which was kept at 38°C for an additional period before it was similarly removed and we determined the distribution of activity in it.

In the example of Fig. 1, 3 hours were allowed after injection of the ganglion before the animal was killed and the control sciatic nerve was removed. The rate of transport was 424 mm/day. This falls within the expected rate distribution of 401 ± 35 mm/day. The nerve which had been left *in situ* in the dead animal for an additional 3 hours did not show much more of a distal displacement of the crest position. The displacement could represent no more than approximately 15 minutes more of downflow. Perhaps it could be accounted for by a variation in the estimation of the rate of transport. The shape of the crest was not altered to any great extent during the additional 3 hours except possibly for the foot of the crest. This finding further removes diffusion as a major factor in transport.

These experiments suggested that the loss of oxygen carried by the blood could have stopped fast axoplasmic transport. This was shown to be the case in a group of six experiments in vitro in which the L7 ganglia on both sides were injected with either [$^3$H]-leucine or [$^3$H]-lycine and the sciatic nerve on one side was removed as a control. After 3 hours the nerve from the opposite side was removed and placed in a chamber filled with oxygen. The distribution of activity in the control nerve (Fig. 2) moved 49 mm from the ganglion giving a rate of fast transport of 392 mm/day. This is within the range expected for a 3-hour downflow. The nerve placed in the chamber containing oxygen and kept moist with a lactate-Ringer solution at 38°C for an additional 3 hours showed continued fast transport in this in vitro preparation by the distal displacement of the crest. Activity had moved 96 mm from the ganglion giving a rate of fast transport for the total time of 6 hours of 384 mm/day in the oxygen-treated nerve. This rate falls within the range expected of a normal fast transport.

In other such experiments it was shown that the somas were not required for transport in vitro. Fast transport was maintained after the somas had been excluded by cutting the nerves just distal to the ganglion prior to placing them in the chamber.

In five similar experiments in vitro, nerves removed from the animals 3 hours after ganglion injection were exposed to nitrogen in the chamber for an additional 3 hours. In such cases there was no evidence of a further movement of the crest of activity beyond that seen in the initial 3-hour period. The two distributions of activity were particularly close, which shows that the failure of fast transport occurred well within 15 minutes in the anoxic nerves.

Thus, the mechanism underlying fast axoplasmic transport is closely dependent on oxidative metabolism. Mammalian nerves lose their excitability within 10 to 35 minutes after an interruption of their oxygen supply (*5*). This is the case also for single mammalian nerve fibers (*6*). The similarity of the two failure times suggests the exhaustion of a common metabolic pool supporting both the fast transport system and the mechanisms underlying excitability of the nerve membrane during anoxia. Alternatively, the metabolically driven fast transport system could maintain excitability by supplying some essential materials to the nerve membrane.

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## Nucleotide Sequence of an RNA from Cells Infected with Adenovirus 2

Abstract. *The principal nucleotide sequence of an RNA in KB cells infected with adenovirus 2 has been determined. As isolated, the molecule shows some terminal and internal heterogeneity. The sequence permits extensive base pairing and contains prominent repeating sequences. A portion of the sequence resembles a sequence found in several transfer RNA's.*

After being infected with adenovirus 2, KB cells synthesize and accumulate in their cytoplasm a stable, low-molecular-weight RNA (*1*) species (VA RNA) whose primary structure is different from that of the principal RNA species found in the cytoplasm of uninfected cells (*2*). We have determined the nucleotide sequence of this RNA (Fig. 1). We prepared $P^{32}$-labeled VA RNA from infected cells and purified it by electrophoresis on an acrylamide gel. The methods for sequence determination have been described (*3, 4*). In particular we obtained useful, large fragments by digestion of the RNA with ribonuclease T1 (1 unit per milligram of RNA) and by fractionation of the products on heated DEAE cellulose and DEAE Sephadex columns. Purified fragments were then extensively digested with ribonuclease T1, spleen acid ribonuclease (*5*), and pancreatic ribonuclease, and the products were fractionated and analyzed by two-dimensional electrophoresis (*4*).

The analyses were complicated by the existence of several points of heterogeneity in the molecule. Thus a portion of the molecule lacked one pU at the 3′-OH end. This type of hetero-

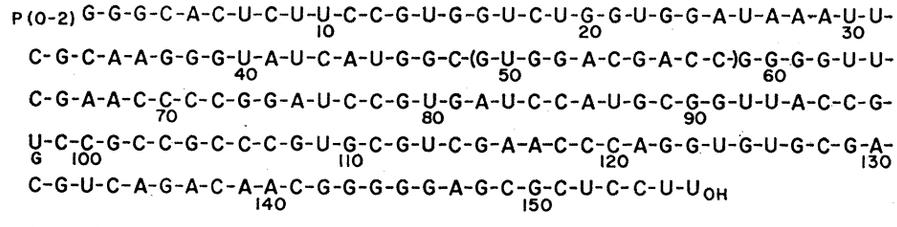
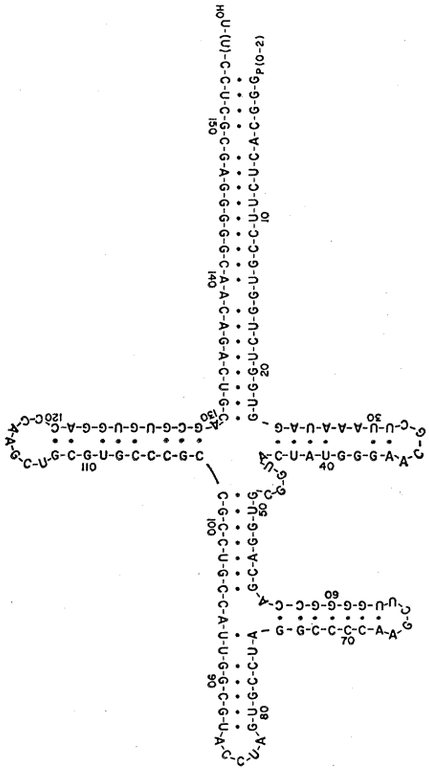



Fig. 1. A principal nucleotide sequence of adenovirus 2 VA RNA. Parentheses indicate a region of the molecule in which alternative sequences may also occur.



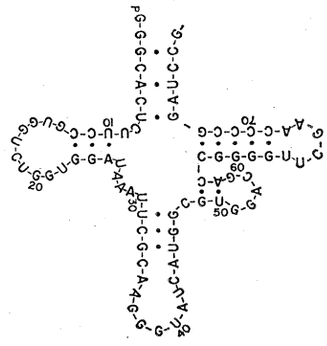
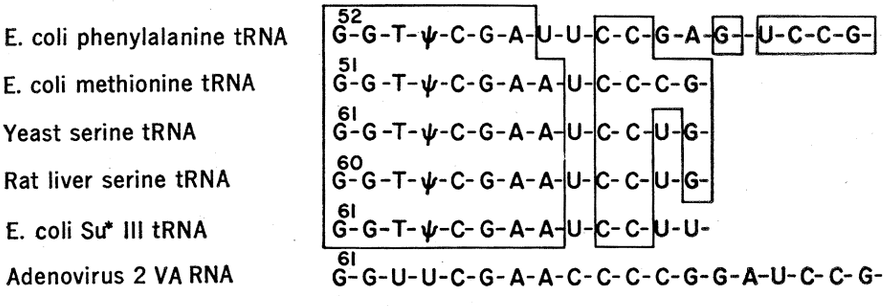


Fig. 2 (left). One possible model for base pairing in VA RNA. Watson-Crick base pairs (G to C and A to U) as well as G to U base pairs are indicated by solid dots.

Fig. 3 (above right). An alternative model for base pairing of one half of VA RNA.

Fig. 4 (below). Resemblance between a nucleotide sequence from VA RNA and sequences from several tRNA's. The tRNA sequences homologous to VA RNA are enclosed in boxes.




geneity has been previously found in K-B cell and mouse-5*S* RNA (*6*). Also, some of the VA RNA molecules had no phosphate attached to the terminal 5′-OH, and other molecules had an extra negative change, possibly due to an additional phosphate. Approximately one-half of the molecules had an alternative sequence in the region of residues 80 to 104, probably a substitution of Gp for Up at residue 98. More than one base change apparently occurs in a portion of the VA RNA molecules in the region between residues 47 and 60. For this reason, analysis of the sequence of nucleotides in this region was somewhat more difficult than in most of the remainder of the molecule. Difficulties were also experienced because of the occurrence of rather similar sequences in different portions of the molecule.

The nucleotide sequence of VA RNA permits fairly extensive base pairing, provided that occasional G • U, and very occasional A • C pairs, are accepted when interspersed between G • C or A • U base pairs. The model illustrated in Fig. 2 is one possible pattern of folding which is consistent with the susceptibility of different phosphodiester links to digestion by ribonuclease T1 and other ribonucleases. The pairing of the 5′- and 3′-ends is similar to that in other RNA molecules whose sequences are known (*7*). Overall, a large portion of the bases in the model are involved in base pairing.

Repeating sequences have been found in *Escherichia coli* 5S, 16S, and 23S ribosomal RNA's and in KB cell 5*S* RNA (*7*, *8*). The VA RNA also has prominent repeating sequences. For example, the nucleotides between residues 64 and 74 differ from those between residues 113 and 123 by only a single Ap to Cp change. These repeating sequences are separated by considerably less than one half of the molecule. There is some sequence homology in the three loops which are drawn between residues 113 and 119, between residues 30 and 36, and between residues 63 and 68 in the model shown in Fig. 2.

Valine is a possible initiating amino acid for protein synthesis in animal cells. Two possble valine codons (GUG and GUC) occur adjacent to one another near the 5′ terminus of VA RNA molecule, and they are not in phase with any known nonsense codon in the remainder of the molecule. However, this may be coincidence, particularly since the molecule has a low content of adenylic acid so that the terminator codons UGA, UAA, and UAG would occur infrequently if the sequence were random.

An alternative pattern of base pairing can be drawn for the first 78 nucleotides of VA RNA that has some resemblance to the cloverleaf model of tRNA (Fig. 3). Prominent differences include the "bulge" between nucleotides 26 and 31 in the model of VA RNA, and the incomplete and weakly paired "stem" of the VA RNA model. There is also homology between portions of VA RNA and certain tRNA sequences (*7*, *9*), particularly between "GpTpψpCpGp loop" of several tRNA's and the nucleotides in a similar position in VA RNA (Fig. 4). The homology of a sequence of VA RNA to a part of the gene of at least one tRNA from animal cells may provide one part of the explanation of our observation that VA RNA preparations would anneal with both host and virus DNA (*10*). The evidence that VA RNA is the product of a virus gene is not definitive (*10*). This is a significant point, because even a short area of homology between virus and host DNA possibly could be a favorable site for recombination or integration of virus DNA with the host cell genome (*11*).

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**References and Notes**

1. Abbreviations: RNA, ribonucleic acid; tRNA, transfer RNA; A, adenine; C, cytidine; G, guanine; U, uracil; T, ribothymidine; ψ, dihydrouracil; p and — indicate a phosphate group, on the left side a 5′-phosphate and on the right side a 3′-phosphate; and DEAE, diethylaminoethyl.
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## ST-Feline Fibrosarcoma Virus: Induction of Tumors in Marmoset Monkeys

Abstract. *Two newborn marmosets, inoculated with a cell-free extract of feline fibrosarcomas, developed multiple sarcomas and died within 46 days of inoculation, whereas two of these animals inoculated with a crude homogenate developed no tumors. This susceptibility to a mammalian RNA sarcoma virus suggests that marmosets may be particularly suitable for attempts to isolate infectious agents from man.*

The cell-free transmission of a feline fibrosarcoma between kittens, and from kittens to newborn dogs and rabbits, was described by Snyder and Theilen (*1*). We describe here the inoculation of marmoset monkeys (*Saguinus fuscicollis*) with tumor homogenates and cell-free extracts of feline fibrosarcomas and their subsequent development of multiple sarcomas.

A tumor homogenate was prepared from a pool of four experimentally induced feline fibrosarcomas (*2*). A crude 25 percent tumor suspension was prepared in tris-ethylenediaminetetraacetate buffer at *p*H 7.0, homogenized in a mortar with sterile sand, and centrifuged at 600*g* for 15 minutes. The supernatant was inoculated intraperitoneally (0.25 ml) and subcutaneously (0.25 ml) into both a newborn and a 3-day-old marmoset. One animal died with bacterial enteritis at 4 weeks after inoculation without signs of tumor development; the other animal is alive and normal 14 weeks afterward.

A cell-free extract was prepared from the same pool of feline fibrosarcomas by Moloney's technique (*3*). The extract, in 0.5 g tumor equivalents per animal, was inoculated intraperitoneally and subcutaneously in divided doses into a newborn and into a 3-day-old marmoset. Both animals developed palpable masses in the right inguinal area by 3½ to 4 weeks after inoculation. They died 4 weeks and 46 days, respectively, after inoculation, and in both animals inguinal and multiple intraabdominal tumors were found. The largest tumor, 3.0 cm in diameter, was found in the animal that died 46 days after inoculation. It was located on the left side of the abdomen, contralateral to the intraperitoneal inoculation site and extended from the stomach anteriorly to the kidney posteriorly. In both animals lesions were absent at the sites of subcutaneous inoculation.

Tumor tissues were collected at necropsy for light and electron microscopy and for in vitro culture. The densely cellular tumors were classified as fibrosarcomas (Fig. 1), although they were composed of two cell types, fusiform and polygonal. Mitoses were numerous in both cell types. Fusiform cells with variable amounts of intercellular collagen comprised 80 to 90 percent of the tumor masses in the animal with the longer course, and in this animal neoplastic tissue had invaded stomach, urinary bladder, and skeletal muscle surrounding the inguinal mass. In the other animal, polygonal-shaped mononuclear cells predominated in the tumors and diffuse mesothelial cell hyperplasia was seen along visceral and parietal peritoneal surfaces. These hyperplastic mesothelial cells were identical histologically to the polygonal-shaped tumor cells. Hemorrhage and necrosis were present in some tumors, but no inflammatory cells were detected within the tumor masses. Electron microscopic study of 100 tumor cells from the animal with the shorter course revealed no viral particles. Tumor cells from both animals are growing in in vitro cell culture and are currently in the 6th and 14th serial cell passages, respectively. They have a modal chromosome number of 46, the normal diploid number for marmosets, and the karyotype is essentially normal. Although a mixture of large mononuclear and fibroblastic cells is still present in the cell cultures, the proportion of fibroblastic cells has increased.

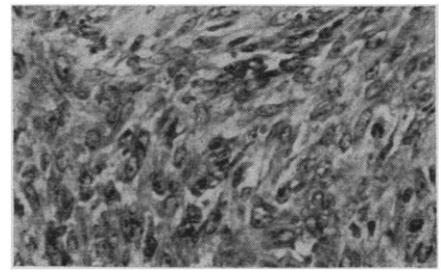

Fig. 1. Photomicrograph of an abdominal tumor from a marmoset that died 46 days after inoculation with ST-feline fibrosarcoma virus (hematoxylin and eosin stain; ×220).

In addition to the susceptibility of marmosets to a mammalian RNA sarcoma virus reported here, it was shown previously that marmosets are more susceptible to certain avian sarcoma viruses (*4–6*) than other nonhuman primates (*7, 8*). The reason for this is unknown, but, even before we understand the reason, marmosets seem to be a prime choice as experimental animals in attempts to isolate similar agents from human malignancies.

*Note added in proof*: Since this report was submitted, tumors have been induced in four more marmosets, and C-type virus particles have been demonstrated in cultured tumor cells by electron microscopy.

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