assays indicated that these RNAs were present in the cytoplasm during the late phase of infection at <10 copies per cell (Table 1). It is unlikely that this value represents a significant underestimate, for comparison of signals generated in blotting assays by infected-cell E2E RNA II and known quantities of the *in vitro* synthesized pol III transcript yielded estimates of 5 copies per cell. The low cytoplasmic concentrations of these E2E RNA species does not appear to be the result of a low rate of pol III transcription: the results of preliminary experiments suggest that pol II and pol III transcribe 5′ terminal sequences of the E2E transcription unit at comparable rates and that small cytoplasmic E2E RNAs turn over more rapidly than exon 1-containing E2E mRNA.

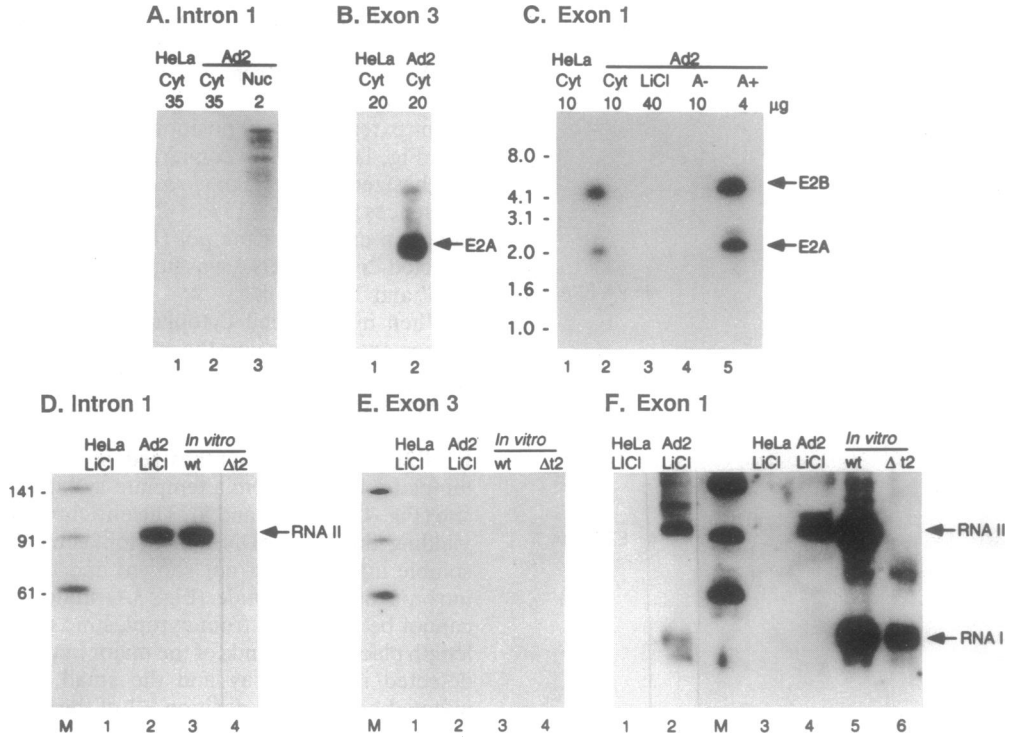

FIG. 3. Adenovirus-infected cells contain small E2E RNAs. Large (*A–C*) and small (*D–F*) E2E RNA species were examined by blotting. The quantities (μg) and/or nature of the RNA samples loaded in each lane are listed at the top. Cyt, cytoplasmic; Nuc, nuclear; A−, poly(A)-lacking RNA; A+, poly(A)-containing RNA. Samples designated LiCl were cytoplasmic RNA soluble in 2.5 M LiCl. RNA synthesized by pol III *in vitro* from the wild-type E2E promoter or a derivative lacking the t2 termination site is indicated by wt or Δt2, respectively. Ethidium bromide staining (data not shown) of rRNAs (*A–C*) or tRNAs (*D–F*) indicated that comparable quantities of each cytoplasmic RNA preparation were run in the gels used to make the membranes. Membranes were hybridized to 5′ end-labeled intron 1, exon 3, or exon 1 oligonucleotides (see *Materials and Methods*). The membrane shown in lanes 1 and 2 of *F* was washed under conditions of low stringency. The lengths of denatured ^{32}P-labeled DNA (*A–C*) or RNA (*D–F*) markers (lanes M) are listed at left. Positions of E2E transcripts are indicated at right.

DISCUSSION

Superposition of promoters specifying *in vivo* transcription by more than a single eukaryotic RNA polymerase has not been observed previously, although several transcriptional control regions can direct transcription by both pol II and pol III *in vitro* (see Introduction). This set includes the subgroup C adenovirus E2E pol II promoter (11), which we now show is also transcribed by pol III in adenovirus-infected cells. E2E transcription exhibiting the same response to increasing concentrations of α-amanitin as transcription from the pol III VA-RNA$_I$ promoter produced discrete RNAs identical in length to those made by pol III *in vitro* (Fig. 2). Furthermore, infected-cell cytoplasmic RNA preparations contain an ≈91-nt RNA that is identical in length, sequence content, and location of 5′ and 3′ termini to E2E RNA II transcribed by pol III *in vitro* (Figs. 3 and 4). These properties, which place its 3′ terminus within the T-rich t2 sequence (Fig. 1*B*) characteristic of pol III termination sites (22), in conjunction with the synthesis of this E2E RNA species by pol III in nuclei from infected cells, demonstrate that the E2E promoter is recognized by pol III in adenovirus-infected cells.

Although the results of run-on (Fig. 2*B*) and RNase protection (Fig. 4*B*) assays suggested that E2E RNA I (see Fig. 1*A*) was also made *in vivo*, this RNA was difficult to observe when infected-cell RNA preparations were examined by blotting (Fig. 3), even though E2E RNA I made by pol III *in vitro* registered well in this assay (Fig. 3). The results of blotting (Fig. 3) and RNase protection (Fig. 4*B*) assays suggested, however, that E2E RNA I might be subject in infected cells to cleavage reactions that do not operate efficiently *in vitro*.

Because adenovirus exploits normal cellular mechanisms, it seems likely that the superposition of pol II and pol III promoters described here is also characteristic of certain cellular transcription units. Mammalian c-*myc* promoters presently represent the best cellular candidates for such an arrangement (9, 12). As in the case of the E2E promoter, pol III transcription from c-*myc* promoters in experimental systems is restricted to 5′ sequences of the pol II transcription unit, terminates at T-rich sequences in the template, and depends on TATA elements of the pol II promoters (9, 12). pol III transcription with these properties has not, however, been reported in isolated nuclei, nor have c-*myc* pol III transcripts been detected in steady-state RNA populations. However, if products of pol III c-*myc* transcription, like the small E2E RNAs (Table 1), attained only low intracellular concentrations, they would easily escape detection.

Certain replication proteins encoded by the E2 pol II transcription unit function stoichiometrically in viral DNA synthesis (23) and must be produced in large quantities during the period of active viral DNA replication. As pol III synthesizes RNA from the E2E promoter during this period (e.g., Fig. 2), activation of the E2 late pol II promoter (see Fig. 1) at the beginning of the late phase (24, 25) may be a device to circumvent limitations upon pol II E2E transcription imposed by competition from the pol III system. What, then, is the virtue of superposition of pol II and pol III E2E promoters in the viral genome? This arrangement might represent a device to maximize utilization of the limited capacity of the viral genome. Alternatively, pol III transcription from the E2E promoter might downregulate pol II transcription, as originally proposed for pol III c-*myc* transcription (9). The similar rates of E2E transcription sequences by pol II and pol III observed in preliminary experiments, and the low intracellular concentrations attained by small E2E RNAs (Table 1), are consistent with such a regulatory function. Moreover, as

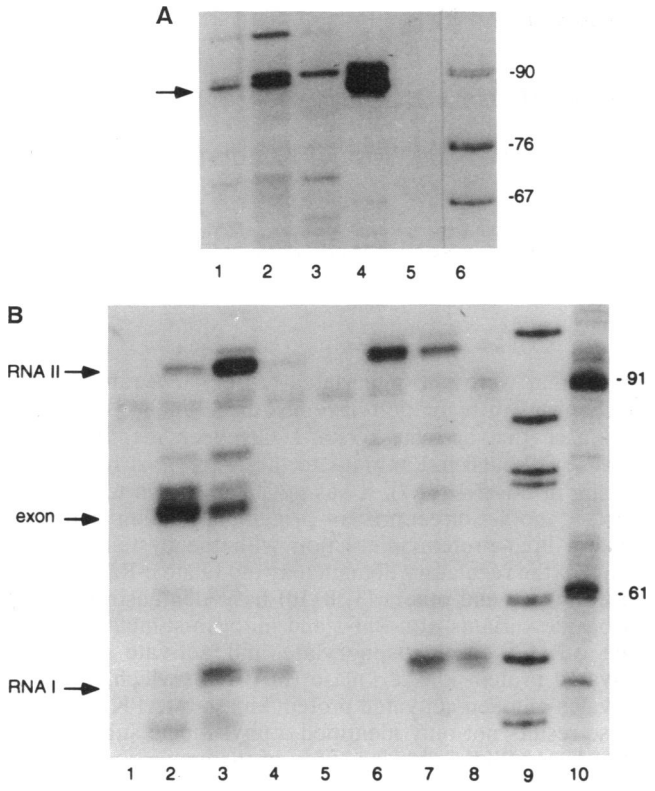

FIG. 4. Mapping 5′ and 3′ ends of small E2E transcripts. (*A*) Primer extension reactions used 15 or 30 μg of cytoplasmic RNA from adenovirus-infected HeLa cells harvested 14 hr after infection (lanes 1 and 2, respectively), 30 μg of HeLa cell cytoplasmic RNA (lane 3), or the products of pol III transcription from a wild-type or t2 termination site-deleted E2E promoter (lanes 4 and 5, respectively). The lengths of ^{32}P-labeled DNA markers (lane 6) are listed at right, and the 89-nt cDNA described in the text is indicated at left. (*B*) RNase T1 protection assay mixtures contained 25 μg of cytoplasmic RNA from uninfected HeLa cells (lane 1) or cells isolated 14 hr after Ad2 infection (lane 2), LiCl-soluble RNA prepared from 125 μg of the same infected-cell cytoplasmic RNA preparation (lane 3), the poly(A)-lacking (lane 4) and poly(A)-containing (lane 5) RNA soluble in LiCl from 50 μg of an independent preparation of infected-cell cytoplasmic RNA, or *in vitro* pol III transcripts from a wild-type E2E promoter (lane 7) or its derivatives lacking the t1 or t2 termination sites (lanes 6 and 8, respectively). DNA and RNA markers, whose lengths are listed at right, were run in lanes 9 and 10, respectively. Positions of the exon 1 and E2E RNA I and RNA II protection products are indicated at left.

the E2 72-kDa DNA-binding protein is lethal when overexpressed (26), its overproduction during the early phase might preclude successful completion of the adenovirus reproductive cycle. Be that as it may, the superimposed pol II and pol III adenovirus E2E promoters provide the opportunity to explore the physiological consequences of competition between these two transcription systems, as well as potential regulatory functions of pol III transcription.

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Table 1. Concentration of small E2E RNAs during the late phase of adenovirus infection

E2E RNA	No. of copies per cell		
	Exp. 1	Exp. 2	Exp. 3
I	4.2	7.2	6.5
II	4.4	6.2	7.0

The radioactivity recovered in RNase T1 protection products when cytoplasmic RNA from Ad2-infected cells harvested 14 hr after infection was hybridized to saturating concentrations of E2E antisense RNA end-labeled at position +2 was measured with a Molecular Dynamics model 400E PhorphorImager and appropriate standards. These values were used with the specific activities of the probes, and measurement of the yield of cytoplasmic RNA from infected HeLa cells, to calculate concentrations of the small E2E RNAs. Experiments 1, 2, and 3 represent independent measurements with two preparations of 3′ end-labeled probe.

1. Greenblatt, J. (1991) *Cell* **66,** 1067–1070.
2. Cormack, B. P. & Struhl, K. (1992) *Cell* **69,** 685–696.
3. Schultz, M. C., Roeder, R. H. & Hahn, S. (1992) *Cell* **69,** 697–702.
4. Sharp, P. A. (1992) *Cell* **68,** 819–821.
5. Rigby, P. W. J. (1993) *Cell* **72,** 7–10.
6. Murphy, S., Moorfield, B. & Pieler, T. (1989) *Trends Genet.* **5,** 122–126.
7. Dahlberg, J. E. & Lund, E. (1991) *Science* **254,** 1452–1453.
8. Hernandez, N. (1992) in *Transcriptional Regulation*, eds. McKnight, S. L. & Tjian, R. (Cold Spring Harbor Lab. Press, Plainview, NY), pp. 281–313.
9. Chung, J., Sussman, O. J., Zeller, R. & Leder, P. (1987) *Cell* **51,** 1001–1008.
10. Mitchell, M. T., Hobson, G. M. & Benfield, P. A. (1992) *J. Biol. Chem.* **207,** 1995–2005.
11. Pruzan, R., Chatterjee, P. K. & Flint, S. J. (1992) *Nucleic Acids Res.* **20,** 5705–5712.
12. Bentley, D. L., Brown, W. L. & Groudine, M. (1989) *Genes Dev.* **3,** 1179–1189.
13. Flint, S. J., Gallimore, P. H. & Sharp, P. A. (1975) *J. Mol. Biol.* **96,** 47–68.
14. Uhlenbeck, O. & Gumport, R. J. (1982) in *The Enzymes*, ed. Boyer, P. D. (Academic, New York), Vol. 15B, pp. 31–60.
15. Greenberg, M. E. & Bender, T. P. (1987) in *Current Protocols in Molecular Biology*, eds. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. (Wiley, New York), pp. 4.10.1–4.10.9.
16. Maxam, A. M. & Gilbert, W. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 560–564.
17. Riley, D. & Flint, S. J. (1993) *J. Virol.* **67,** 3586–3595.
18. Hayes, B. W., Telling, G. C., Myat, M. M., Williams, J. F. & Flint, S. J. (1990) *J. Virol.* **64,** 2732–2742.
19. Church, G. M. & Gilbert, W. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 1991–1995.
20. Chen, H. & Flint, S. J. (1992) *J. Biol. Chem.* **267,** 25457–25465.
21. Sharp, P. A. (1984) in *The Adenovirus*, ed. Ginsberg, H. S. (Plenum, New York), pp. 173–204.
22. Geiduschek, P. & Tocchini-Valentini, G. P. (1988) *Annu. Rev. Biochem.* **57,** 873–914.
23. Challberg, M. D. & Kelly, T. J. (1989) *Annu. Rev. Biochem.* **58,** 671–717.
24. Chow, L. T., Broker, T. R. & Lewis, J. (1979) *J. Mol. Biol.* **134,** 265–303.
25. Goldenberg, C. J., Rosenthal, R., Bhaduri, S. & Raskas, H. J. (1981) *J. Virol.* **38,** 932–939.
26. Klessig, D. F., Brough, D. E. & Cleghorn, V. (1984) *Mol. Cell. Biol.* **4,** 1354–1362.
27. Mattaj, I. W., Dathan, N. A., Parry, H. D., Carbon, P. & Krol, A. (1988) *Cell* **55,** 435–442.