LOCALIZATION OF A DELETION IN THE DNA OF A BIOLOGICALLY ACTIVE SIMIAN VIRUS 40 STRAIN

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Introduction

Different strains of SV40 have been compared with regard to their restriction endonuclease cleavage patterns. Initially differences were noticed without, however, allocating them on the physical map [1]. Subsequently a number of methods have been devised to identify non-essential regions on the SV40 genome that may be dispensible and may not be required for vegetative growth [2–4]. It has been possible to assign such regions (not exceeding 200 base pairs) to specific areas of the physical map of the map of SV40 genome [2–4].

We shall describe in this paper a wild type SV40 strain which in comparison to other strains is missing 75 ± 10 base pairs within the close vicinity of the initiation site for DNA replication. This strain is fully infectious and it is capable of transforming cells [5].

2. Materials and methods

2.1. *Virus strains*

All SV40 strains had been plaque purified and were passaged at low multiplicities of infection (0.01 plaque forming units per cell). The following strains were used: Rh911 [5], Wt436 and TsA30 provided by Peter Tegtmeyer, and a small plaque strain sp777, provided by Allan Fried.

2.2. *Cells*

CV-1 cells were used for virus propagation. They were grown in minimum essential medium supplemented with 5% fetal bovine serum.

2.3. *Purification of SV40 DNA*

Radioactive labeling with [^{3}H] thymidine and purification of the selectively isolated DNA were as described [6] recently.

2.4. *Restriction enzyme analysis*

The protocol for digestion of the DNA with Endo R. Hind, Endo R. Hpa and Endo R. EcoR_I was a published [6].

2.5. *Gel electrophoresis*

DNA samples (50–100 μl) were electrophoresed in 1.4% agarose (30 × 9 × 0.3 cm) at 70 V for 18 h in a cold room as described [6]. The gels were stained with ethidium Gromide and photographed under ultraviolet light. Densitometer tracings using the photographs were performed with a Joyce-Loebl chromoscan. The distribution after electrophoresis of radioactively labeled DNA was determined by cutting the frozen gels into 1 mm slices and determining the radioactivity in a liquid scintillation counter.

3. Results

The DNA of four different SV40 strains was digested with Endo R. Hind III which cleaves SV40 DNA into six fragments [7]. It may be seen in fig.1 that fragments Hind III B and C are rather similar in size and apparently coelectrophorese in the case of strains sp777, Wt436 and TsA30 forming in the tracing only one peak. Only in the case of strain Rh911 is it possible to resolve both fragments owing to the smaller size of fragment Hind III C.

The electrophoretic mobility of fragment Hind III C relative to that of fragment Hind III B permits the

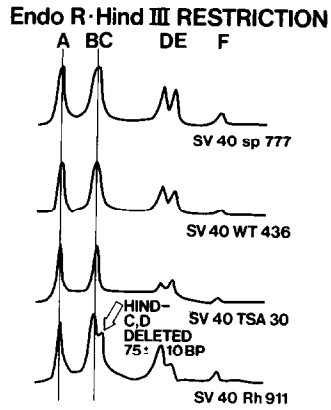

Fig.1. Agarose-gel electrophoresis of the Endo R. Hind III restricted superhelical FO I DNAs of four different SV40 strains. The gels were stained with ethidium bromide, photographed under ultraviolet light, and densitometer tracings of the photographs were taken with a Joyce-Loebl chromoscan. The respective Hind III fragments are designated A–F.

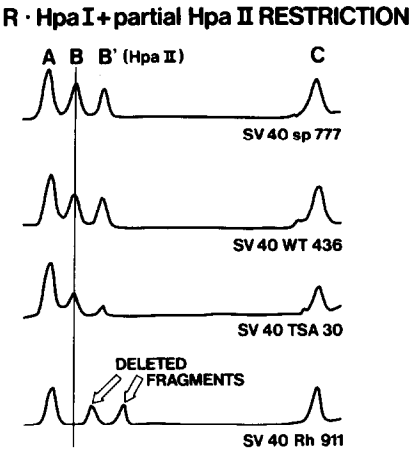

Fig.2. Agarose-gel electrophoresis of the Endo R. Hpa I + partially Endo R. Hpa II restricted superhelical FO I DNAs of four different SV40 strains. The respective Hpa I fragments are designated A–C. The large Hpa II fragment derived from Hpa I B is designated B'.

estimate that the size of the deletion comprizes 75 ± 10 base pairs. Fragment Hind III C comprizes 21% of the SV40 genome and can be resolved after cleavage with Hind II + III endonucleases into two fragments termed Hind C + D [7]. There are two more fragments between C and D on the genome which are termed Hind L and M, respectively [8]. They are, however, owing to their small size (only 1.0–1.5% of the viral genome) not discernable in the gels.

To determine whether the deletion is contained in fragment Hind C or D, Endo R. Hpa I was used which cleaves SV40 FO I DNA into three large segments [9]. In this case Hind D is contained in the largest Hpa I A fragment, while Hind C is contained in fragment Hpa I B. The deletion in strain Rh911 can be allocated within fragment Hind C as may be seen in fig.2d by the increased mobility of fragment Hpa I B relative to the same fragment of the other strains (fig.2a–c). In the experiment described in fig.2 a second restriction endonuclease Endo R. Hpa II was employed at a concentration which produced, unlike Hpa I, only a partial digest. Endo R. Hpa II cleaves the Hpa I B fragment into one large and one small segment and can, therefore, be employed for a more precise localization of the deletion. The third peak from the left in fig.2 represents the large segment derived from the Hpa I B fragment after cleavage with Endo R. Hpa II. The second generated by Endo R. Hpa II is not revealed here owing to its small size which is much less than the size of the Hpa I C fragment. It is clear, however, that the deletion is contained within the larger rather than within the smaller fragment that was obtained after digestion of the Hpa I B fragment with Endo R. Hpa II (fig.2d, third peak from left).

The size of the deletion can be estimated (from the increased mobility of the Hpa I B fragment in fig.2d relative to the Hpa I A fragment) to comprize as shown already above (see fig. 1) 75 ± 10 base pairs. A deletion of 75 base pairs should render the FO I DNA of strain Rh911 smaller than the FO I DNA of the other three strains. To test this prediction, the superhelical DNA of strains Rh911 and sp777 which is contained in the dense bands of CsCl–EtBr density gradients was cleaved open with Endo R. $EcoR_I$ to linear rods of unit length (FO III) and coelectrophoresed in a double-labeling experiment in an agarose gel (fig. 3). It may be seen that that Rh911 FO III runs, as expected, faster than the larger sp777 FO III.

2. Discussion

A naturally occurring deleted SV40 strain Rh911 [5] is described in this paper whose genome is 75

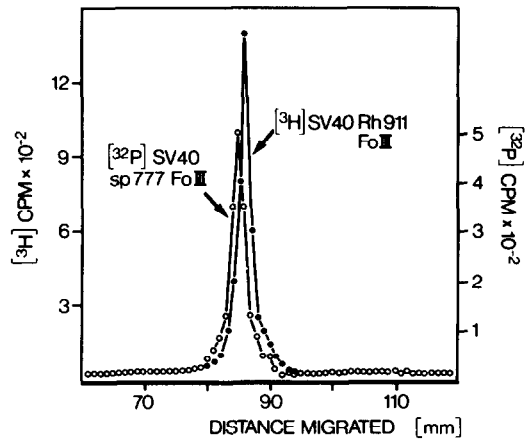

Fig.3. Agarose-gel coelectrophoresis of the linear FO III DNAs of the SV40 strains sp777 and Rh911 obtained after cleavage with Endo R. $EcoR_I$. The gel was sliced with a set of razor blades after electrophoresis and the radioactivity contained in each slice was determined as described [6].

base pairs shorter than the genomes of other SV40 strains (such as Wt436, TsA30, sp777). The deletion is localized close to the origin of replication somewhere between 0.68 and 0.73 map units on the SV40 map (fig.4). This region has been described also by others, who have used artificially generated mutants as being dispensible. Mertz and Berg [10] have isolated viable deletion mutants of SV40 where the Endo R. Hpa II site at the position 0.735 is missing. Further viable deletion mutants were described recently by Shenk et al. [4] who used circularly permuted linear SV40 DNA for infection of cells. In some cases the linear DNA was shortened by mild λ 5′-exo-nuclease digestion. A number of their deletions mapped within the same region described by us and comprized up to 170 base pairs without impairing the vegetative growth of the virus. This dispensible part of the genome is contained in the 'late' region, which is transcribed in lytically infected cells after the onset of viral DNA replication [11]. In view of the small size of the SV40 genome which consists of 5500 base pairs coding for 4–5 polypeptides it is surprising that substantial regions are, nevertheless, dispensible without affecting both vegetative growth and the transforming ability of the virus.

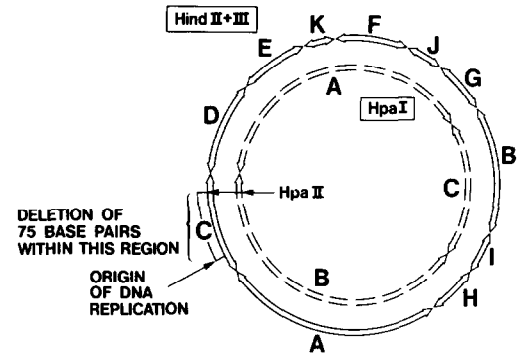

Fig.4. Localization of the deletion comprizing 75 ± 10 base pairs in the physical map of SV40.

Acknowledgement

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