Volume 12 Number 19 1984 Nucleic Acids Research

Adenovirus mutants with DNA sequence perturbations in the intragenic promoter of VAI RNA gene allow the enhanced transcription of VAII RNA gene in HeLa cells

Ramesh A.Bhat and Bayar Thimmappaya*

Department of Microbiology and Immunology and Northwestern University Cancer Center, Northwestern University Medical School, 303 E. Chicago Ave., Chicago, IL 60611, USA

Received 11 July 1984; Revised and Accepted 13 September 1984

ABSTRACT

Ad2 VAI gene strongly competes for transcription with VAII gene *in vitro*. It has been suggested that this competition may be a basis for the large excess of VAI gene transcription in virus infected cells at late times. We have studied the effect of the DNA sequence perturbations of the intragenic promoter of the VAI gene on transcription of VAII gene at the level of viral chromosome. Several Ad5 mutants with mutations in the promoter of VAI gene were constructed and transcription of their VAI and VAII genes were analyzed in the infected cells. It was found that transcription of VAII gene increased dramatically when either Box A or Box B promoter sequences of VAI gene were mutated or when the entire VAI gene was replaced by a DNA segment with an unrelated DNA sequence. Thus, at late times, active transcription of VAI gene appears to partially repress transcription of VAII gene. Those mutants which synthesized large quantities of VAII RNA only grew more slowly yielding a titer which was 1/10 of that of their parent but 5 to 6 fold higher than that of an Ad5 mutant lacking both VAI and VAII genes.

INTRODUCTION

Cells infected by adenoviruses (Ad) 2 or 5 synthesize large amounts of two low molecular weight RNAs designated virus-associated RNAs I and II (VAI and VAII RNAs, ref. 1,2). The genes coding for these RNAs are located between 29.0 and 31.0 map units (m.u.) on the conventional adenoviral map (2,3,4). The VA RNA genes are transcribed by RNA polymerase III (2,5). The nucleotide sequences of the two VA RNAs and the DNA sequences that encode the RNAs have been determined (6,7,8). The VAI RNA is heterogenous both at 5' and 3' ends and it is 157 to 162 nucleotides long. Transcription of VAI gene is initiated at two closely located sites on the genome separated by three nucleotides (VAI-A and VAI-G species, ref. 7). The VAII RNA is initiated with a G residue and it is 162 nucleotides long. Both VAI and VAII genes are transcribed by independent promoters and they are separated by a 98 base pair (bp) spacer segment. Using *in vitro* transcription systems, it was shown that the DNA sequences required for the transcription of the VAI gene were located within its coding sequence (from +10 to +69 ref. 9,10,11; the numbering here is relative to the starting point of the VAI-G RNA). DNA sequences required for the VAII gene transcription have not been determined; but they are very likely to be in a similar location within the coding sequences of VAII gene. The VAI gene has a split promoter (11). Two DNA sequence blocks within the 59 bp control region of VAI gene that are indispensable for its *in vitro* transcription have been identified (11). Nucleotide

© IRL Press Limited, Oxford, England.

sequences +10 to +18 (Block A) and +54 to +69 (Block B) form two control elements of this promoter and they are separated by a 35 bp DNA segment (11). Mutations in Block A or Block B abolish transcription. Mutations in the segment separating these two blocks have various effects on in vitro transcription; deletion mutations almost abolish transcription whereas substitution and insertion mutations allow near-normal levels of transcription.

The VAI RNA functions to enhance translation of viral mRNAs at late stages in the adenovirus lytic cycle (12). This species is synthesized in much larger quantities than VAII RNAs, the relative proportion of VAI to VAII RNAs at late times being about 40:1 (ref. 12, and our unpublished results). Biosynthesis of the two VA RNAs follows different kinetics (4). Both VAI and VAII RNAs are synthesized at early times after infection in roughly equal amounts. The synthesis of VAII RNA, however, levels off after about 8 to 10 hours whereas the synthesis of VAI RNA continues to rise as the infection cycle progresses, leading to widely different levels of the two species at late times (4). The molecular basis of this differential synthesis of the two VA species is not clear. In in vitro transcription assays, the VAI gene was shown to compete effectively for transcription of the VAII gene (9). These results led to a suggestion that the differential level of the transcription in vivo may be due to this competition effect, viz. the VAI gene competing strongly with the VAII gene for one or more transcriptional factors, thus limiting the transcription of VAII gene (13). In in vitro transcription assays, DNA sequences from +54 to +69 of VAI gene promoter are capable of competing for transcription with another VAI gene or VAII gene (10).

In this paper we have examined the differential regulation of the two VA genes in vivo using a genetic approach. We have asked which nucleotide sequences of the VAI promoter are responsible for the suppresion of transcription of VAII promoter in vivo. Several adenovirus mutants were constructed in which the promoter sequences of the VAI gene were mutated in different regions. Transcription of the VAII gene was analyzed at late times in human cells infected by these mutants. It was found that mutations in either A or B block sequences of the VAI promoter had identical effect and led to a dramatic increase of VAII RNA synthesis.

MATERIALS AND METHODS

Cells and viruses. The 293 cell line (Ad5-transformed human embryo, ref. 14) and HeLa cells (obtained from T. Shenk) were maintained in Dulbecco's modified minimal essential medium containing 10% calf serum. Mutant dl703 is a variant of wild type (WT) Ad5. This variant has a single XbaI site and a single EcoRI site at 29.0 and 30.0 m.u. respectively. The EcoRI site at 30.0 m.u. is a new EcoRI site introduced through recombinant DNA route. Dl704 is identical to dl703 except that it has a 17 bp deletion within the intragenic promoter of the VAII gene. The DNA sequence from XbaI (29.0 m.u.) to HindIII (31.5 m.u.) of dl704 is of Ad2 origin. Dl703 and dl704 are $VAI^+/VAII^+$ and $VAI^+/VAII^-$ respectively. The construction of these two variants was described in an earlier report (15). Both dl703 and dl704 grow to WT levels. Sub722 is a derivative of dl703 in which the 230 bp DNA fragment coding for VAI RNA, between XbaI and EcoRI (29.0 to 30.0 m.u.) was replaced by a 434 bp DNA fragment from 88.3 to 89.7 m.u. of the Ad2 DNA. This mutant is therefore $VAI^-/VAII^+$. The construction of dl705 is similar to the protocol described in an earlier report (similar

to dl330, described in ref. 12). DNA sequences from XbaI (29.0 m.u.) to HindIII sites (31.5 m.u.) of dl705 are of Ad2 origin. The mutant lacking VAI and VAII genes (double mutant) was constructed as follows: the DNA fragment from 0 to 30.0 m.u. of sub722 was ligated to 30.0 to 100.0 m.u. fragment of dl704 ($VAI^+/VAII^-$) taking advantage of the unique EcoRI sites between the two VA genes of these variants. The ligated DNA sample was introduced into human cells by DNA transfection (15). Cell lysate prepared from those cells was used to infect monolayers again. Cytopathic effect was observed after the fourth passage. The resulting mutant was $VAI^-/VAII^-$ (dl-sub720). Deletion mutations in VAI and VAII genes of dl-sub720 were confirmed by digesting the viral DNA with various restriction endonucleases. Polyacrylamide gel analysis of ^{32}P-labelled RNA isolated from HeLa cells infected with dl-sub720 showed no detectable VAI or VAII RNAs, further confirming that both VAI and VAII genes of the double mutant were transcriptionally inactive. The remaining mutant viruses were constructed as described below:

Construction of mutant VAI genes. Plasmid pA2-113 contains a 230 bp fragment from the HindII site to the EcoRI site of dl704 (29.0-30.0 m.u.) cloned between the PvuII and EcoRI sites of pBR322. We have previously introduced deletion, substitution and insertion mutations into the 59 bp intragenic promoter of VAI gene of this plasmid by a variety of site directed mutagenesis procedures (11). Deletion mutation in dl726 was obtained by a D-loop mutagenesis procedure (16,17). The mutant dl-sub723 has DNA sequences of VAI gene from -6 joined to +27 with a HindIII linker. Dl-sub724 has nucleotide sequences of VAI gene, +19, +20 and +22 to +25 substituted with HindIII linker sequences. This gene also has a single base pair deletion at +26. Mutational alterations of the mutant VAI genes described above were confirmed by DNA sequence analysis (18).

Transcription analysis. HeLa cells were infected with various mutant viruses and labelled with ^{32}P-inorganic phosphate at 2 hrs after infection for varying periods of time. At the end of the labelling period, cytoplasmic RNA was prepared by treating the cells with NP40 as described earlier (12). The labelled total RNA was subjected to electrophoresis on a 8% denaturing polyacrylamide gel containing 8M urea. For the quantitation of VA RNAs, the appropriate bands were excised from the gel and their radioactivity was determined. Fingerprint analysis of the gel purified RNA samples was performed as described previously (11).

The restriction endonucleases, T_4 polynucleotide kinase, T_4 DNA ligase and HindIII linkers were purchased from New England Biolabs. All of the enzymes were used as recommended by the suppliers.

RESULTS

Construction of mutant viruses.

The mutant viruses described here were constructed in two steps. In the first step we cloned a 230 bp DNA fragment coding for the VAI gene into a derivative of pBR322 (see Materials and Methods). Mutations were introduced into the promoter segment of the VAI gene present on this plasmid by site directed mutagenesis procedures (described under Methods). The mutant VAI genes were then reintroduced into dl703 between XbaI (29.0 m.u.) and EcoRI (30.0 m.u.). The location of two VA RNA genes on the adenoviral chromosome and the strategy used to introduce mutant VAI genes into viral chromosome are shown in Fig. 1.

In order to study the competition effect of VAI gene promoter on the expression of VAII gene, four Ad5 mutants with mutations in the 59 bp promoter

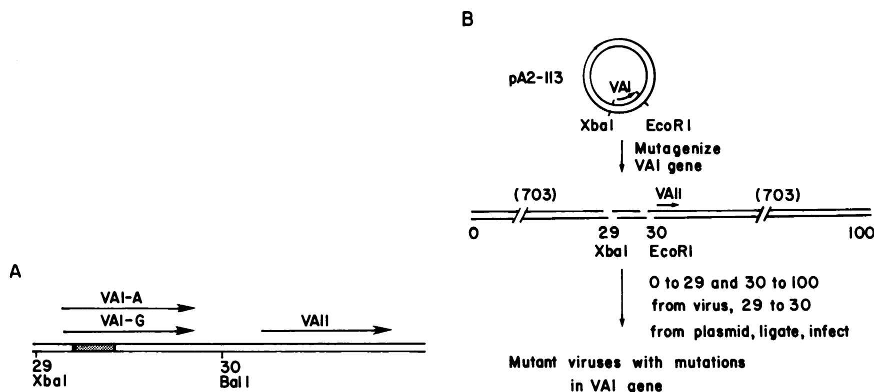

Fig. 1. A. Location of the two VA genes on the Ad2 or Ad5 chromosome. The restriction sites are positioned on the Ad2 physical map. Arrows represent VAI and VAII RNAs. The shaded area represents the intragenic promoter of the VAI gene.

B. Diagram of the strategy used to construct Ad5 mutants with mutations in VAI gene. See Materials and Methods for the construction of plasmid pA2-113. The XbaI and EcoRI sites of the plasmid pA2-113 correspond to XbaI and EcoRI sites at 29.0 and 30.0 m.u. respectively of dl704.

sequence block of VAI gene were constructed. We have previously shown that the 59 bp intragenic promoter sequence of the VAI gene can be subdivided into two non-contiguous blocks; Block A, from +10 to +18 and Block B, from +54 to +69. These two control sequences are separated by a 35 bp DNA segment (11). Mutant dl-sub723 has DNA sequences of VAI gene from -6 joined to +27 with a HindIII linker. As a result, 28 bps overlapping the promoter and the 5' flanking sequences are deleted eliminating the entire A block control sequence and an additional 8 bps from the anterior side of the promoter sequence. Dl-sub724 has a mutant VAI gene in which DNA sequences at +19, +20 and +22 to +25 were substituted by a HindIII linker sequence. This mutant also has a single bp deletion at +26. The mutant dl705 has a 29 bp deletion, from +46 to +76; this deletion eliminates the entire B block control sequence (nucleotide sequences preceding and following the deletion are numbered). Mutant dl726 is a deletion mutant in which 7 bp sequence, from +57 to +65 of the VAI gene deleted. This deletion therefore removes only a part of the B block control sequence. Finally, the mutant sub722 is a mutant in which the 230 bp DNA fragment from XbaI to EcoRI site of dl703 was replaced by an Ad2 DNA segment from 88.3 to 89.7 m.u. (434 bp). This mutant, therefore, has none of the VAI promoter sequences. The mutational alterations of Ad5 mutants described here were also confirmed by digesting the viral DNA with various restriction endonucleases (data not shown). The mutations of the VAI gene described above are shown in Fig. 2B and are also diagrammed in Fig. 3B.

Mutants dl-sub724 synthesizes altered VAI RNA while the rest of the mutants are $VAI^-/VAII^+$. The mutational alteration of VAI RNA of mutant dl724 was confirmed by fingerprint analysis of the gel purified RNA (data not shown).

Transcription analysis.

In order to evaluate the effect of the promoter mutations of VAI gene on

A

(10619) +10 +20 +30 +40 +50 +60 (10679) +70

BOX A SEGMENT C BOX B

CTTCCGTGGTCTGGTGGATAAATTCGCAAGGGTATCATGGCGGACGACCGGGGTTCGAACCCCGG

POL III CONSENSUS SEQUENCE RRYNNARYGG G(T/A)TCRANNC

B

dl-sub 723 dl-sub 724 dl 705 dl 726

AA CTTG●

CTTCCGTGGTCTGGTGGATAAATTCGCAAGGGTATCATGGCGGACGACCGGGGTTCGAACCCCGG

+27 (10636) +46 (10655) +57 (10666) +65 (10674)

Fig. 2. A. Nucleotide sequences of the Ad2 genome corresponding to the intragenic promoter of the VAI gene. The single stranded sequence shown here reads as the sense strand for the VAI RNA. The nucleotide sequences shown in the boxes correspond to two transcription control elements.

B. Deletion and substitution mutations shown on the DNA sequence of the VAI gene promoter. DNA sequences shown above the promoter sequence substitute for the corresponding nucleotides in dl-sub724. Solid circle represents a deletion. The numbers shown in parenthesis represent the nucleotide sequence positions published by Gingeras et al (22).

transcription of the VAII gene, HeLa cells were infected with each mutant at a multiplicity of 20 plaque forming units per cell. ^{32}P-labelled cytoplasmic RNA was prepared from these cells after 24 hrs of infection and analyzed on denaturing polyacrylamide gels as described under Materials and Methods. Fig. 3A shows the results of the polyacrylamide gel-analysis of the low molecular weight RNAs synthesized by various mutants described above. As can be seen, mutants sub722, dl-sub723, dl705 and dl726 did not synthesize VAI RNAs. This was expected, because these mutants lack either Block A or Block B control sequences that are essential for transcription (sub722 lacks the entire VAI gene). On the other hand, the synthesis of VAII RNAs in these mutants dramatically increased. Ad5 and Ad2 VAII RNAs migrate at different rates in our gel systems with Ad2 VAII RNA moving much faster than that of Ad5. Because dl705 has Ad2 VAII gene (see Materials and Methods), the VAII RNA of this mutant migrates faster. When we quantitated the VAII RNAs of these mutants, it was found that sub722, dl-sub723, dl705 and dl726 synthesized about 12 fold higher quantities of VAII RNAs than dl703, our WT control. Furthermore, the quantity of VAII RNA synthesized by all of these mutants was approximately equal. Thus, the mutational alterations in Box A (dl-sub723) lead to the same level of enhancement of VAII RNA synthesis as did those in Box B (dl705, dl726).

Because mutations in Box A and Box B showed identical effects, it seemed possible that either one of these control elements could be capable of repressing transcription of the VAII gene in WT infected cells. Although deletion mutations in dl-sub723, dl705 and dl726 resulted in a 12 fold increase in VAII gene transcription, a possibility existed that the Box A or Box B alone

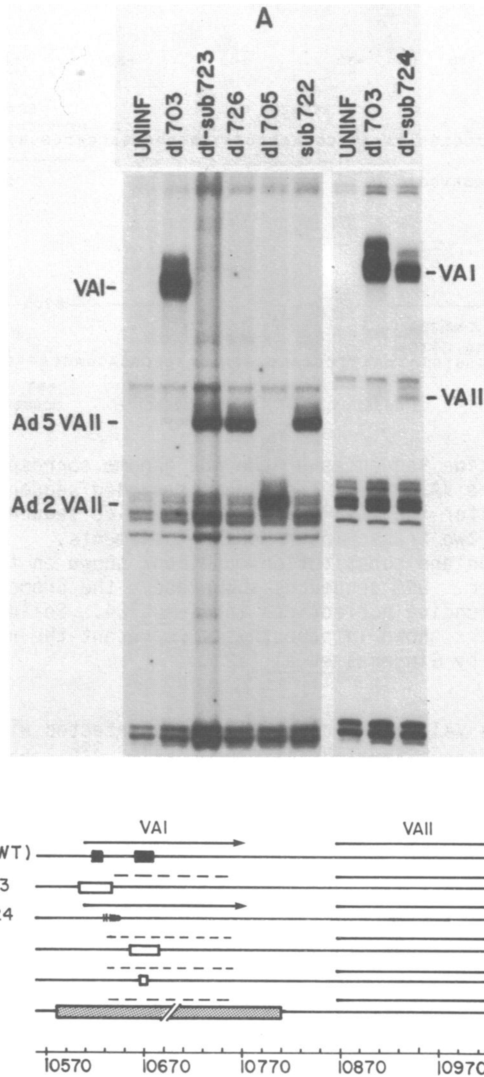

Fig. 3. A. Analysis of the VA RNAs synthesized by dl703 and various adenovirus mutants. HeLa cells were infected at 20 plaque forming units per cell and ^{32}P-labelled total cytoplasmic RNAs from these cells were analyzed on a 8% polyacrylamide/8M urea gel (0.4m x 40cm, 600V in tris-borate buffer) and autoradiographed. Top 8 and bottom 15cm of the autoradiogram are not shown.

B. Schematic representation of the deletion and substitutions of different adenovirus mutants. Solid horizontal lines represent the part of the Ad genome which code for the two VA genes. Solid boxes represent the two transcription control elements of VAI gene promoter. Arrows represent the VAI and VAII RNAs. Only the VAI-G species is shown. Broken lines indicate that the

transcripts are not synthesized. Open boxes show the deletions. Short vertical lines in the case of dl-sub724 represent the DNA sequences substituted. Solid circle in the case of dl-sub724 shows deletion. Shaded box in the case of sub722 indicates substitution. The numbers below the diagram correspond to nucleotides sequence positions published by Gingeras et al (22). The numbers in the parenthesis to the right show the fold increase of VAII RNA synthesis over dl703 VAII RNA in the respective mutants.

could still be inhibiting the synthesis of VAII RNAs as these mutants retain either Box A or Box B sequences. In that case, the level of VAII RNA synthesis although elevated, may not be maximal but would increase further if both the control elements were deleted in a single mutant. Indeed, the earlier in vitro results established the boundary of the DNA sequences of VAI gene promoter that compete for the transcription of VAI gene between +54 and +69 (10). To see if the deletion of entire promoter sequences of VAI gene would further enhance transcription of VAII gene, we constructed an Ad5 mutant in which the entire VAI gene was replaced by a DNA segment with unrelated sequence (sub722). Transcription of the VAII gene of this mutant was quantitated under identical conditions. As can be seen (Fig. 3A), the quantity VAII RNA accumulated in the cytoplasm of this mutant at 24 hr post infection was approximately equal to that of dl-sub723 or dl705 (or dl726). These results demonstrate that the effect of deletions in the Box A or Box B control sequences of VAI promoter on transcription of VAII gene is not additive.

Transcription of the VAII gene is also affected by mutations outside Box A or Box B sequences of the VAI promoter. Dl-sub724, which has DNA sequences between +19 and +25 substituted (see Fig. 2) showed a two and a half fold increase in VAII RNA synthesis. This mutation also overlaps the consensus polymerase III sequence for Box A (RRYNNARYGG, +15 to +24; R represents a purine, Y represents a pyrimidine and N represents any nucleotide; ref. 19). This gene was transcribed to about 20 percent of WT in an in vitro system (11). However, when this gene is introduced into a viral chromosome, it was transcribed to about 50 percent of WT level. This suggests that transcription is much more efficient when this gene is organized in the chromatin structure.

If the control elements of the VAI gene promoter do indeed compete in vivo for transcriptional factors, deletions of one of these control elements should end this competition. In this situation, the synthesis of VAII RNA should rise rapidly with a kinetics similar to that of WT VAI gene. A time course experiment was performed to examine this aspect. Transcription of VAII gene of dl703 and dl726 (deletion in Box B) was monitored after 12 hrs at three hr time intervals up to 32 hrs. An autoradiogram of the gel showing these results is presented in Fig. 4A. Fig. 4B shows a graphic representation of the quantity of VA RNAs synthesized by dl703 and dl726 as a function of time. Transcription of VAI gene of dl703 (WT control) continued to increase up to 21 hrs and declined thereafter. Transcription of VAII gene of this variant stayed more or less at the same level (less than a two fold increase was noted at the 32 hr time point). Transcription of the VAII gene of dl726 showed a steady increase up to 24 hrs and declined at the 32 hr time point. This kinetics is very similar to that of the VAI gene of dl703 (Fig. 4B). Thus in the absence of VAI gene transcription, transcription of VAII gene follows the same pattern as that of VAI gene in WT. At 24 hrs, when the VAII gene is transcribed at maximal level in dl726 infected cells, the quantity of VAII RNA was found to be about 1/2 of that of the VAI RNA

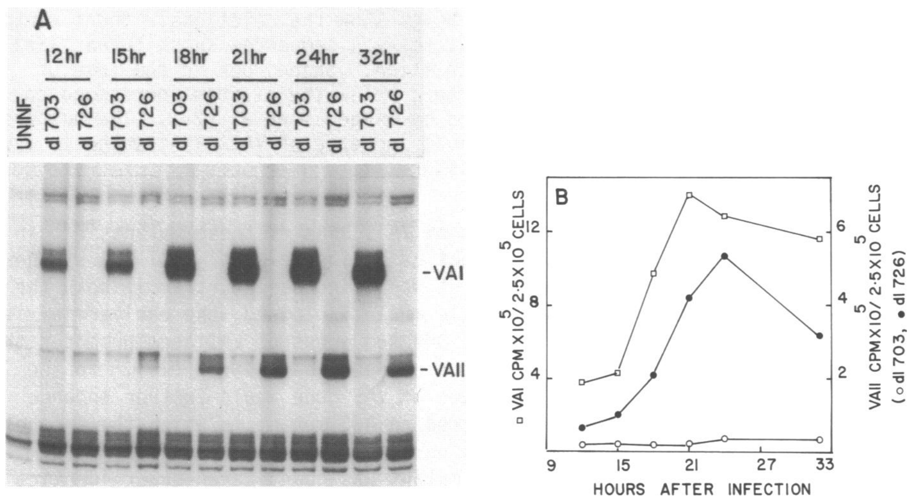

Fig. 4. A. Analysis of VA RNAs synthesized by dl703 and dl726 in HeLa cells infected for different periods of time. HeLa cells were infected with the viral mutants at 20 plaque forming units per cell for indicated periods of time and the total cytoplasmic RNAs were analysed on a denaturing polyacrylamide gel. The conditions for electrophoresis are as described in the legend to Fig. 3A.
B. Graphic representation of the quantity of VAI and VAII RNAs synthesized by dl703 or VAII RNAs synthesized by dl726 shown in A. The bands corresponding to VA RNAs shown in gel in (A) were excised and quantitated by Cerenkov counting.

of dl703 (maximum synthesis of VAI RNA of dl703 was found to be at the 21 hr time point and this value was compared with that of VAII of dl726 synthesized at 24 hrs).

Growth kinetics and yield of Ad5 mutants with VA-specific mutations.

The ability of the mutants described above to grow on HeLa cells was examined by growth kinetics experiments. These results are shown in Fig. 5. Mutants dl-sub723, dl726 and sub722 which synthesized approximately equal quantities of VAII RNA grew more slowly with more or less similar kinetics and yielded titers about 1/10 of that of dl703. Mutant dl705, although it synthesizes equal quantities of VAII RNA compared to dl-sub723 or dl726, grew about two fold better than these mutants (dl-sub723 or dl726). The VAI specific mutation of this gene is identical to that of dl330 described earlier (12). Earlier studies have shown that dl330 grows more slowly yielding a titer about 1/20 of that WT in 293 cells (12). The reasons for the increased yield of this mutant in monolayer HeLa cells is not clear at present.

We have recently constructed an Ad5 mutant which lacks functional VAI and VAII genes and found that it is viable (dl-sub720); this mutant grows very poorly with a final yield of about 1/60 of that of WT. To examine whether or not the $VAI^-/VAII^+$ mutants that we constructed grow better than dl-sub720, we compared the growth kinetics of dl726 with that of dl-sub720. Figure 5B shows that under identical conditions, dl726 yields a titer about five times higher than that of dl-sub720. These results suggest that an increased quantity of

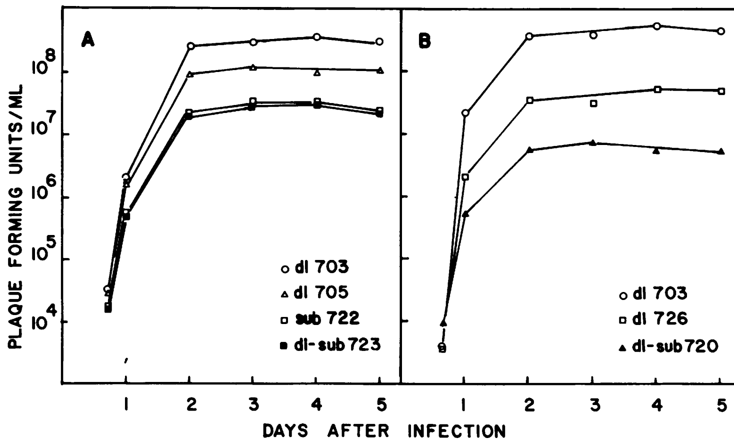

Fig. 5. Growth kinetics of mutant viruses. To monitor the growth kinetics, HeLa cells were infected with various mutants at a multiplicity of 3 plaque-forming units per cell. After one hr incubation, the dishes were washed three times with tris-saline and refed with DME containing 5% calf serum. At the indicated times the virus yield was measured by a plaque assay on HeLa cells.

VAII RNA is able to functionally substitute for the VAI RNA function although at reduced efficiency. Growth kinetics of dl-sub720 is somewhat anomolous for a mutant which yields a titer about 1/60 (3 day time point) to 1/80 (5 day time point) of that of dl703 (Fig. 5B). Instead of a gradual increase in the titer, virus grew rapidly within 48 hrs yielding the final titer on the third day with a slight decrease thereafter. These results were found to be reproducible but we do not know the reasons for this observation.

We have recently undertaken a mutational analysis of the adenovirus VAI gene to determine the nucleotide sequences and structural domains of the VAI RNA required for its biological function. A series of VAII$^-$ adenovirus mutants with mutations in the VAI gene were constructed and their growth properties were analysed. The majority of these mutants grew to WT or near-WT level suggesting that nucleotide sequences in several regions of VAI RNA were despensable for its function. One of these mutants, dl-sub707 which had VAI-specific mutation identical to that of dl-sub724 grew to WT level (manuscript submitted). In agreement with this, we find that dl-sub724 grows to WT level (data not shown).

DISCUSSION

Our results show that the deletion of nucleotide sequences from Box A or Box B of the intragenic promoter of VAI gene lead to maximal expression of VAII gene at late times in the mutant-infected cells. The kinetics of transcription of VAI and VAII genes in the dl703 and dl726 infected cells indicate that (i) the VAII gene is transcribed at least 11 to 12 fold higher level when the VAI gene is not transcribed (in some of our experiments VAII gene of the VAI$^-$/VAII$^+$ mutants is transcribed up to a 18 fold higher level than WT VAII gene) and (ii) the transcription of VAII gene will follow the same kinetics as that of VAI gene in WT virus under these conditions. Maximum levels of

transcription of the VAII gene can occur when either anterior (Block A) or posterior (Block B) sequences of the promoter of the VAI gene are mutated, for the synthesis of VAII RNA does not increase further when all of the VAI sequences are replaced by an unrelated DNA segment (sub722).

Earlier workers have shown that the VAI gene is a strong competitor for transcription of VAII gene (9,10) presumably competing for initiation factors. Deletion mapping studies demonstrated that only nucleotide sequences from +54 to +69 of VAI promoter are capable of competing for transcription with a second VAI gene *in vitro* (10). The mechanism of initiation of transcription of class III genes is being investigated intensely in several laboratories. Lassar *et al* (20) have shown that two transcription factors in conjunction with RNA polymerase III from human cells can initiate transcription of VAI gene *in vitro* (factor B and C). Factor C specifically binds to Block B sequences of the intragenic promoter of VAI gene. This interaction was shown to be stable because preincubation of factor C with VAI promoter eliminated the transcription of a subsequently added second VAI gene. If we relate these studies and the earlier *in vitro* observation that the DNA sequences containing Box A did not compete for the VAI gene transcription (10), one observation remains inconsistent. If the transcription factors bind first to Block B sequences as stable complexes, the mutant dl-sub723 (Box A sequence deleted) would have been inefficient or less efficient in relieving the inhibition of transcription of the VAII gene. That this is not the case is shown by the fact that mutations in A block are as efficient as mutations in B block in enhancing transcription of VAII gene. It is conceivable that transcription factors that may interact with Box A sequences may be present in HeLa cells. Indeed a transcription factor that may specifically interact with Box A sequences during initiation of transcription has been implicated in the case of $tRNA^{Arg}$ gene in homologous cell free extracts (21). It is likely that the entire transcription machinery is diverted to transcribe VAII gene at late stages in the case of $VAI^-/VAII^+$ mutants. The fact that a mutant VAI gene with reduced transcription (dl-sub724) is less efficient in relieving the inhibition of transcription of a VAII gene supports this hypothesis. As suggested earlier, the polymerase III enzyme and the necessary factors may be present in limiting amounts in adenovirus infected cells. If the VAI gene is able to bind more efficiently to these factors, less quantities of these factors will be available for transcription of VAII gene. Therefore, the synthesis of VAII RNA levels off after 8 to 10 hr after infection. Nucleotide sequence differences between VAI and VAII gene in the promoter region may contribute to this effect as DNA sequences of VAI and VAII genes show only a scattered sequence homology (8).

Although mutants with deletions in Box A or Box B produce high levels of VAII RNA, they grow about 1/10 as well as WT. An adenovirus mutant which lacks VAI and VAII genes grows about 1/60 as well as WT. Increased quantities of VAII RNA therefore, can enhance the growth of the virus although less efficiently than VAI RNA. At least in tissue culture cells, virus mutants lacking functional VAII gene show no growth defects (12). Although the VAI and VAII RNAs do not show extensive sequence homology, both of them can fold to form very similar compact stem-loop structures and it has been proposed that they arose via gene duplication (8). The observation that abundant quantities of VAII RNA do not support the growth of the virus to WT level suggest that as the two VA genes diverged, the VAI gene might have evolved as a much better gene suited for its transcription and for its biological function.

Relative strength of the interaction of the polymerase III genes with transcription specific factors has been implicated as at least one of the bases for the differential gene regulation (20). Soderlund et al showed that duringthe lytic cycle of the adenovirus, the level of 5S RNA does not change whereas tRNA synthesis is dramatically reduced (2). It has been suggested that stable and more efficient binding of factor C may preclude the transcription of tRNA genes in the infected cells (20). An analogous differential interaction with transcriptional factors between the two VA genes may explain the excessive transcription of VAI gene compared to VAII gene in adenovirus lytic cycle.

ACKNOWLEDGEMENTS

We wish to thank Noel Bouck for critical reading of the manuscript, Beth Metz and Aubrey Miller for competent technical assistance, and Sonia Lorenzi for excellent secretarial assistance. This work was supported by a grant from National Institute of Health (AI18029). B.T. is an Established Investigator of American Heart Association.

*To whom correspondence should be addressed

REFERENCES

1. Reich, P.R., Baum, S.G., Rose, J.A., Rowe, W.P. and Weissman, S.M. (1966), Proc. Natl. Acad. Sci. USA, 55:336-341.
2. Soderlund, H., Pettersson, U., Vennstrom, B., Phillipson, L. and Mathews, M.B. (1976), Cell, 7:585-593.
3. Pettersson, U., and Phillipson, L. (1975), Cell, 6:1-4.
4. Mathews, M.B. (1976), Cell, 7:557-566.
5. Weinmann, R., Raskas, H.J., and Roeder, R.G. (1974), Proc. Natl. Acad. Sci. USA, 71:3426-3430.
6. Celma, M.L., Pan, J., and Weissman, S.M. (1977), J. Biol. Chem., 252:9043-9046.
7. Thimmappaya, B., Jones, N., and Shenk, T. (1979), Cell, 18:947-954.
8. Akusjarvi, G., Mathews, M.B., Anderson, P., Vennstrom, B., and Pettersson, U. (1980), Proc. Natl. Acad. Sci. USA, 77:2424-2428.
9. Fowlkes, D.M., and Shenk, T. (1980), Cell, 22:405-413.
10. Guilfoyle, R., and Weinmann, R. (1981), Proc. Natl. Acad. Sci. USA, 78:3378-3382.
11. Bhat, R.A., Metz, B., and Thimmappaya, B. (1983), Mol. Cell Biol., 3:1996-2005.
12. Thimmappaya, B., Weinberger, C., Schneider, R.J., and Shenk, T. (1982), Cell., 31:543-551.
13. Weinberg, C., Thimmappaya, B., Fowlkes, D.M., and Shenk, T. (1981), ICN-UCLA Symp. Mol. Cell Biol., 23:507-514.
14. Graham, F.L., Smiley, J., Russell, W.C., and Nairu, R. (1977), J. Gen. Virol., 36:59-72.
15. Bhat, R.A., and Thimmappaya, B. (1983), Proc. Natl. Acad. Sci. USA, 80:4789-4793.
16. Green, C., and Tibbets, C. (1980), Proc. Natl. Acad. Sci. USA, 80:4789-4793.
17. Shortle, D., Koshland, D., Weinstock, G.M., and Bostein, D. (1980), Proc. Natl. Acad. Sci. USA, 77:5357-5379.
18. Maxam, A.M., and Gilbert, W. (1980), Methods in Enzymol., 65:499-559.
19. Ciliberto, G., Castagnoli, L., Melton, D.A., and Cortese, R. (1982), Proc. Natl. Acad. Sci., 79:1195-1199.
20. Lassar, A.B., Martin, P.L., and Roeder, R.G. (1983), Science, 222:740-748.

21. Sharp, S., Dingerman, T., Schaack, J., DeFranco, D., and Soll, D. (1983), J. Biol. Chem., 250:2440-2446.
22. Gingeras, T.R., Sciaky, D., Gelinas, R.E., Bing-Dong, J., Yen, C.E., Kelly, M.M., Bullock, P.A., Parsons, B.L., O'Neill, K.E., and Roberts, R.J. (1982), J. Biol. Chem., 257:13475-13491.