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Transcription of BK Virus DNA by *Escherichia coli* RNA Polymerase: Size and Sequence Analysis of RNA

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Transcription of human papovavirus BK superhelical DNA by *Escherichia coli* RNA polymerase yielded symmetric RNA with an average chain length of 1,300 nucleotides. All regions of human papovavirus BK DNA were equally transcribed. At least four initiation sites were available to the procaryotic enzyme.

Human papovavirus BK (BKV) was first described by Gardner et al. in 1971 (4). A number of studies on the biological and biochemical properties of this virus have since been reported (3, 5, 6, 13, 15, 19). Comparative studies with other members of the papovavirus family have shown that BKV is related to oncogenic simian virus 40 (SV40). Structural proteins of the two viruses have antigenic determinants in common, as shown by immune electron microscopy and by a partial neutralization of SV40 plaque formation by BKV hyperimmune serum (4, 14, 19). Upon infection, the two viruses give rise to immunologically related T and V antigens (6, 18, 19). Furthermore, the "early" products of the two viruses have been shown to be functionally equivalent, since BKV can complement temperature-sensitive early SV40 mutants at the nonpermissive temperature (12). Biochemical analysis of the virion components, on the other hand, has shown that virus-coded polypeptides found in BKV and SV40 virions have different electrophoretic mobilities (2) and different tryptic digest patterns (22). Hybridization analysis have revealed a partial sequence homology between BKV and SV40 DNA. The extent of this homology depends on the technique used for hybrid detection and can vary from 20% under the less stringent conditions to approximately 10% when more restrictive methods are used (5, 7). The shared sequences have been reported to be principally located in the "late" region of SV40 DNA.

Nothing has been known until now about BKV transcription. In vivo studies are complicated by the low efficiency of BKV infection in systems used so far (11). We report here a characterization of in vitro transcription of BKV DNA by *Escherichia coli* RNA polymerase. In view of the partial sequence homology and similar gene organization (7) of BKV and SV40 genomes, it was of interest to compare our results with those previously reported for SV40 in vitro transcription (3, 8, 10, 21). Our data show that in spite of their similarity, the two viral DNAs are transcribed differently by the procaryotic enzyme.

First, we measured the size distribution of BKV transcripts. Superhelical viral DNA (BKV or SV40) was obtained from purified virions in the following way. Virus suspension was heated at 50°C for 30 min in the presence of 10 mM Tris-hydrochloride (pH 7.5)-10 mM EDTA-0.5% sodium dodecyl sulfate (SDS)-50 μg of proteinase K (Merck & Co., Inc.) per ml. Incubation was then continued at 37°C for 3 h. CsCl and ethidium bromide (400 μg/ml of original solution) were then added, and the density was adjusted to 1.600 g/cm^3. After centrifugation for 50 to 60 h at 43,000 rpm and 10°C in a Spinco 50Ti rotor, the band containing the superhelical form of DNA was collected, extracted with isopropanol to eliminate ethidium bromide, and dialyzed against 10 mM Tris (pH 7.5)-1 mM EDTA.

E. coli RNA polymerase was prepared by a procedure to be described in detail elsewhere. Briefly, cells (*E. coli* MRE 600, RNase$^-$) were disrupted by grinding with alumina, and DNA was precipitated with 6% polyethylene glycol in 2 M NaCl. After precipitation with NH_4 sulfate, the sample was passed through an Ultrogel AcA 22 column (LKB Instruments Inc.). Fractions containing RNA polymerase activity were pooled, and proteins were precipitated with NH_4 sulfate. After desalting by Sephadex G-25 filtration, the sample was passed through a DNA-cellulose column (double-stranded calf thymus DNA). After extensive washing with buffer containing 50 and then 150 mM NaCl, RNA polymerase activity was eluted stepwise with an NaCl concentration of 0.75 M. Enzyme purified by this method contained no appreciable DNase activity, as measured by conversion of superhelical

BKV [^{3}H]DNA into the circular relaxed form under our transcription conditions. RNA was synthesized in a 400-μl reaction mixture containing: 40 mM Tris-hydrochloride (pH 7.9); 10 mM $MgCl_2$; 0.15 M NaCl; 0.4 mM EDTA; 4 mM dithiothreitol; 0.4 mM each ATP, GTP, and CTP; 0.1 mM [α-^{32}P]UTP (specific activity, 1×10^6 to 3×10^6 cpm/nmol); and 7 μg of BKV or SV40 superhelical DNA. The reaction was started by the addition of 0.7 U of *E. coli* RNA polymerase and incubated at 37°C for 30 min. EDTA and SDS were then added to 20 mM and 1%, respectively, and the sample was passed through a Sephadex G-75 column equilibrated with 10 mM Tris-hydrochloride (pH 7.5)–1 mM EDTA–0.2% SDS. Fractions containing RNA were pooled, mixed with 50 μg of purified yeast tRNA, and extracted with a phenol-chloroform mixture (50:50, wt/wt). After ethanol precipitation, RNA was dissolved in 10 mM Tris-hydrochloride (pH 7.5)–1 mM EDTA, heat denatured in formamide, and analyzed by sedimentation through a sucrose gradient in 80% formamide (Fig. 1a). Under these conditions, RNA sediments as a relatively heterogeneous population of molecules, with an average chain length of approximately 1,300 nucleotides (relative to rRNA markers). A very similar pattern was obtained when SV40 DNA was used as the template (data not shown). This sedimentation behavior is also similar to that reported by Westphal (21) for SV40 DNA transcription by *E. coli* RNA polymerase. Fried and Sokol (3) found SV40 in vitro RNA chains of up to 3×10^6 daltons, but in their case synthesis was carried out for 80 min. When the NaCl concentration in our synthesis reaction was increased to 0.3 M, longer RNA molecules were obtained (Fig. 1b), with chain lengths of as high as 4×10^3 nucleotides (BKV DNA contains approximately 5×10^3 base pairs).

We then determined the self-complementarity of BKV and SV40 transcripts. SV40 DNA is known to be transcribed asymmetrically by *E. coli* RNA polymerase, the majority of RNA being complementary to the E (early) strand (21). By contrast, very little strand selection occurs during BKV DNA transcription, as indicated by the high self-complementarity of the RNA produced. Figure 2 shows the time course of self-hybridization of RNAs synthesized in vitro on BKV DNA and SV40 DNA under identical conditions. It can be seen that, with increasing time, over 60% of BKV RNA became RNase resistant. When BKV RNA made in the presence of 0.3 M KCl was self-hybridized, essentially the same plateau value was obtained (data not shown). RNA transcribed from SV40 DNA, on the other hand, became about 15% RNase resistant. Similar values have been reported previously by others (10, 21). Thus, initiation sites for transcription in both directions are available on BKV DNA, whereas on SV40 DNA all of the identified initiation sites give rise to transcription in the same (leftward) direction (9).

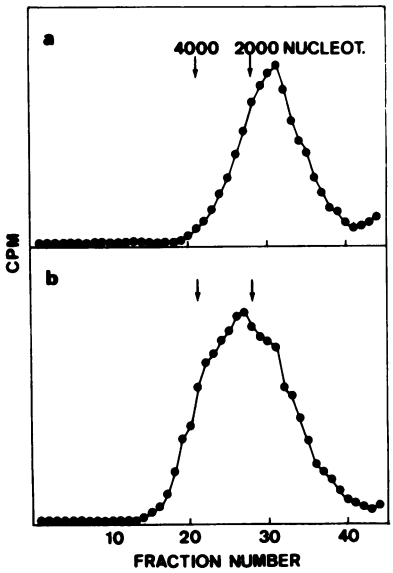

FIG. 1. *Sedimentation analysis of in vitro BKV RNA. Superhelical BKV DNA was transcribed by E. coli RNA polymerase in the presence of [α-^{32}P]UTP, and RNA was purified as described in the text. BKV RNA and ^{3}H-labeled rRNA from HEp-2 cells in a total volume of 10 μl were mixed with 70 μl of 95% formamide (purified as described by Tibbets et al. [20]) in 10 mM Tris-hydrochloride (pH 7.9)–2 mM EDTA–0.2% SDS and heated at 65°C for 6 min. The samples were then layered on 5 to 20% sucrose gradients in 80% formamide in the same buffer. Centrifugation was for 380 min at 58,000 rpm and 24°C in a Spinco SW60 rotor. (a) RNA made in the presence of 0.15 M NaCl; (b) RNA made in the presence of 0.30 M NaCl.*

All regions of BKV DNA are transcribed at approximately the same efficiency, as shown by hybridization to BKV DNA restriction fragments. Restriction endonuclease *Hin*dIII cleaves BKV DNA at four sites, giving fragments of 1.5 $\times 10^6$, 1.2×10^6, 0.41×10^6, and 0.34×10^6 daltons (5). When RNA synthesized under the above conditions was hybridized to the separated DNA fragments as described by Southern (17), hybridization to all four fragments was detected (Fig. 3a). The extent of hybridization to each fragment (46, 45, 5, and 4%, respectively, from 1 to 4) was roughly proportional to the molecular weight of the fragment. Since DNA was in excess over RNA during hybridization, this result shows that DNA regions corresponding to re-

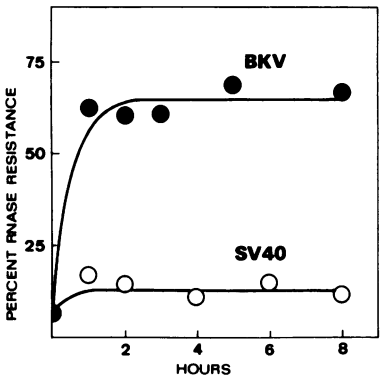

FIG. 2. *Self-complementarity of in vitro BKV and SV40 RNAs. Purified RNA (0.15 μg; see text) was incubated at 70°C in 0.3 M NaCl–30 mM sodium citrate (2× SSC). Portions of 20 μl were withdrawn at the indicated times and diluted in 2× SSC. One half of the dilution was then spotted on a GF/C filter, whereas the other half was digested with pancreatic and T1 RNases at 37°C for 15 min before applying to GF/C filters. Filters were washed twice in 5% trichloroacetic acid containing 1% $NaPP_i$ and once in ethanol, dried, and counted. Zero time points were obtained by boiling the whole hybridization mixture for 7 min and quenching on ice before withdrawing the first portion. Points represent for each time the percentage of RNase resistance relative to the untreated (100%) portion of each sample. The 100% values remained practically constant throughout the experiment.*

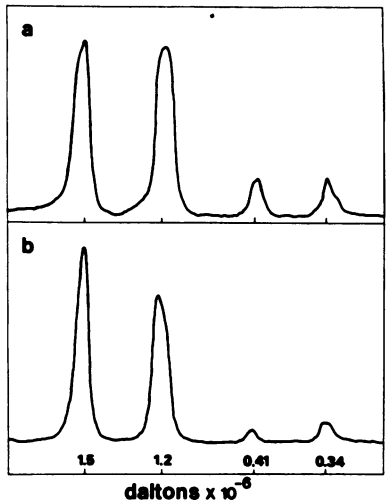

FIG. 3. *Hybridization of in vitro BKV RNA to BKV DNA HindIII fragments. (a) RNA was synthesized on superhelical BKV DNA as described in the text. About 3 × 10^5 cpm (0.13 μg) were put in a glass scintillation vial in 2 ml of 0.45 NaCl–45 mM sodium citrate (3× SSC). A nitrocellulose strip, to which BKV DNA HindIII denatured fragments had been transferred by the method of Southern (17), was then added to the vial, which was then sealed and incubated at 70°C for 20 h. The strip was then washed in 6× SSC, treated with pancreatic and T1 RNases, washed in 2× SSC, dried, and laid against Kodak Industrex film for autoradiography. (b) BKV DNA was digested with HindIII restriction endonuclease (Miles Laboratories, Inc.) was described by Howley et al. (5). Fragmented DNA was purified by phenol-chloroform extraction (50:50, wt/wt) and precipitated by ethanol. Digestion and recovery were checked by agarose gel electrophoresis (16). The fragment mixture was then transcribed by E. coli RNA polymerase as described for undigested superhelical DNA (see text) and hybridized to BKV DNA HindIII fragments as in (a). Tracings were obtained as densitometric scans of developed films. Note that before transfer to nitrocellulose, agarose bands containing fragments were cut and set apart to obtain cleaner hybridization patterns. Relative positions of bands, therefore, are not representative of the actual migration of fragments.*

striction fragments were equally transcribed by *E. coli* RNA polymerase.

If BKV DNA is digested with *Hin*dIII before transcription, only fragments containing initiation sites will be transcribed. Random or end-specific initiation does not occur under these conditions, as proven by experiments with other viral DNAs, in which some restriction fragments were never transcribed by *E. coli* RNA polymerase (our unpublished data). Hybridization of RNA made on BKV DNA *Hin*dIII fragments to the separated fragments (Fig. 3b) showed that all four fragments had been at least partly transcribed. In this experiment, the amount of RNA hybridized to each fragment depends among other factors on the position and orientation of initiation sites within each fragment. Relative intensities of hybridization bands, therefore, cannot be quantitatively interpreted in a straightforward way. However, hybridization to all fragments indicates that each one of them contains at least one initiation site for *E. coli* RNA polymerase.

It can be concluded from our results that in spite of the relatedness of BKV and SV40, their DNAs are not transcribed in the same way by *E. coli* RNA polymerase. BKV transcripts are complementary to both DNA strands. There are at least four initiation sites for the procaryotic enzyme on BKV DNA, and they must be of both the leftward and the rightward kinds. Sequence specificity is therefore an important feature of transcription of these small, superhelical DNAs by *E. coli* RNA polymerase. However, it is hard to evaluate the relevance of this specificity to viral transcription in infected cells. One would expect that viruses so similar in their structure and biological properties should have similar transcription patterns during infection, as suggested by the striking similarity between SV40

and polyoma virus transcription (for a review, see reference 1). In this respect, the fact that the E strand of SV40 is preferentially transcribed by *E. coli* RNA polymerase should be regarded as a lucky biochemical accident rather than the in vitro reproduction of early transcription in SV40-infected cells. The finding by Lebowitz et al. (9) that none of the five identified SV40 in vitro initiation sites corresponds to 5′ termini of in vivo viral mRNA's further supports this conclusion.

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LITERATURE CITED

1. **Acheson, N. H.** 1976. Transcription during productive infection with polyoma virus and simian virus 40. Cell **8**:1-12.
2. **Barbanti-Brodano, G., G. Minelli, G. Portolani, L. Lambertini, and M. Toppini.** 1975. Structural proteins of a human papovavirus (BK virus): a comparison with the structural proteins of SV40. Virology **64**:269-271.
3. **Fried, A. H., and F. Sokol.** 1972. Synthesis *in vitro* by bacterial RNA polymerase of simian virus 40-specific RNA: multiple transcription of the DNA template into a continuous polyribonucleotide. J. Gen. Virol. **17**:69-79.
4. **Gardner, S. D., A. M. Field, D. V. Coleman, and B. Hulme.** 1971. New human papovavirus (B.K.) isolated from urine after renal transplantation. Lancet **i**:1253-1257.
5. **Howley, P. M., G. Koury, J. C. Byrne, K. K. Takemoto, and M. A. Martin.** 1975. Physical map of the BK virus genome. J. Virol. **16**:959-973.
6. **Howley, P. M., M. F. Mullarkey, K. K. Takemoto, and M. A. Martin.** 1975. Characterization of human papovavirus BK DNA. J. Virol. **15**:173-181.
7. **Koury, G., P. M. Howley, C. Garon, M. F. Mullarkey, K. K. Takemoto, and M. A. Martin.** 1975. Homology and relationship between the genomes of papovaviruses, BK virus and simian virus 40. Proc. Natl. Acad. Sci. U.S.A. **72**:2563-2567.
8. **Koury, G., and M. A. Martin.** 1972. Comparison of SV40 DNA transcription *in vivo* and *in vitro*. Nature (London) New Biol. **238**:4-6.
9. **Lebowitz, P., R. Stern, P. H. Ghosh, and S. M. Weissman.** 1977. Specificity of initiation of transcription of simian virus 40 DNA I by *Escherichia coli* RNA polymerase: identification and localization of five sites for initiation with [γ-^{32}P]ATP. J. Virol. **22**:430-445.
10. **Mandel, J. L., and P. Chambon.** 1974. Animal DNA-dependent RNA polymerases. Analysis of the RNA synthesized on simian virus 40 superhelical DNA by mammalian RNA polymerases AI and B. Eur. J. Biochem. **41**:379-395.
11. **Maraldi, N. M., G. Barbanti-Brodano, M. Portolani, and M. La Placa.** 1975. Ultrastructural aspects of BK virus uptake and replication in human fibroblasts. J. Gen. Virol. **27**:71-80.
12. **Mason, D. H., and K. K. Takemoto.** 1976. Complementation between BK human papovavirus and a simian virus 40 tsA mutant. J. Virol. **17**:1060-1062.
13. **Mullarkey, M. F., J. F. Hruska, and K. K. Takemoto.** 1974. Comparison of two human papovaviruses with simian virus 40 by structural protein and antigenic analysis. J. Virol. **13**:1014-1019.
14. **Penney, J. B., and O. Narayan.** 1973. Studies of the antigenic relationships of the new human papovaviruses by electron microscopy agglutination. Infect. Immun. **8**:299-300.
15. **Portolani, M., G. Barbanti-Brodano, and M. La Placa.** 1975. Malignant transformation of hamster kidney cells by BK virus. J. Virol. **15**:420-422.
16. **Sharp, P. A., B. Sugden, and J. Sambrook.** 1973. Detection of two restriction endonuclease activities in *Haemophilus parainfluenzae* using analytical agarose-ethidium bromide electrophoresis. Biochemistry **12**:3055-3063.
17. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503-517.
18. **Takemoto, K. K., and M. A. Martin.** 1976. Transformation of hamster kidney cells by BK papovavirus DNA. J. Virol. **17**:247-253.
19. **Takemoto, K. K., and M. F. Mullarkey.** 1973. Human papovavirus, BK strain: biological studies including antigenic relationship to simian virus 40. J. Virol. **12**:625-631.
20. **Tibbets, C., K. Johansson, and L. Philipson.** 1973. Hydroxyapatite chromatography and formamide denaturation of adenovirus DNA. J. Virol. **12**:218-225.
21. **Westphal, H.** 1970. SV40 DNA strand selection by *Escherichia coli* RNA polymerase. J. Mol. Biol. **50**:407-420.
22. **Wright, P. J., and G. Di Mayorca.** 1975. Virion polypeptide composition of the human papovavirus BK: comparison with simian virus 40 and polyoma virus. J. Virol. **15**:828-835.