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# Effect of Upstream Reading Frames on Translation Efficiency in Simian Virus 40 Recombinants

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**In a previous report (S. Subramani, R. Mulligan, and P. Berg, Mol. Cell. Biol. 1:854–864, 1981), it was shown that mouse dihydrofolate reductase (DHFR) could be efficiently expressed from simian virus 40 recombinant viruses containing the DHFR cDNA in different locations in the viral late region. This was true even in the case of the SVGT7dhfr26 recombinant, which had the DHFR coding sequence 700 to 800 nucleotides from the 5′ end of the mRNA, where it was preceded by the VP2 and VP3 initiator AUGs and a number of other noninitiator AUGs. To investigate the process of internal translation initiation in mammalian cells, we constructed a series of SVGT7dhfr recombinants in which the upstream VP2 and VP3 reading frame was terminated in various positions relative to the DHFR initiation codon. The efficient production of DHFR in infected CV1 cells depended on having the terminators of the VP2-VP3 reading frame positioned upstream or nearby downstream from the DHFR initiation codon. These results reinforce the notion that mammalian ribosomes are capable of translational reinitiation.**

Based on analysis of the nucleotide sequences of many eucaryotic mRNAs and direct experiments on the mechanism of translation initiation, Kozak proposed the scanning hypothesis and suggested that translation is almost invariably initiated at the first AUG downstream from the 5′ capped end of the mRNA (13). However, we found numerous instances in which protein-coding segments of DNA that had been inserted at several locations in the simian virus 40 (SV40) genome or into expression plasmids were efficiently translated even though there were several AUG triplets upstream of the protein-coding sequence. Thus, the SV40 recombinant genome SVGT7dhfr26, which contains the mouse dihydrofolate reductase (DHFR) cDNA, produces high levels of DHFR protein even though its coding sequence is preceded in the mRNA by the initiation codons for VP2 and VP3, as well as by a number of noninitiator AUGs (see Fig. 5) (30). The bacterial DNA segment (*gpt*) that encodes xanthine-guanine phosphoribosyl transferase (23) and the gene from the transposon Tn*5* plasmid (*neo*) that specifies neomycin-kanamycin-G418 phosphotransferase (29) are expressed in mammalian cells from mRNAs that contain two and seven noninitiator AUGs, respectively, upstream of the protein-coding sequences (24, 29). Indeed, the many exceptions to the first-AUG rule have resulted in modifications of the original scanning hypothesis to account for initiation of translation at internal AUGs (14–18).

In the accompanying report (27), we demonstrated that translation could be initiated at an internal AUG provided that the translation that initiates at an upstream AUG codon is terminated close to an internal AUG codon. In this paper, we extend our earlier analysis of the expression of DHFR with SV40-*dhfr* recombinant viruses by altering the sequence upstream of the *dhfr* initiator AUG to reveal signals required for efficient internal translation initiation. The results reinforce the suggestion that translation of mammalian mRNAs can be reinitiated at internal AUG codons if translation that had been initiated upstream is terminated in the vicinity of the internal AUG. Our results are also consistent with those recently reported by others (6, 10, 11, 20).

## MATERIALS AND METHODS

**Construction and modification of SV40 vectors containing the mouse DHFR cDNA sequence.** The SV40 vectors containing the mouse DHFR cDNA segment at various locations in the late region and the types of viral mRNAs that contain the *dhfr* sequence are shown in Fig. 1. Various modifications within the *dhfr* segment and alterations of the nucleotide sequences at the junction of the *dhfr* and SV40 sequences of the SVGT7 and SVGT9 recombinants were performed in plasmid forms of the SV40 recombinants constructed by inserting pBR322 sequences at the *Pst*I restriction site in the SV40 early region and propagating them in *Escherichia coli*. After introduction of the various modifications, the plasmids were cloned in *E. coli* and their structures were verified by nucleotide sequencing (21). The recombinant viral genomes were excised from the plasmids by digestion with *Pst*I, recircularized by ligation at low DNA concentrations, and transfected into CV1P cells with *ts*A58 DNA as helper (22).

Figure 2 summarizes the structures of several SVGT7dhfr derivatives at the junction of the viral and *dhfr* sequences. Each contained various lengths of DNA in the 5′ untranslated region of the *dhfr* cDNA segment. The SVGT7dhfr28, -22, and -7 recombinants were constructed with *dhfr* cDNA clones that lacked different amounts of sequence 5′ to the initiator AUG. The resulting deletions and frameshifts altered the position of termination of the VP2 and VP3 reading frame relative the *dhfr* initiation codon. SVGT7dhfr22X was constructed by filling in the *Hin*dIII site at the SV40-*dhfr* junction with DNA polymerase I and inserting a synthetic *Xba*I linker sequence. The net result is the insertion of a total of 12 nucleotides and the introduction of a UAG triplet in the VP2/3 reading frame. Another construction, pSVGT7 dhfr22ter3, also restores upstream termination by the insertion of a 25-base-pair sequence containing terminators in all

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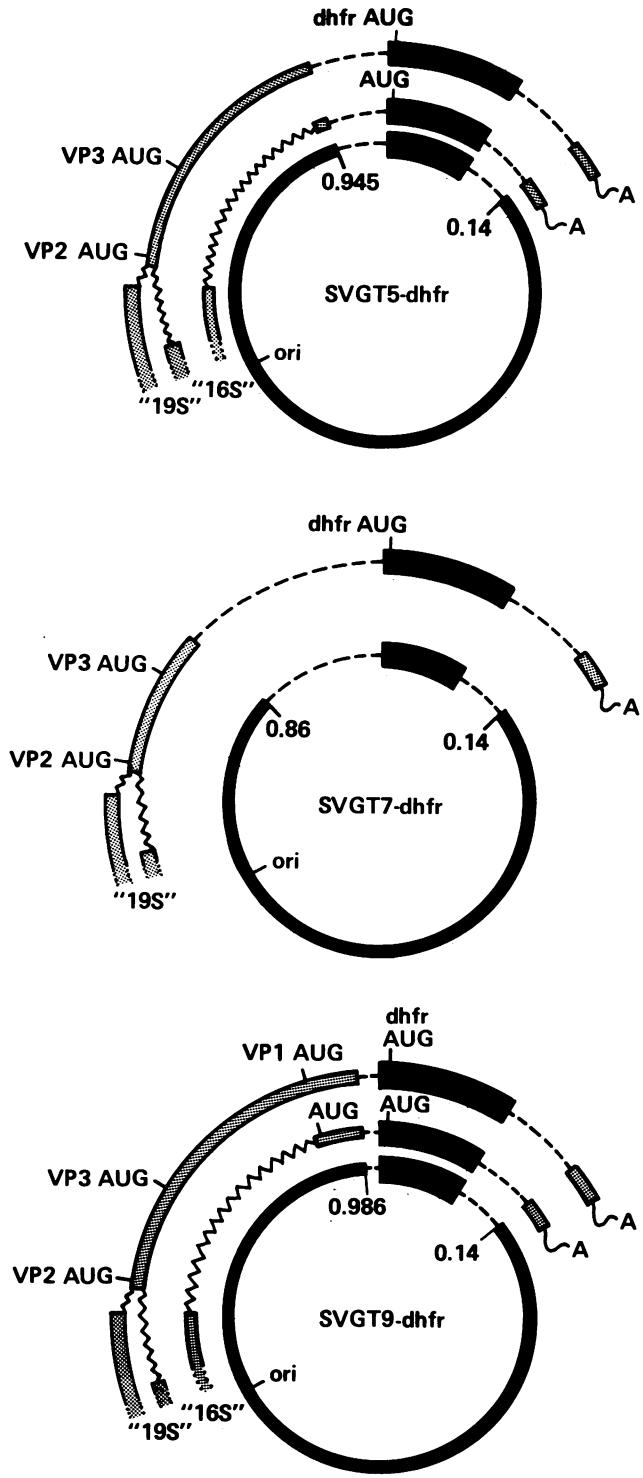


FIG. 1. Structures of the SVGT5, SVGT7, and SVGT9 *dhfr* recombinants and the structures of the mRNAs they produced. Zigzag lines indicate portions of the primary transcripts that were removed by splicing to produce the mature 16S- and 19S-like species. Solid bars represent the *dhfr* sequence. Stippled bars are portions of the SV40 late region that are retained in the mature transcripts from the recombinant. Dashed lines indicate portions of SV40 deleted in the recombinant. Map coordinates for the insertion sites of the *dhfr* sequence are indicated.

three reading frames. (We thank T. Chappell of Stanford University for providing the ter3 fragment.)

In the SVGT9dhfr recombinants, the DHFR cDNA segment lay between the *Hin*dIII site at map position 0.985 and the *Bam*HI site at 0.14 in the SV40 genome (Fig. 1). The modified derivatives SVGT9dhfr26FH and SVGT9dhfr7FH were prepared from the plasmid forms of SVGT9dhfr26 and SVGT9dhfr7 by filling in the *Hin*dIII site at the SV40-*dhfr* junction with DNA polymerase I, followed by recircularization with T4 DNA ligase. This converted the sequence AAGCTT to AAGCTAGCTT, introducing a UAG triplet into the VP1 reading frame upstream of the *dhfr* initiator AUG. The structure of the recombinants was confirmed by nucleotide sequencing.

**Analysis of protein and RNA extracted from infected cells.** CV1 cells were infected with recombinant viruses (1 to 10 PFU/cell), and protein was extracted at 48 h postinfection as described previously (30). Extracts were fractionated on 15% polyacrylamide gels in the presence of sodium dodecyl sulfate (SDS) (19) and then transferred to a nitrocellulose membrane by the method of Burnette (4). DHFR was detected by incubation of the membrane with antibody to DHFR (the gift of R. Schimke, Stanford University) and $^{125}$I-protein A, followed by exposure to Kodak X-Omat AR film.

RNA was extracted at 48 h postinfection (25) and analyzed by the S1 nuclease protection method (3, 31) with probes labeled with [$\gamma$-$^{32}$P]ATP and polynucleotide kinase (21) at a *Hin*fI site within the *dhfr* coding sequence (see Fig. 4A). After hybridization and treatment with S1 nuclease, the protected fragments were separated by electrophoresis in agarose gels containing methylmercuric hydroxide (1) and visualized by autoradiography of the dried gel.

## RESULTS

**SVGT7dhfr recombinants.** In the recombinants used in this study, various segments of the SV40 late regions were replaced with cDNAs containing different amounts of *dhfr* 5′ leader sequences. Since the recombinants lacked essential viral functions, their genomes were defective, and they were propagated in the presence of *ts*A58 as helper. The basic structures of these vectors and the mRNA species they produced are illustrated in Fig. 1. The SVGT5dhfr recombinants lacked the *Hin*dIII to *Bam*HI fragment between map units 0.945 and 0.14; it was replaced with the *dhfr* cDNA sequence. This recombinant retained all the late mRNA splicing signals, and analogs of the normal 16S and 19S mRNAs were produced in infected cells. Since the *dhfr* sequence almost exactly replaced the VP1 coding region, DHFR was produced in the large quantities typical of the major virion protein.

SVGT7dhfr recombinants had a more extensive portion of the SV40 genome replaced, extending from the *Hin*dIII site at map coordinate 0.86 to the *Bam*HI site at 0.14. Here, the 3′ splice site for the 16S mRNA species was deleted and only 19S-like mRNAs were produced. This places the *dhfr* coding sequence downstream of the VP2 and VP3 initiation codons, about 800 nucleotides from the 5′ end of the mRNA. In this arrangement a number of AUG triplets occurred between the 5′ end of the mRNA and the beginning of the *dhfr* coding sequence. In spite of this apparently unfavorable arrangement, SVGT7dhfr26 produced DHFR at levels similar to those produced by SVGT5dhfr26 (30) (see Fig. 5). The *dhfr26* cDNA segment contained about 90 nucleotides of 5′ leader sequence with termination codons in two reading

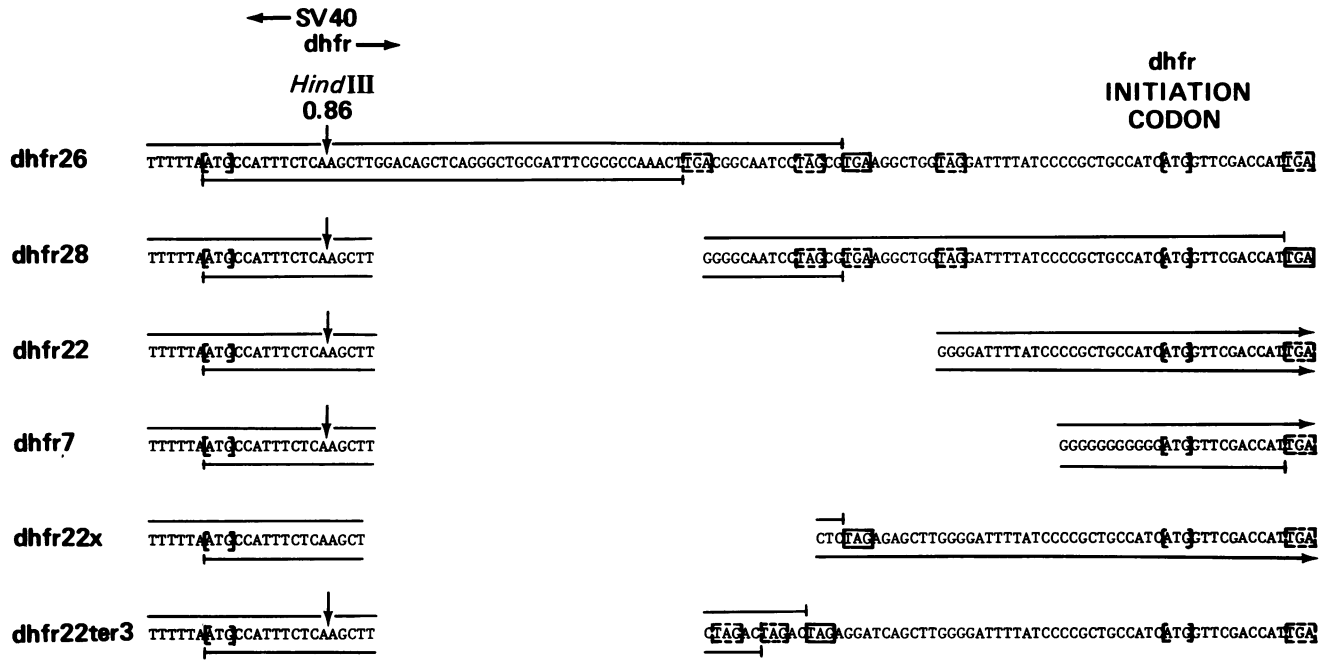


FIG. 2. Nucleotide sequence at the junction of SV40 and 5′ *dhfr* sequences in the SVGT7dhfr recombinants. Gaps in the sequence indicate deletions. Initiation codons are bracketed, and termination codons in the VP2/3 reading frame are boxed with solid lines. Terminators in other frames are boxed with dashed lines. The bar over each sequence illustrates the VP2/3 frame and the position at which it terminates. In SVGT7dhfr22 and -22X the bar below the sequence shows that the upstream AUG has been brought into frame with the *dhfr* coding region.

frames. Another termination codon in the third frame was located 13 nucleotides downstream of the *dhfr* initiation codon (Fig. 2). Thus, in SVGT7dhfr26, translation that initiated at the VP2 and VP3 initiation codons terminated at a UGA triplet in the *dhfr* leader, about 35 nucleotides upstream of the *dhfr* initiation codon. Initially, we believed that the high level of DHFR expression by this recombinant might be the consequence of efficient translational reinitiation, but an alternative explanation was that DHFR synthesis resulted from the failure of some scanning ribosomes to recognize the upstream AUGs as initiation codons. To explore this question we constructed a series of SVGT7 recombinants with various amounts of the sequence just upstream of the *dhfr* initiator AUG (Fig. 2). These derivatives contained progressively larger deletions of the *dhfr* leader, but the upstream SV40 sequences were unchanged. The deletions eliminated termination codons and introduced frameshifts, changing the position of termination of the VP2/3 reading frame relative to the *dhfr* initiator AUG. The arrangements of the VP2/3 and *dhfr* reading frames in the 19S-like mRNAs produced by these recombinants after infection of monkey cells are shown schematically in Fig. 3.

S1 nuclease analyses were performed to verify that the predicted mRNA species (Fig. 3) were indeed produced. In this experiment, probes were labeled at their 5′ ends at a *Hin*fI site within the *dhfr* sequence as illustrated in Fig. 4A. These probes measured the intactness of the mRNA from a position about 140 nucleotides downstream of the *dhfr* initiator AUG up to the 3′ splice site of the SV40 late 19S mRNA. For comparison, similar analyses were performed with SVGT5dhfr26 and with SV40 itself. All the SVGT7dhfr recombinants produced predominantly the expected 19S-like species and a small amount of unspliced RNA (Fig. 4B).

The consequences of the deletions in the *dhfr* leader for DHFR expression were determined by protein blotting analysis (Fig. 5) and are summarized in Fig. 3. SVGT5dhfr26, which was included for comparison, produced DHFR from a 16S-like mRNA in which the *dhfr* coding region replaced that of VP1 (Fig. 1). It produced enough DHFR to be easily visualized by Coomassie blue staining of whole-cell extracts fractionated by SDS-polyacrylamide gel electrophoresis (30). SVGT7dhfr26 produced the same amount of DHFR in spite of the presumably unfavorable location of *dhfr* sequences in the mRNA. In SVGT7dhfr28, 34 nucleotides of the 5′ leader sequence of the *dhfr* cDNA were deleted (Fig. 2) and the VP2/3 reading frame was terminated at a UGA triplet 13 nucleotides 3′ to the *dhfr* initiation codon. This recombinant also produced high levels of DHFR. SVGT7dhfr22 had a further deletion of 25 nucleotides and terminated VP2/3 translation at a UGA triplet 137 nucleotides beyond the *dhfr* initiator. In this case, DHFR expression dropped to about 5% of that produced by the other recombinants. In addition, there was another species that was slightly larger than DHFR. Note also the presence of a faint band between these two; this represents endogenous monkey DHFR, which was present even in uninfected cells. The formation of the longer species probably resulted from initiation at an AUG in the SV40 sequence near the junction with the *dhfr* segment (Fig. 2). This AUG was within the VP2/3 coding sequence but not in the VP2/3 reading frame. However, in SVGT7dhfr22, this AUG fell in the DHFR reading frame and its location, 14 codons upstream of the *dhfr* initiation codon, would explain the increased size of the additional species. Further evidence of the identity of this species is presented below. Considering the two DHFR species together, expression from SVGT7dhfr22 was about 20% of the level observed with SVGT7dhfr26 and SVGT7dhfr28.

In SVGT7dhfr7, a further deletion removed an additional 20 nucleotides, but nine G's that were remnants of the

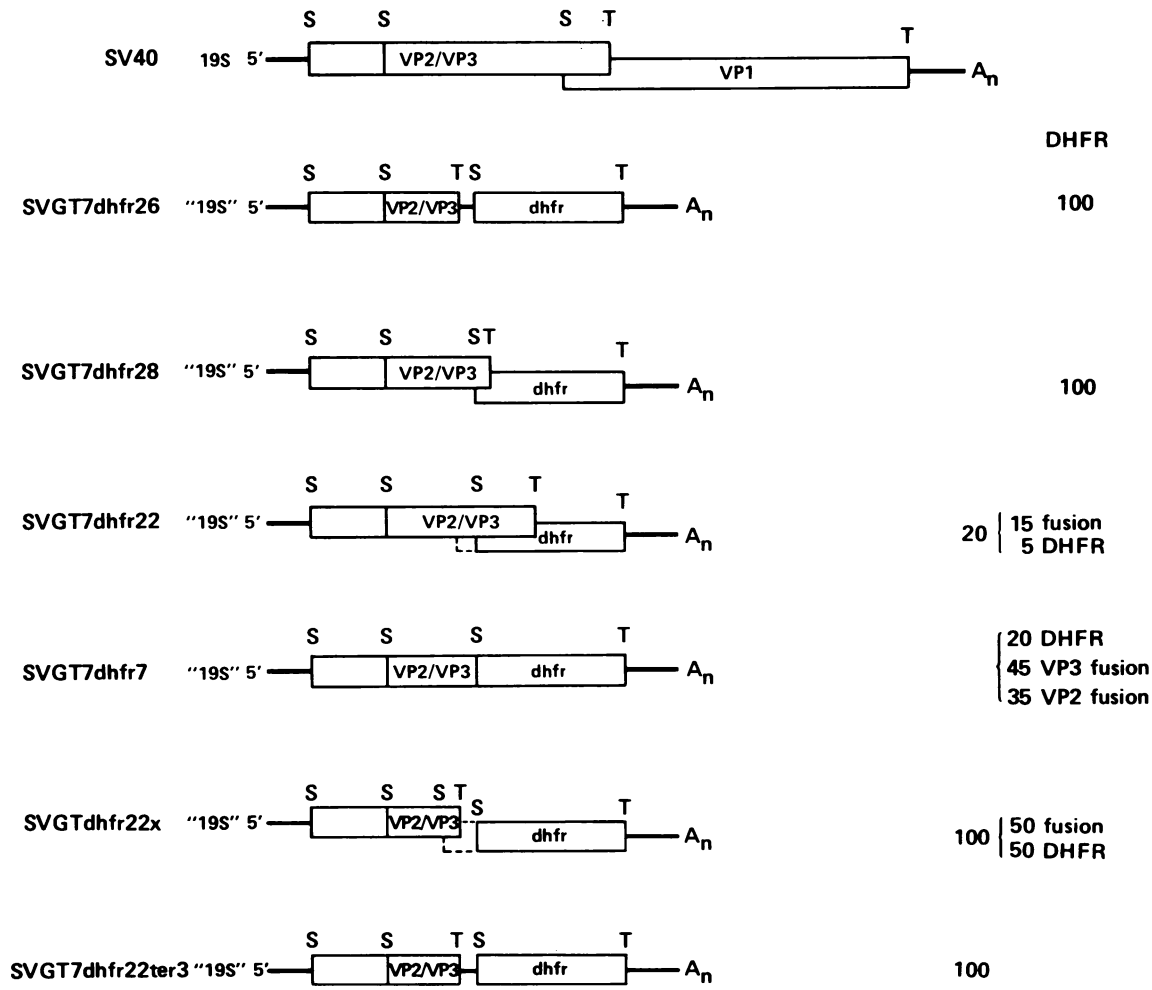


FIG. 3. Structures of the late mRNAs produced by the SVGT7dhfr recombinants and the relationship of the VP2/3 reading frame to the *dhfr* reading frame. S, Translation start site; T, terminator of an open reading frame. The relative percentages of DHFR produced by each recombinant are listed at right, with the amount of DHFR produced by SVGT5dhfr26 arbitrarily defined as 100%.

cloning of the *dhfr7* cDNA (5) were included immediately adjacent to the initiator codon (Fig. 2). In this recombinant, the VP2/3 reading frame was fused to the DHFR coding sequence. Consequently, initiation at the VP2 and VP3 initiation codons continued through to the UAA termination codon at the end of the DHFR coding sequence. SVGT7dhfr7 produced two species that corresponded in size to the predicted VP2-DHFR and VP3-DHFR fusion proteins, while the normal form of DHFR was produced at about 20% of the level synthesized by SVGT7dhfr26.

In the SVGT7dhfr26, -28, -22, and -7 recombinants, the termination codon for translation in the VP2/3 reading frame was moved from a position upstream of the *dhfr* initiator codon to locations increasingly distant downstream. These deletions also shortened the *dhfr* leader sequence. Therefore, it was possible that the reduction in DHFR levels observed with SVGT7dhfr22 and -7 was not due to the changed position of VP2/3 termination, but to the loss of a sequence in the *dhfr* leader needed to capture scanning ribosomes and direct them to the initiation site. In this view, translation of the *dhfr* coding sequence is presumed to result from ribosomes that fail to recognize the VP2 and VP3 initiators and continue scanning until they encounter a signal in the *dhfr* leader sequence that mediates efficient recognition of the initiator AUG. To distinguish between these possibilities, two additional recombinants were constructed from SVGT7dhfr22. One, SVGT7dhfr22X, was made by inserting a synthetic oligonucleotide containing the recognition sequence for the *Xba*I restriction endonuclease at the *Hin*dIII site that joins the SV40 and *dhfr* sequences (Fig. 2). This introduced a UAG triplet into the VP2/3 reading frame upstream of the *dhfr* initiator AUG. SVGT7dhfr22ter3, another derivative of SVGT7dhfr22, contained a 25-base-pair fragment which placed termination triplets in each of the three reading frames at the same *Hin*dIII site (Fig. 2). In each case, insertion of a termination codon in the VP2/3 frame upstream of the *dhfr* initiation site restored efficient DHFR synthesis (Fig. 5). Cells infected with SVGT7dhfr22X also produced high levels of a second DHFR-related species which probably resulted from initiation at the same upstream AUG triplet that produced the larger species in SVGT7dhfr22. The *Xba*I linker insertion in SVGT7dhfr22X resulted in the addition of four amino acids in the amino-terminal portion of the aberrant protein. Thus, it was 18 amino acids longer than normal DHFR and 4 amino acids longer than the corresponding SVGT7dhfr22 fusion species. The mobility shifts observed on SDS-polyacrylamide gels were consistent with the predicted changes in the molecular

sizes of these proteins. SVGT7dhfr22X produced both the fusion and normal forms of DHFR at high levels, apparently because termination of VP2/3 translation occurred only 21 nucleotides downstream of the alternative initiation site. The results with SVGT7dhfr28 have already shown that ribosomes can reinitiate translation efficiently at a site upstream of the terminator if the distance is not too great.

Infection with SVGT7-dhfr22X also produced a third, still larger DHFR fusion protein. Its size suggested that it was the product of initiation at either of two SV40 AUGs just 3′ to

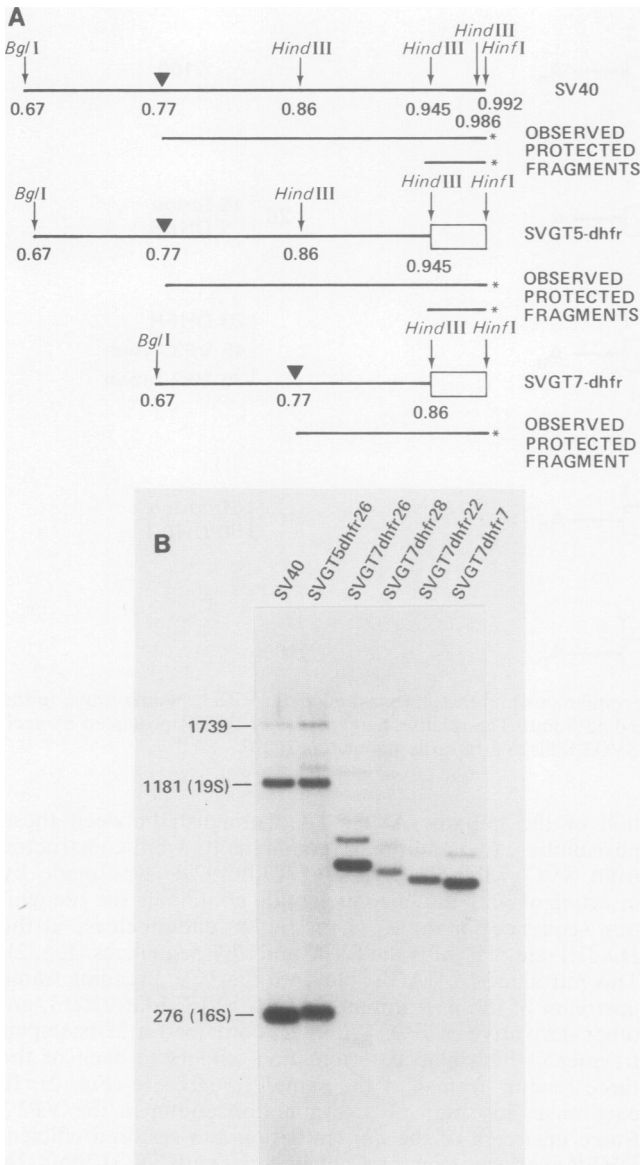


FIG. 4. S1 nuclease analysis of *dhfr*-containing mRNAs produced by the SVGT7dhfr recombinants. (A) Schematic illustration of the probes used in this experiment. All were labeled with [$\gamma$-$^{32}$P]ATP and polynucleotide kinase at *Hin*fI sites as indicated. SV40 sequences are shown as straight lines, and the inserted *dhfr* sequence is illustrated as a box. The extent of the fragments protected by the mRNA from S1 nuclease digestion is indicated below each probe. Asterisks indicate the position of the $^{32}$P label. (B) Analysis of protected fragments by electrophoresis in an agarose gel containing methylmercuric hydroxide. Bands were visualized by autoradiography. Sizes are indicated on the left (in nucleotides).

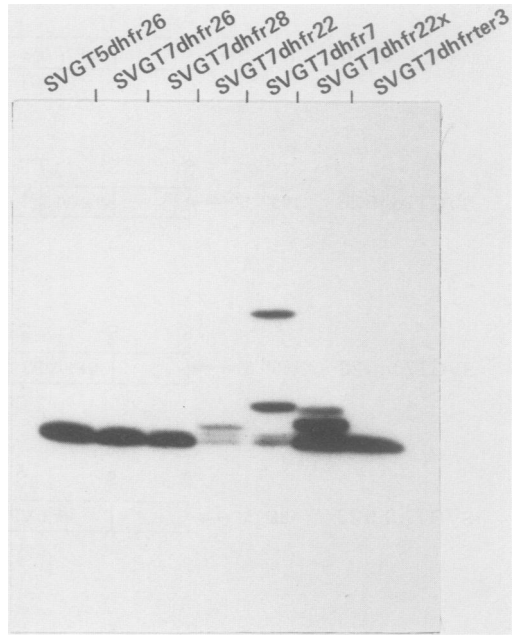


FIG. 5. Western blot analysis of DHFR and DHFR-related proteins produced in cells infected with the SVGT7dhfr recombinants. Crude cell lysates were fractionated by SDS-polyacrylamide gel electrophoresis, and after transfer to a nitrocellulose membrane, DHFR was detected with antibody and $^{125}$I-labeled protein A.

the VP3 initiation codon and about 140 nucleotides upstream of the *dhfr* initiator. This would produce a form of DHFR containing an additional 46 or 49 amino acids at its amino terminus.

**SVGT9dhfr recombinants.** In another series of recombinants, termed SVGT9dhfr, the *dhfr26* and *dhfr7* cDNA sequences were inserted between the *Hin*dIII site at map position 0.986 and the *Bam*HI site at coordinate 0.14 in the SV40 genome (Fig. 1). All the late region splice sites were preserved intact in SVGT9dhfr7 and -26, and the *dhfr* analogs of the 16S and 19S viral mRNAs were produced. Their structures are diagrammed in Fig. 6. In SVGT9dhfr26, translation that began at the VP1 initiator terminated before reaching the *dhfr* initiation triplet, whereas in SVGT9dhfr7 translation of VP1 terminated at a site 137 nucleotides downstream of the *dhfr* initiator. The recombinant called SVGT9dhfr7FH was derived from SVGT9dhfr7 as described in Materials and Methods; a UAG triplet was introduced into the VP1 reading frame 17 nucleotides upstream of the *dhfr* initiator codon. For comparison a similar derivative of SVGT9dhfr26 was constructed and called SVGT9dhfr26FH.

After infection with these recombinants, the predicted 16S- and 19S-like mRNAs diagrammed in Fig. 6 were identified by S1 nuclease protection assays as described above. No aberrant species of mRNA were detected (data not shown).

The production of DHFR by these recombinant viruses was analyzed by the protein blotting technique (Fig. 7). After infection with SVGT9dhfr26 and -26FH, DHFR was produced at levels somewhat lower than those with SVGT5dhfr26 (compare with Fig. 5). The production of DHFR by these recombinants was not surprising since both of them contained terminators in the VP1 reading frame upstream of the *dhfr* initiation codon (Fig. 6). However, in SVGT9dhfr7, VP1 translation terminated 137 nucleotides

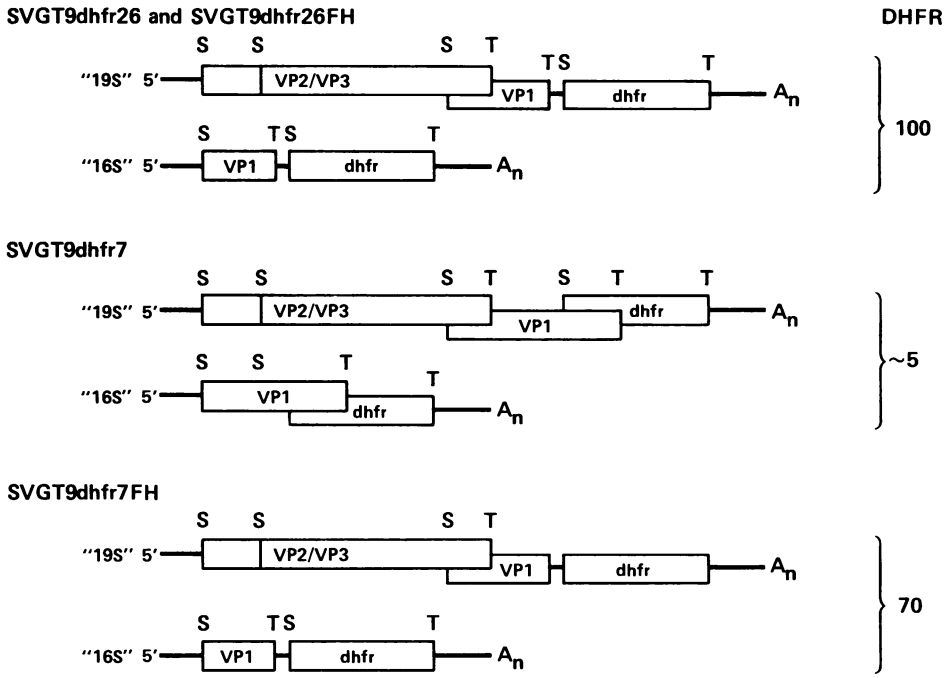


FIG. 6. Structures of the late mRNAs produced by the SVGT9dhfr recombinants and the positions of the various reading frames. Relative percentages of DHFR produced are shown at the right. For symbols, see the legend to Fig. 3.

beyond the *dhfr* AUG. As a consequence, DHFR synthesis was abolished. When a termination codon was reintroduced in SVGT9dhfr7 to produce SVGT9dhfr7FH, DHFR synthesis was restored to a level nearly equivalent to that produced by SVGT9dhfr26.

## DISCUSSION

The results presented in this paper are consistent with our previous observations (27) and support the model that translation initiation can occur at an internal AUG if the translation beginning upstream is terminated before or just after the internal initiation codon. The synthesis of DHFR by the recombinant viruses described in this paper was strongly dependent on the presence of a termination codon in the appropriate reading frame either upstream or at a nearby downstream location relative to the *dhfr* initiator AUG. Thus, DHFR is synthesized efficiently in the SVGT7 recombinants when translation begun in the VP2/3 reading frame terminates upstream of the *dhfr* initiation codon. However, if the translation is terminated at a point 137 nucleotides or more downstream of the *dhfr* AUG, DHFR expression is significantly reduced.

These experiments also support our earlier contention (27) that ribosomes can reinitiate translation at sites upstream of a termination codon. This was the case for SVGT7dhfr28 and for the alternative forms of DHFR produced by SVGT7dhfr22X. Thus, if termination occurs about 15 to 20 nucleotides downstream of an initiation codon, reinitiation occurs about as efficiently as when the termination codon is a similar distance upstream of the internal initiation site. In SVGT7dhfr22X, the termination codon for VP2/3 translation lies between the two major initiation sites, and each appears to be utilized with about equal efficiency. The production of the third and largest DHFR-related species by SVGT7dhfr22X suggests that "reach-back" reinitiation may even be possible over distances of 80 to 90 nucleotides. Our previous experiments indicated that reach-back reinitiation could occur over distances of at least 50 nucleotides. Although the maximum distance over which a ribosome may reach back to reinitiate has not been precisely determined, these results show that ribosomes may be able to scan bidirectionally.

The low levels of DHFR synthesis by SVGT7dhfr7 and -22 indicate that in addition to termination-reinitiation, there may be another way to initiate translation at an internal AUG. Perhaps some of the scanning ribosomes fail to recognize the VP2 and VP3 initiator codons and continue their migration along the mRNA until they encounter the *dhfr* initiator AUG. The same mechanism might also account for the fusion protein produced by SVGT7dhfr22. Kozak has referred to such a process as relaxed scanning (14). She has compared the sequences flanking many eucaryotic initiation

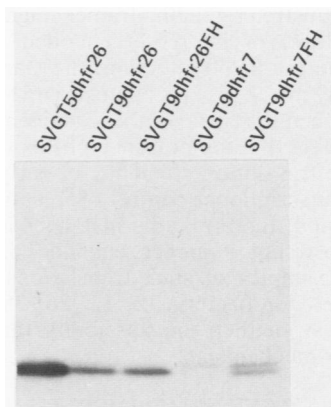


FIG. 7. Western blot analysis of DHFR produced by SVGT5 and SVGT9 *dhfr* recombinants.

codons and finds a preference for the sequence AXXAUGG (14–17). The sequence around the VP2 translation initiation site is UCCAUGG, whereas translation of VP3 is initiated with GGAAUGG. Since both are less than ideal fits to the consensus sequence, it may be that the VP2 and VP3 initiation codons are weakly recognized and permit some ribosomes to initiate translation at a downstream AUG. In fact, the synthesis of VP3 is itself an apparent example of relaxed scanning, since its initiation codon is located downstream of the VP2 initiation site on all the abundant forms of 19S RNA. Alternatively, residual DHFR synthesis may represent a low level of direct internal initiation at the DHFR initiator AUG or long-range reach-back reinitiation.

While the dependence of *dhfr* translation on the position of terminators of upstream reading frames is consistent with the termination-reinitiation model that we (27) and others (10, 11, 18, 20) have proposed, it must be emphasized that alternative mechanisms have not been ruled out categorically. For example, a fraction of scanning ribosomes may fail to recognize any of the several upstream AUGs and bypass them to gain access to the internal *dhfr* initiation codon. If active translation from the VP2 and VP3 AUGs proceeding through the *dhfr* initiation codon inhibits the ability of the remaining scanning ribosomes to utilize the internal site, a similar dependence on upstream termination would be observed. This is probably an unlikely explanation of our results, since we would have expected a similar steric inhibition of DHFR synthesis in recombinants like SVGT7dhfr28, in which the VP2/3 frame terminates a shorter distance downstream of the *dhfr* initiator. In addition, such a "relaxed scanning-ribosome occlusion" model predicts that in SVGT7dhfr7, VP2/3 translation reading through the *dhfr* AUG reduces its initiation efficiency to 20% of the unoccluded levels observed with SVGT7dhfr26. In other words, 80% of the scanning ribosomes that arrive at the *dhfr* initiator AUG in SVGT7dhfr7 should fail to initiate there. This model therefore predicts a reduction of almost one-half in the total quantity of DHFR and DHFR fusion products in SVGT7dhfr7 compared with the amounts produced by SVGT7dhfr26. This is a difference that would be detected by our methods. However, the sum of the amounts of VP2-DHFR, VP3-DHFR, and DHFR synthesized in cells infected with SVGT7dhfr7 was equal to the DHFR levels present in SVGT7dhfr26 infections.

Regardless of the mechanism, however, it is clear that upstream reading frames can profoundly influence the level of internal translation initiation depending on where they are terminated relative to the internal AUG. This raises the possibility that upstream reading frames might be utilized to modulate the level of internally encoded products. For example, late during SV40 infection, the start site for transcription of early mRNA is shifted upstream so that an AUG triplet is introduced upstream of the T antigen initiator AUG (7–9). It is possible that the presence of this upstream AUG may reduce the translation efficiency of early mRNA, providing a posttranscriptional control of T antigen synthesis. On the other hand, there may be instances in which translation of an upstream sequence enhances initiation at an internal site. Examples of such translational coupling are well documented for bacteria (2, 12, 26, 28). The results presented here strengthen the possibility that animal cells may use similar mechanisms.

## ACKNOWLEDGMENTS

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