Adenovirus 2 VA RNAI synthesized in SP6 system

Alexandra G.Evstafieva, Biragyn Arya, Bela Pragai¹ and Miklos I.Toth*

Institute of Biochemistry, Biological Research Center, Hungarian Academy of Sciences, Szeged PO Box 521 and

¹Institute of Microbiology, Medical University, Szeged Dom ter 10, Hungary

Submitted January 12, 1988

The adenovirus (Ad) VA RNAI is a small, 156 base pair (bp) long transcript with an extended hairpin configuration synthesized by RNA polymerase III late after infection. It is required for the translation of Ad mRNAs (1), and prevents the activation of the P_i (eIF-2) kinase by dsRNA (2). The availability of large amounts of pure VA RNAI would facilitate further analyses. Therefore we cloned the 976 bp XbaI-HindIII (position 10579 to 11555) fragment of Ad2 DNA carrying the VA RNAI gene into pSP65 (Amersham, UK). Most of the *in vivo* untranscribed region at the 5' end was deleted by Bal 31 digestion resulting in pSPVA Δ 16 (Fig. A). This plasmid was cleaved at the HhaI site (5 bp upstream of the *in vivo* termination site), and used to synthesize RNA by SP6 RNA polymerase. One μ g plasmid yielded 0.2 μ g RNA. Although the RNA contains extra bases at the 5' end and has truncated 3' end, its secondary structure is similar to that of the *in vivo* counterpart. The *in vitro* synthesized VA RNA inhibits the activation of P_i kinase by dsRNA in a similar way as viral VA RNA I (Fig. B, ref. 2). The availability of the *in vitro* synthesized RNA made possible to determine, that VA RNAI activates the 2-5A synthetase as efficiently as polyI:polyC, but in contrast to other dsRNAs it is unable to induce IFN in mouse cells.

Fig. (A). Structure of pSPVA Δ 16.

(B). Autoradiogram. Phosphorylation of P_i is inhibited by SP6 transcripts. The kinase activity was tested by the polyI:polyC binding assay (3).

*Present address: Institut für Genetik, Universität zu Köln, Weyertal 121, 5000 Köln, FRG

REFERENCES

1. Kaufman et al. (1985) P.N.A.S. 82, 689-693.
2. Kitajewski et al. (1986). Cell 45, 195-200.
3. O'Malley et al. (1986) Cell 44, 391-400.