Tumor Virus Ribonucleic Acid Directed Deoxyribonucleic Acid Synthesis: Nucleotide Sequence at the 5′ Terminus of Nascent Deoxyribonucleic Acid†

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ABSTRACT: An analysis has been made of the nucleotide sequence at the 5′ terminus of DNA transcribed from the 70S RNA genome of Rous sarcoma virus by the viral polymerase. At least 94% of the transcripts begin with the sequence: d(A-A-T-G-A-A-G-C). This sequence has been found with three strains of Rous sarcoma virus (Schmidt-Ruppin A, B77-C, and Prague C) and also with Rous associated virus II. The data are discussed in terms of the mechanism by which the viral genome is transcribed into DNA.

In Rous sarcoma virus (RSV)[1] a 4S RNA molecule can serve as primer for the transcription of the viral genome into DNA (Canaani and Duesberg, 1972; Faras *et al.*, 1973). Using several avian RNA tumor viruses we and others have found that the 3′ terminus of the RNA primer is adenosine and that in all initiations of DNA synthesis the first deoxynucleoside incorporated is deoxyadenosine (Verma *et al.*, 1971; Taylor *et al.*, 1973).[2] DNA synthesis can be blocked by the replacement[3] in the reaction mixture of TTP with the analog d_2TTP or araUTP. In this situation, the only nucleoside incorporated other than the analog is dA (Faras *et al.*, 1973). The initial DNA sequence is therefore dA-dA_n-T where n could be either zero or a small positive number (Faras *et al.*, 1973).

We have now further characterized the initial sequences of DNA that are covalently linked to the primer during transcription by detergent-disrupted virions of the Schmidt-Ruppin strain of RSV. Our intent was to determine the number of different DNA sequences that exist on the 5′ terminus of the nascent DNA, and thereby deduce the minimum number of sites for the initiation of DNA synthesis on the tumor virus genome. Our results indicate that at least 94% of all initiation sequences are identical out to the eighth nucleotide. The sequence of this octanucleotide has been determined and an identical sequence has been found in two other strains of Rous sarcoma virus as well as in an avian leukemia virus.

Materials and Methods

Reagents. Unlabeled deoxynucleoside triphosphates were from Calbiochem. [^{3}H]dATP (9 Ci/mmol) was from New England Nuclear. α-^{32}P-labeled nucleoside triphosphates (10–20 Ci/mmol) were from International Chemical and Nuclear Corp. [α-^{32}P]dATP (100–400 Ci/mmol) was also prepared by a modification of the method of Symons (1966). d_2TTP was a gift of D. Brutlag. Uracil and cytosine arabinoside triphosphates and also [α-^{32}P]araCTP (10 Ci/mmol) were gifts from D. Shannahoff and S. Hendler. dA, 2′-Ap, 3′-Ap, and A-A were purchased from P-L Biochemicals.

Enzymatic Reaction. The standard reaction mixture (Faras *et al.*, 1972) contained: 0.1 M Tris-HCl (pH 8.1), 0.01 M $MgCl_2$, 0.01% (v/v) Nonidet P-40, 2% (v/v) β-mercaptoethanol, 100 μg/ml of actinomyciin, and 200–400 μg of protein/ml of purified virus (Bishop *et al.*, 1970). Deoxynucleoside triphosphates were present at 10 μM. All reactions were for 1 hr at 37°.

Isolation of Product. As previously described (Taylor *et al.*, 1972), the reaction product was extracted with sodium dodecyl sulfate and Pronase, isolated as a 70S complex by rate-zonal centrifugation, and finally passed through a column of G-50 Sephadex to remove unincorporated nucleoside triphosphates.

Characterization of Product. Where indicated, the reaction products were isolated and treated with alkali (0.3 N NaOH, 16 hr, 37°) to hydrolyze the RNA. Conditions for electrophoresis (on Whatman DE-81 or 3MM) have been described previously (Faras *et al.*, 1973; Taylor *et al.*, 1972). One-dimensional homochromatography was performed on thin-layer plates of DEAE-cellulose (40 × 20 cm, DEAE (1): cellulose (7.5), Analtech) by the methods of Barrell (1971), using "Homomix C" prepared with 3% RNA digested for 30 min with KOH. Homochromatography was for 8 hr at 60° in a sealed chamber. The nearest neighbor analysis of DNA products was as previously described (Taylor *et al.*, 1972).

Results

Initial Trinucleotide Sequence. The initial sequence(s) of DNA synthesized onto the RNA primer during transcription of Rous sarcoma virus is dA-dA_n-T (Faras *et al.*, 1973). We now show that the unknown n has a single value, 1.

Two enzymatic reactions were carried out using detergent-disrupted virions. dGTP and dCTP were omitted and TTP was replaced by d_2TTP. In one reaction [^{3}H]dATP was the ra-

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[1] Abbreviations used are: RSV, Rous sarcoma virus; d_2TTP, dideoxythymidine triphosphate; araUTP, uracil arabinoside triphosphate; araCTP, cytosine arabinoside triphosphate.

[2] Flügel *et al.* (1973) have obtained similar results for DNA synthesis by avian RNA tumor virus particles disrupted by NP-40. However, when the disruption is achieved with ether they observe an additional initiation: dC to U. Neither we nor D. Baltimore (personal communication) has been able to confirm their finding.

[3] The use of the analogs circumvents potential artifacts due to either contamination of the other deoxynucleotide triphosphates or the presence of deoxynucleoside triphosphates in virions of RSV (Mizutani and Temin, 1971).

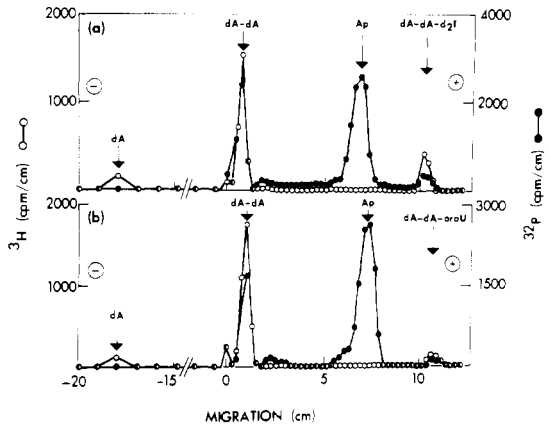

FIGURE 1: Separation by electrophoresis of oligodeoxyribonucleotides synthesized in the presence of analogs of TTP. DNA was synthesized under standard conditions except that TTP was replaced in (a) by d_2TTP and in (b) by araUTP. The only labeled nucleoside triphosphate present was either [^{3}H]dATP (10 μM, 5 Ci/mmol in a 5-ml reaction volume) or [α-^{32}P]dATP (0.3 μM, 400 Ci/mmol in a 0.5-ml reaction volume). Aliquots of the products synthesized in the presence of [^{3}H]dATP or [α-^{32}P]dATP were combined, treated with alkali, and analyzed by electrophoresis on paper (Whatman 540) at pH 3.5 for 1 hr at 90 V/cm. Optical density markers of dA, Ap, and A-A were used. The sequences noted by arrows represent deductions as discussed in the text. The electrophoretic path was then cut into strips and counted in a liquid scintillation counter. Two different strip widths were used and so the measured radioactivity is expressed in the figure as cpm/cm.

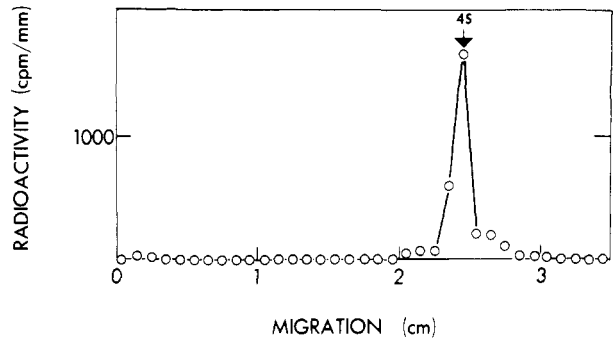

FIGURE 2: Polyacrylamide gel electrophoresis of covalently linked primer-product complex. DNA was synthesized in a standard reaction from which dCTP was omitted and in which [α-^{32}P]dATP was the only labeled nucleoside triphosphate present. The product was isolated as described in Materials and Methods, denatured with 90% dimethyl sulfoxide, and analyzed by electrophoresis in a 10% polyacrylamide gel for 2 hr at 6 mA/gel. The gel was cut into 1-mm slices and radioactivity assayed as Čerenkov radiation in a liquid scintillation counter. The marker indicated is 4S tRNA.

dioactive precursor and in the other [α-^{32}P]dATP. After synthesis the ^{3}H- and ^{32}P-labeled DNA products were mixed, isolated, treated with alkali to hydrolyze RNA (including the primer RNA–DNA linkage (Faras *et al.*, 1973)), and analyzed by electrophoresis on paper (Figure 1a). The identities of the products are indicated in the figure, and were established as follows. (1) "Ap" migrated with marker Ap during electrophoresis. It was unlabeled when [^{3}H]dATP was the sole radioactive precursor, but contained ^{32}P when reactions were carried out with [α-^{32}P]dATP. These observations conform to previous demonstrations that the 5′-terminal dA of nascent DNA is covalently linked by a phosphodiester bond to 3′-terminal adenosine of primer. (2) "dA" derives from initiations where only a single residue of deoxynucleotide is added to primer (Faras *et al.*, 1973). It migrates with marker dA during electrophoresis, and is labeled in reactions with [^{3}H]dATP but not those with [α-^{32}P]dATP. (3) "dA-dA" migrated with marker A-A during electrophoresis at pH 3.5 (the deoxydinucleotide monophosphate marker was not available). Hydrolysis with a combination of spleen phosphodiesterase and micrococcal nuclease produced equal amounts of dAp and dA, identified by their electrophoretic mobility and content of [α-^{32}P] and/or [^{3}H]dA (data not shown). (4) dA-dA-d_2T: the ratio of ^{3}H to ^{32}P in this component is identical with that of dA-dA, but it has an additional negative charge at pH 3.5. If d_2TTP is omitted from the initial reaction, only this peak is absent from the electropherogram. Also if the only nucleoside triphosphates present in the initial reaction are [^{3}H]TTP and unlabeled dATP, only this peak is labeled, and subsequent digestion with micrococcal nuclease and spleen phosphodiesterase releases [^{3}H]T as the only radioactive product (data not shown).

Our interpretation of these results is that DNA synthesis is initiated with the trinucleotide sequence dA-dA-T and that the presence of dA and dA-dA is a consequence of incomplete chain elongation. It is possible in the present experimental situation that dA and dA-dA are precursors for other sequences. However, subsequent experiments on the sequence beyond the initial trinucleotide render this possibility unlikely (see below).

In similar studies with araUTP rather than d_2TTP (Figure 1b), we have observed four components with electrophoretic mobilities similar to those components obtained when d_2TTP is used. We deduce that the oligonucleotide with the same mobility as dA-dA-d_2T is dA-dA-araU because it is absent when araUTP is omitted and the enzymatic reaction carried out with radioactive dATP as the sole precursor.

In both of these experiments (Figure 1a and b) the reaction with [α-^{32}P]dATP was at low nucleoside triphosphate concentration (0.3 μM) and hence most initiations terminate at the first residue (Faras *et al.*, 1973). This accounts for the large amount of Ap. The reaction with [^{3}H]dATP was at much higher precursor concentration (10 μM) and the reaction terminates predominantly at the second nucleotide since the third nucleotide is an analog and is not readily incorporated. Therefore, the amounts of differentially labeled products are not directly comparable.

We have also analyzed transcription from RSV 70S RNA by purified polymerase of RSV. When TTP is replaced by araUTP in the reaction, the electrophoretic pattern of the oligonucleotides synthesized is qualitatively identical with that of Figure 1b. Quantitative differences are found which depend on the ratio of enzyme to RNA in the reaction mixture. Table I summarizes the amount of radioactivity in each component for a series of reactions in which the ratio is varied from enzyme excess[4] (1 and 3 μg/ml of RNA) to template excess (9 and 27 μg/ml). From these data it is possible to compute the relative number of moles of each of the three possible terminations: dA, dA-dA, and dA-dA-araU. The relative amount of the trinucleotide synthesized decreases as the ratio of enzyme to template decreases. The DNA synthesized by detergent-disrupted virions has been analyzed in the same manner (Table I). The results are comparable to those obtained with purified polymerase in the presence of an excess of template.

Initial Octanucleotide Sequence. DNA synthesis by detergent-disrupted virions can be interrupted at a point distal to the above-mentioned trinucleotide by the omission of dCTP. Characterization of the DNA synthesized under these conditions has been used to establish the initial DNA sequence to the eighth residue. DNA was synthesized in a standard reaction which contained [α-^{32}P]dATP, dGTP, and TTP, and from

[4] Conditions of enzyme excess are those in which the amount of DNA synthesized is directly proportional to the concentration of template.

TABLE I: Relative Amounts of the Oligonucleotides Synthesized in the Presence of araUTP.

Enzymatic Reaction[a]	Distribution of Radioactivity (cpm)[b]			Molar Distribution (%)[c]		
	Ap	dA-dA	dA-dA-araU	N_{75}-dA	N_{75}-dA-dA	N_{75}-dA-dA-araU
Purified polymerase						
70S RNA concentration (μg/ml)						
1	627	348	179	16.0	55.4	28.6
3	1102	664	290	13.4	60.3	26.3
9	3179	2065	251	27.1	64.9	7.9
27	3825	1822	120	49.1	47.7	3.2
Detergent-disrupted virions	7469	3828	447	41.1	53.1	5.8

[a] DNA was synthesized under standard conditions except that the only nucleoside triphosphates present were [α-^{32}P]dATP and araUTP. The reaction mixture using purified enzyme contained 0.2 unit of enzyme/ml (Faras *et al.*, 1972), 100 μg/ml of actinomycin, 0.1 M Tris-HCl (pH 8.1), 0.01 M $MgCl_2$, and 2% (v/v) β-mercaptoethanol. The nucleoside triphosphates, including the analog, were at 10 μM. [b] The DNA product was isolated and analyzed by electrophoresis on paper as in Figure 1. Radioactivity was located by autoradiography and counted as Čerenkov radiation in a liquid scintillation counter. [c] Here N_{75} is used to designate the RNA primer, the 3′ terminus of which is A. The identification of the three possible DNA products shown here is given in the text. The percentages shown in the table refer to the relative number of molecules of each of the three terminations, and include allowance for the fact that alkaline hydrolysis transfers ^{32}P from the oligonucleotide to Ap.

which dCTP was omitted. Purified product was denatured with dimethyl sulfoxide (to disrupt hydrogen bonding between the template and the primer–product complex) and analyzed on a 10% polyacrylamide gel (Figure 2). The electrophoretic mobility of the product is the same as that of a 4S RNA marker. Similar results were obtained from the product of a reaction in which dCTP was replaced by araCTP. In both instances the majority of the DNA constituent of the 4S nucleic acid is less than nine nucleotides long (see below). The present results therefore conform to the earlier finding (Faras *et al.*, 1973) that DNA synthesis is initiated on a 4S RNA primer.

Each product was hydrolyzed with alkali and then analyzed both by electrophoresis on DEAE-cellulose paper (Figure 3) and by homochromatography (Figure 4). The electrophoretic pattern for the product of the reaction without araCTP shows that the majority of the radioactivity migrates as an oligonucleotide designated I (Figure 3a). There is radioactivity in 2′-Ap and 3′-Ap and a small amount (6.8%) at the origin. The product of the reaction performed in the presence of araCTP gives a very similar pattern (Figure 3b) except that an additional oligonucleotide, designated II, occurs.

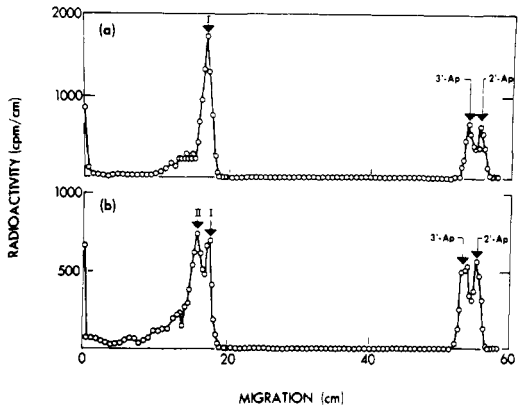

FIGURE 3: Separation by electrophoresis of oligodeoxyribonucleotides synthesized in the absence of dCTP: (a) DNA was synthesized in a standard reaction from which dCTP was omitted and in which [α-^{32}P]dATP was the only labeled nucleoside triphosphate; (b) the enzymatic reaction was identical except that araCTP was present. In each case the DNA products were isolated, treated with alkali, and applied to DEAE paper (Whatman DE-81). Electrophoresis was for 15 hr at 10 V/cm in the presence of 7% formic acid. Optical density markers of 2′-Ap and 3′-Ap were used and located as indicated. The electrophoretic path was cut into strips and assayed as Čerenkov radiation in a liquid scintillation counter.

The homochromatograms (Figure 4) show essentially the same qualitative features as the above electrophoretic analysis but with better separation of oligonucleotides I and II. Also, there is no radioactivity at the origin when homochromatography is used. When DNA is synthesized in the presence of araCTP, small amounts are detected of some oligonucleotides that are longer than oligonucleotide II because they have a lower mobility (16–22 cm in Figure 4b).

The data illustrated in Figures 3 and 4 indicate that most of the short DNA chains produced are either oligonucleotides I or II. The nucleotide sequence of these two oligonucleotides was determined as follows. Four separate DNA syntheses were performed in the presence of dATP, dGTP, and TTP, and with dCTP replaced by araCTP. In each reaction one of the four

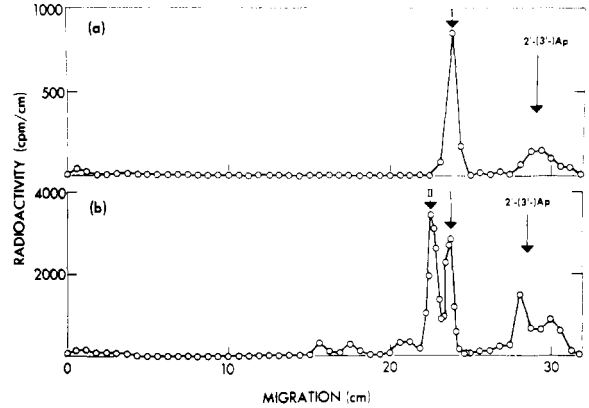

FIGURE 4: Separation by homochromatography of oligodeoxyribonucleotides synthesized in the absence of dCTP. DNA products (a) and (b) were prepared as in Figure 3a and b, respectively. Homochromatography was performed as described in Materials and Methods. The chromatographic path was cut into strips by carefully scraping the cellulose from the supporting glass plate. The radioactivity was assayed as Čerenkov radiation in a liquid scintillation counter.

TABLE II: Nearest Neighbor Analysis of Oligodeoxynucleotides Synthesized in the Absence of dCTP.

Oligonucleotide[a]	α-^{32}P-Labeled Deoxynucleoside Triphosphate Present during DNA Synthesis[b]	Nearest Neighbors in DNA Product[c] dAp	dGp	Tp	Deduced Sequence[d]
I	dATP	1.99	1.00	0.16	2A[A],G[A]
	dGTP	1.00	0.05	1.19	A[G],T[G]
	TTP	1.00	0.03	0.04	A[T]
II	dATP	1.86	1.00	0.07	2A[A],G[A]
	dGTP	1.00	0.04	1.18	A[G],T[G]
	TTP	1.00	0.11	0.07	A[T]
	araCTP	0.06	1.00	0.03	G[C]

[a] Four enzymatic reactions were carried out. For each the DNA product was isolated and analyzed as described for Figure 3b. The peaks of radioactivity designated I and II were located by autoradiography, eluted from the paper with 30% triethylamine bicarbonate, and evaporated to dryness. [b] As indicated, in each reaction only one of the four nucleoside triphosphates present (dATP, dGTP, TTP and araCTP) was labeled. [c] For each reaction, the oligonucleotides I and II were isolated and subjected to nearest neighbor analysis using standard procedures (Taylor *et al.*, 1972). [d] First letter indicates the "nearest neighbor" of the nucleotide shown in square brackets, *e.g.*, 2Ap[A] indicates that 2 mol of Ap are labeled when the α-^{32}P nucleoside triphosphate precursor is dATP.

TABLE IV: Analysis of Oligodeoxyribonucleotides Separated by Electrophoresis on DEAE-Cellulose.[a]

Labeled Products after Alkaline Hydrolysis	Distribution of Radioactivity (cpm)	Deduced Relative Molar Distribution (%)
Ap	19362	60.1
d(A-A)	310	1.6
d(A-A-T)	203	1.0
d(A-A-T-G)	209	1.1
d(A-A-T-G-A)	332	1.7
d(A-A-T-G-A-A)	873	4.5
d(A-A-T-G-A-A-G)	3782	19.5
d(A-A-T-G-A-A-G-C)	2011	10.4

[a] DNA was synthesized under standard conditions except that dCTP was replaced by araCTP. [α-^{32}P]dATP was the only labeled nucleoside triphosphate present. The reaction was terminated at 30 min. The DNA product was isolated, hydrolyzed with alkali, and analyzed by electrophoresis on DEAE-cellulose as in Figure 3. Radioactivity was located by autoradiography and assayed as Čerenkov radiation in a liquid scintillation spectrometer. The sequences designated for the DNA products were deduced from experiments similar to those described in Table II (data not shown). The molar amounts of each DNA sequence were calculated as described for Table I. In line one, 60.1% refers to initiations which involve the addition of pdA only.

nucleoside triphosphates present was labeled with α-^{32}P. In each case the DNA products were analyzed as in Figure 3b, and the oligonucleotides I and II were isolated and a nearest neighbor analysis was performed. The results are in Table II. Of the possible nucleotide sequences consistent with this analysis for oligonucleotide I (Table III), only one has the initial trinucleotide sequence, dA-dA-T, which was shown in the previous section to occur in all initiations. This sequence is the heptanucleotide d(A-A-T-G-A-A-G) (Table III, line 3). Oligonucleotide II has nearest neighbor composition identical with that of I, except that it is labeled with [α-^{32}P]araCTP. This observation is consistent with dC being the 3′-terminal residue of II. The nearest neighbor of araC, the penultimate residue, is dG. Therefore, II is the octanucleotide d(A-A-T-G-A-A-G-C).

Oligonucleotides smaller than the heptanucleotide have been observed in small and variable amounts. For example, in Figure 3 the amount of such oligonucleotides is so low as to be imperceptible. In other experiments the amount has been sufficient to allow isolation and subsequent nearest neighbor analyses (data not shown). The oligonucleotides have been found to represent intermediates in the synthesis of the octanucleotide II. These findings are therefore additional evidence for the assigned octanucleotide sequence. The distribution of radioactivity among these intermediates is presented in Table IV. On the basis of the determined nucleotide sequence we have calculated the relative number of moles of each intermediate.

Another experimental approach has been used to verify that the eighth residue is dC. DNA was synthesized in a reaction from which dCTP was omitted. The complex of template-primer and nascent DNA was isolated intact (*i.e.*, not treated with dimethyl sulfoxide or alkali), and used as template-primer in a second reaction with purified polymerase and unlabeled araCTP. The final product was isolated, treated with alkali, and analyzed by homochromatography. The results showed that the second reaction caused about 30% of the labeled oligonucleotide I to migrate in the position of II. Thus II represents the addition of araC to I.

By the selective omissions of specific nucleoside triphosphates from the second reaction we have established that the ninth and tenth residues of the newly synthesized DNA are dC and T, respectively. We cannot exclude the possibility that sequences other than this exist beyond the eighth nucleotide because reinitiation of DNA synthesis occurs on only about 30% of the initial DNA product.

Fraction of Nascent DNA Molecules that Begin with the Specific Octanucleotide. From studies with the heptanucleotide I, we can determine what fraction of all initiations begin with that sequence. Of the labeled DNA with a mobility less than or equal to that of I, 94% migrates as I (Figure 3a). That is, 94% of all observed initiations which proceed beyond the simple addition of one nucleoside of dA have the same nucleotide sequence out to the 7th residue.

The studies in which the octanucleotide is synthesized cannot be analyzed in the same manner because in the presence of

TABLE III: Possible Nucleotide Sequences for Oligonucleotide I That Are Consistent with the Nearest Neighbor Analyses Shown in Table II.[a]

1. d(A-A-A-T-G-A-G)	4. d(A-A-G-A-A-T-G)
2. d(A-A-A-G-A-T-G)	5. d(A-T-G-A-A-A-G)
3. d(A-A-T-G-A-A-G)	6. d(A-G-A-A-A-T-G)

[a] Previous work (Taylor *et al.*, 1973) excludes those sequences for which the 5′-terminal nucleoside is not dA.

araCTP about half of the DNA synthesis stops at the heptanucleotide rather than incorporating araCTP to make octanucleotide (Figures 3b and 4b).

Initiation of DNA Synthesis by Different Avian RNA Tumor Viruses. The above studies were made with the Schmidt-Ruppin strain (subgroup A) of RSV. The identical octanucleotide has also been observed with the B77 strain (subgroup C), with a cloned isolate of the Prague C strain (subgroup C), and with Rous associated virus II, an avian leukosis virus that is unable to transform cells in culture.

Discussion

Transcription of the viral RNA into a DNA copy of provirus (Temin, 1971) is presumably a mandatory step in the intracellular replication of an RNA tumor virus. Viruses which possess a temperature-sensitive RNA directed DNA polymerase can neither replicate nor induce cellular transformation at a restrictive temperature (Linial and Mason, 1973). Likewise, viruses which are genetically deficient in the polymerase are not infectious (Hanafusa and Hanafusa, 1971). It is therefore important to understand the process by which the viral RNA is transcribed into DNA.

The present studies relate to the *in vitro* transcription of RSV RNA into DNA. It has been found that 94% of DNA synthesized by detergent-disrupted RSV is initiated with the octanucleotide: d(A-A-T-G-A-A-G-C). We estimate that the 6% of the DNA which is larger than the heptanucleotide (Figure 3a) is 14–20 nucleotides long. It cannot be excluded that DNA synthesis could proceed this far before requiring a single residue of dC. An alternative interpretation is that this 6% represents read-through beyond the first dC. Another qualification is that while 94% of the DNA initiates with this common sequence, we cannot exclude the possibility that the remaining 6% could be of more relevance to the process by which the entire RSV genome is transcribed into DNA.

Several avian RNA tumor viruses have been observed to initiate DNA synthesis with the same octanucleotide sequence. This is consistent with the finding that the genomes of the viruses studied possess extensive sequence homology (H. E. Varmus, personal communication).

The transcription *in vitro* of tumor virus RNA into DNA occurs primarily from limited regions of the template, and the DNA produced is small (average of 4 S) (Taylor *et al.*, 1972). In the presence of actinomycin, as much as 20% of the product DNA represents a uniform transcript (Garapin *et al.*, 1973). Nevertheless, the average size of the DNA remains small and the small DNA contains nucleotide sequences representing the entire genome (unpublished observations of the authors). These facts imply the existence of multiple initiation sites (as many as several hundred) on each molecule of viral RNA. By contrast, transcription of DNA from 70S RNA is primed by specific 4S RNAs of which there are only 2–4 per 70S molecule (Faras *et al.*, 1973; Dahlberg *et al.*, 1974). The homogeneity of the nucleotide sequence at the 5′ terminus of nascent DNA as observed in the present findings further suggests that initiation occurs at a limited number of sites on the viral genome. We cannot presently resolve this paradox.

We have used triphosphates of nucleoside analogs to block the elongation of DNA chains. When TTP is omitted during DNA synthesis, the viral polymerase will terminate DNA chains with d_2TTP and to a lesser extent araUTP (Figure 1a and b). Also, when dCTP is omitted, the polymerase will terminate synthesis with araCTP but this only occurs in about half of the nascent chains. Most of the remaining chains stop at the preceding nucleotide (Figures 3b and 4b). In the present studies we have not observed a significant incorporation of analogs into *internucleotide* bonds. Such incorporation, if present, is small relative to the blockage of nascent DNA chains. This is comparable to the finding of Momparler (1972) using araCTP in synthesis with calf thymus DNA polymerase.

Acknowledgments

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