COMMON STRUCTURAL FEATURES IN DNA AROUND THE REPLICATION ORIGIN OF PAPOVA VIRUS, MOUSE POLYOMA VIRUS, SIMIAN VIRUS 40 AND HUMAN BKV

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Received 28 February 1979

1. Introduction

The replication origin of mouse polyoma virus DNA has been located at a unique site of the genome which has been mapped at 71 map units, taking a single cleavage site by the restriction endonuclease *Eco*RI as zero map unit [1]. The DNA replication proceeds bidirectionally along the circular DNA and terminates at the opposite site of the origin [2,3]. Both the 5′-termini of early and late mRNAs are also located near the origin of DNA replication [4]. Tumor antigens, which react immunologically with antisera raised in animals bearing tumor induced by the virus [5,6], are involved in the initiation of the viral DNA replication and in the expression of the late genes [7]. One of the tumor antigens, designated by large T-Ag, is a product of the gene A of the viral genome [25]. It is known that T-Ag of the related virus SV40 (Simian virus) binds preferentially to the replication origin of [14,15]. There are many evidences that Papova viruses including SV40, polyoma virus and BKV (human virus) are very similar to each other in the organization of their genetic functions [8]. Since the nucleotide sequences around the replication origins of SV40 [9] and BKV [10] have been worked out, it is of interest to compare them with the sequence in the corresponding region of polyoma virus in order to seek the common structure with the identical function and to manifest their evolutionary relationship. We have determined the nucleotide sequence of DNA restriction fragment (*Hap*II-5) near the origin of DNA replication [11] and demonstrated the similarity of the sequence between SV40 and polyoma virus [12]. Here we report the nucleotide sequence of the DNA fragment (*Hap*II-3) adjacent to the *Hap*II-5 fragment reported [11]. Both the fragments are generated by cutting at 70.8 map units with restriction endonuclease *Hap*II, so that the replication origin and the recognition sites with other functions mentioned above may be covered with either or both the fragments. We found that the nucleotide sequence homologous to SV40 and BKV DNAs was located beyond the *Hap*II-5–3 junction and into the *Hap*II-3 fragment. Based on this fact, the homology in the aspects of evolution of Papova viruses is also discussed here.

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2. Materials and methods

Polyoma DNA and α-^{32}P-labeled dXTPs were prepared by the method in [11]. The DNA was digested with *Hap*II endonuclease, the digestion products were labeled with d[α-^{32}P]GTP and T_4-induced DNA polymerase, and subjected to electrophoresis on a 4% polyacrylamide gel. *Hap*II-3 fragment separated from the gel was eluted electrophoretically into a dialysis bag and redigested with *Alu*I restriction endonuclease. Two subfragments labeled at the 3′-termini were isolated from a 10% acrylamide gel after electrophoresis. The longer fragment coming from the *Hap*II-3–5 junction was analyzed by the method in [13] to elucidate the nucleotide sequence.

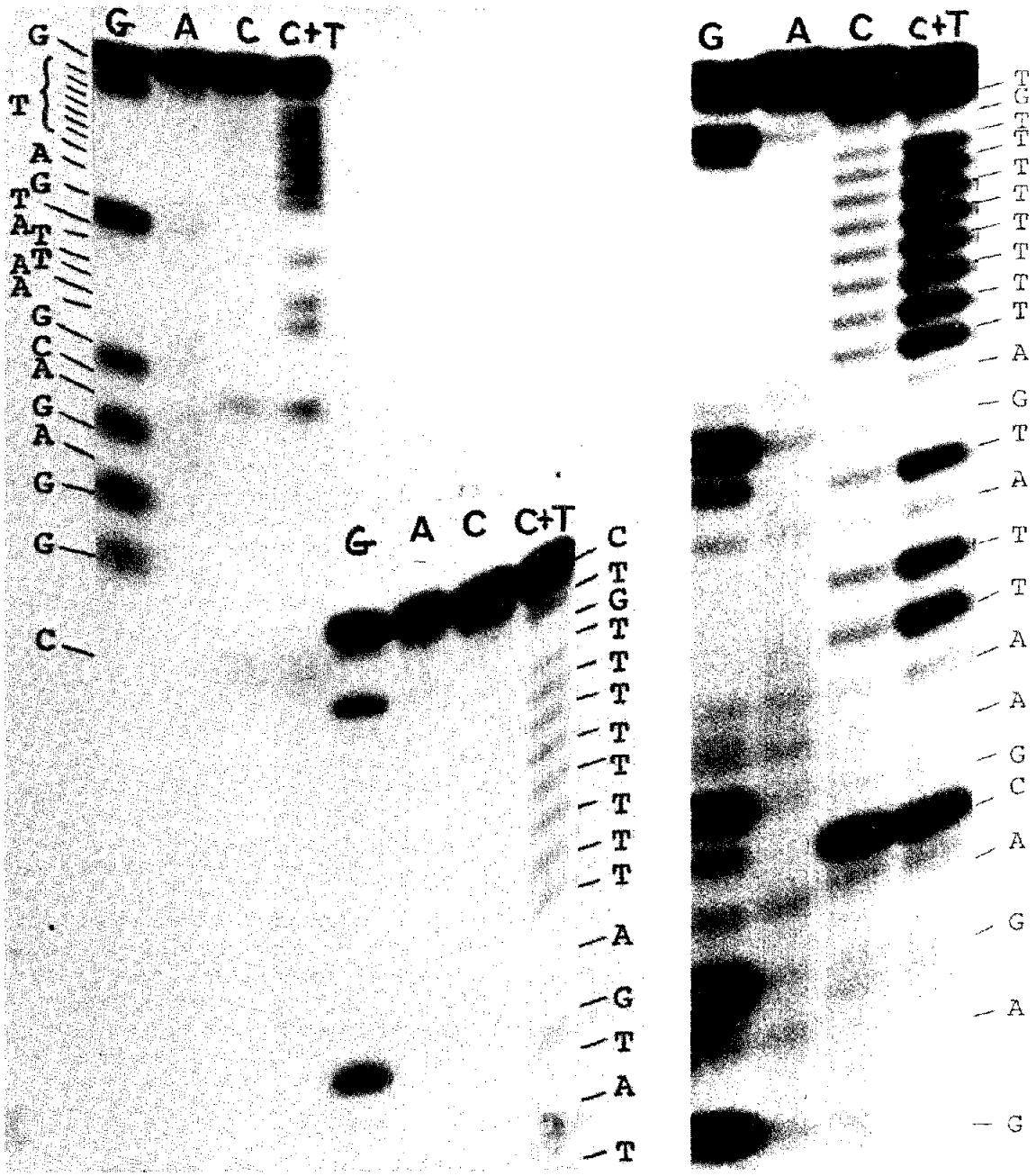

Fig.1. Sequencing of the *Hap*II-3/*Alu*I fragment around the origin of DNA replication. The total *Hap*II digestion products labeled at the 3′-termini with d[α-^{32}P]CTP and T_4-induced DNA polymerase were redigested with *Alu*I endonuclease. A fragment of the *Hap*II-3/*Alu*I was isolated from the redigestion products by electrophoresis in polyacrylamide slab gels. It was submitted to sequencing by the method in [13] as in [12].

3. Results and discussion

In [12], *Hap*II-5 fragment of polyoma DNA was cleaved by *Alu*I endonuclease and the subfragment (*Hap*II-5/*Alu*I) derived from the *Hap*II-3–5 junction of polyoma DNA was analyzed. Here, we determined the nucleotide sequence of another subfragment derived from the same junction which were generated from ^{32}P-labeled *Hap*II-3 fragment followed by digestion with *Alu*I endonuclease. The partial digest of the subfragment was analyzed according to [13], as shown in fig.1. The successive 8-T tract terminated with A is conspicuous.

The nucleotide sequence beyond the *Hap*II-3–5 junction was constituted by jointing the sequences of *Hap*II-5/*Alu*I and *Hap*II-3/*Alu*I as shown in fig.2. Then we compared with the sequences of SV40 [9] and BKV DNAs [10] which are presumed to contain the origins of DNA replication (fig.3). Some gaps are left to maximise the homology. Remarkable similarities among these nucleotide sequences were found, which contain several identical nucleotides at the corresponding positions. It is significant that an 8-T cluster terminated with A and a sequence AGAGGCCG are common to these 3 viral DNAs, because such long fragments with definite sequences can appear at a very low frequency as expected from the random distribution of nucleotides in such small sizes as these viral DNAs. The G–C rich regions containing AGAGGCCG have a dyad symmetry. The fact that such long homologous sequences with common structural feature are located near the origins of DNA replication may reveal an evolutionary relationship of these viruses and the presence of the immobile nucleotide sequences for specific functions during evolution. These sequences must function as recognition sites involved in biological events such as the viral DNA replication, processing of early and late mRNAs, and the expression of late mRNA. In SV40 and probably in the other 2 viruses, large T-Ag binds

Fig.2. The nucleotide sequence of *Hap*II-3/*Alu*I neighbouring the fragment *Hap*II-5.

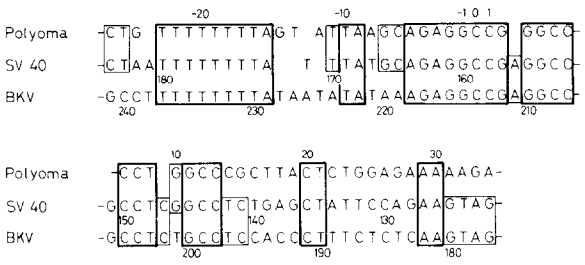

Fig.3. Comparison of the nucleotide sequences around the replication origins of DNA from polyoma virus, SV40 [9] and BKV [10]. Some gaps have been inserted between homologous regions. Common nucleotide sequences are indicated in boxes.

preferentially to this region of SV40 [14,15]. Protein, closely related to SV40 large T-Ag, has been reported [16] to bind in a sequential manner to tandem recognition sites which lie over the homologous sequence of SV40 mentioned above. It is interesting to note that large T-Ag of SV40 can bind the region near the origin of DNA replication of polyoma virus (Tjian, personal communication) though polyoma T-Ag did not crossreact immunologically with sera against SV40 T-Ag. The complete nucleotide sequence of polyoma virus has been determined (E. S. et al. in preparation) and the presumed amino acid sequences of the large T-Ags between SV40 and polyoma virus are quite homologous, particularly extensive in the regions which tsA mutants, related to the viral replication, of both viruses have been localized [17–19]. T-Ags of SV40 and BKV are closely related as evidenced by a high level immunological crossreactivity [20,21] and the presence of many common tryptic peptides between them [22]. This evidence may support the general prediction in evolution that the recognition sites on the genome must be preserved as well as proteins recognizing them because it is difficult that preferable mutations occur simultaneously on either the recognition site and the gene of the protein recognizing it in order to adapt to a new environment.

Here, we have compared the sequence around the DNA replication origin of polyoma virus with that of rat mitochondria determined [23]. As shown in fig.4, if a palindrome sequence is written in a hairpin structure, these two DNAs show similar features: there are A (or T) clusters on both sides of the G–C-rich hair-

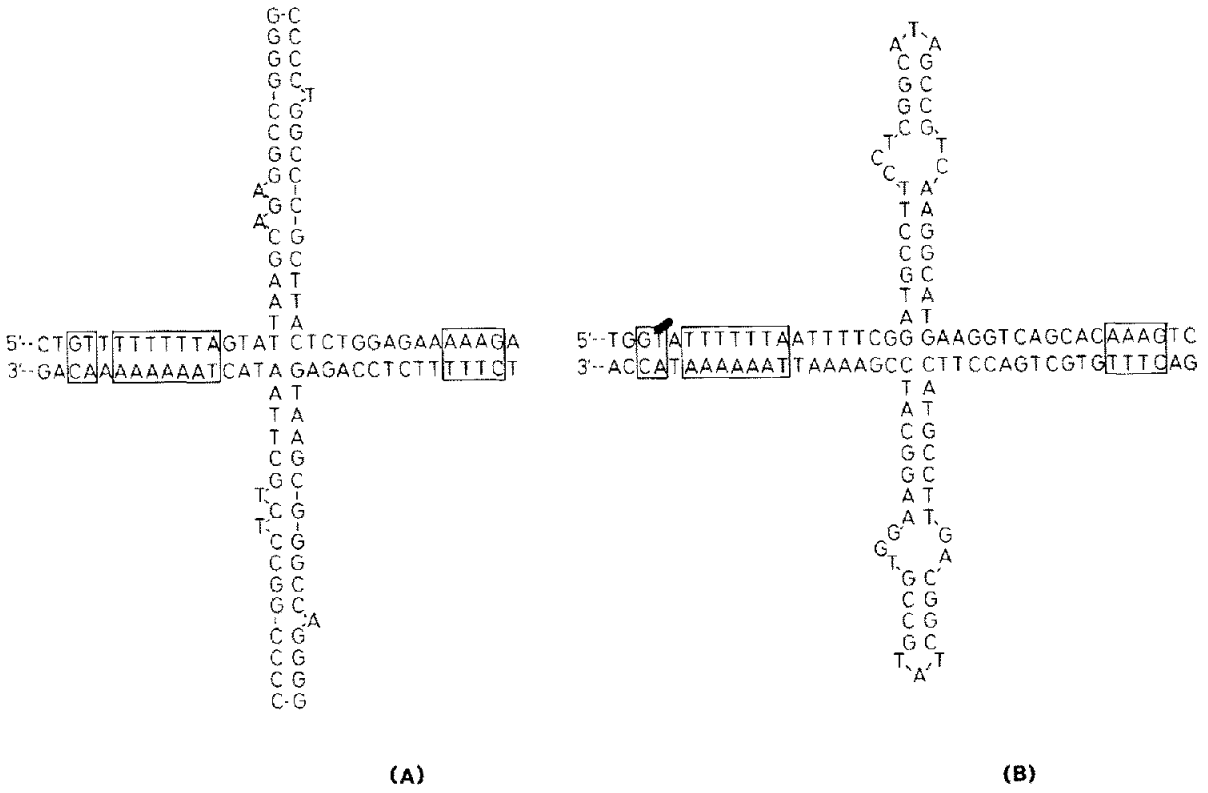

Fig.4. A possible double hairpin loop structure for the DNA fragment around the replication origin from mouse polyoma virus (A) and rat mitochondria (B). Common nucleotide sequences are indicated in boxes.

pin structure. These features are also common with the sequence around the DNA replication origin of SV40 and BKV, proposed [9,10].

While this paper was in preparation, the sequences in this region of polyoma virus DNA have been published [24]. Our DNA sequences are almost in agreement with this, but a dinucleotide sequence TC in [24] was not found at the location between 13 and 14 in our experiments.

Acknowledgements

The authors are grateful to Professor Genki Kimura for preparation of polyoma virus. This work was supported partly by Grants-in-Aid from the Ministry of Education, Science and Culture of Japan.

References

[1] Folk, W. R. and Wang, H.-C. E. (1974) Virology 61, 140–155.
[2] Griffin, B. E., Fried, M. and Cowei, A. (1974) Proc. Natl. Acad. Sci. USA 71, 2077–2081.
[3] Crawford, L. V., Robbins, A. K., Nicklin, P. M. and Osborn, K. (1974) Cold Spring Harb. Symp. Quant. Biol. 39, 219–225.
[4] Kamen, R. and Shure, H. (1976) Cell 7, 361–371.
[5] Greenblatt, J. F., Allet, B., Weil, R. and Ahmad-Zadeh, C. (1976) J. Mol. Biol. 108, 361–379.
[6] Ito, Y., Spurr, N. and Dulbecco, R. (1977) Proc. Natl. Acad. Sci. USA 74, 1259–1263.
[7] Franke, B. and Eckhardt, W. (1973) Virology 55, 127–135.
[8] Fried, M. and Griffin, B. E. (1977) Adv. Cancer. Res. 24, 67–108.
[9] Subramanian, K. N., Dhar, R. and Weissman, S. M. (1977) J. Biol. Chem. 252, 355–367.
[10] Dhar, R., Lai, C.-J. and Khoury, G. (1978) Cell 13, 345–358.

[11] Soeda, E., Miura, K., Nakaso, A. and Kimura, G. (1977) FEBS Lett. 79, 383–389.
[12] Soeda, E., Kimura, G. and Miura, K. (1978) Proc. Natl. Acad. Sci. USA 75, 162–166.
[13] Maxam, A. M. and Gilbert, W. (1977) Proc. Natl. Acad. Sci. USA 74, 560–565.
[14] Reed, S. I., Ferguson, J., Davis, R. W. and Stark, G. R. (1975) Proc. Natl. Acad. Sci. USA 72, 1605–1609.
[15] Jessel, D., Landau, T., Hudson, J. Lator, T., Tenen, D. and Livingston, D. M. (1976) Cell 8, 535–545.
[16] Tjian, R. (1978) Cell 13, 165–170.
[17] Feunteun, J., Sompayrac, L., Fluck, M. and Benjamin, T. (1976) Proc. Natl. Acad. Sci. USA 73, 4169–4173.
[18] Miller, L. and Fried, M. (1976) J. Virol. 18, 824–832.
[19] Tegtmeyer, P., Schwartz, M., Collins, J. K. and Rundell, K. (1975) J. Virol. 16, 168–178.
[20] Shah, K. V., Daniel, W. R. and Strandberg, D. J. (1975) J. Natl. Cancer Inst. 54, 945–950.
[21] Takemoto, K. and Martin, A. M. (1976) J. Virol. 17, 247–253.
[22] Simmons, D. T., Takemoto, K. K. and Martin, M. A. (1977) J. Virol. 24, 319–325.
[23] Koike, K., Kobayashi, M. and Sekiya, T. (1979) Cold Spring Harb. Symp. Quant. Biol. in press.
[24] Friedmann, T., LaPorte, P. and Esty, A. (1978) J. Biol. Chem. 253, 6561–6567.
[25] Paulin, D. and Cuzin, F. (1975) J. Virol. 15, 393–397.