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Pietro Volpe, Tamilla Eremenko

Timing of integration of SV40 DNA into 3T3 cell DNA

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Biochimica. — *Timing of integration of SV40 DNA into 3T3 cell DNA* (*). Nota di PIETRO VOLPE e TAMILLA EREMENKO, presentata (**) dal Corrisp. A. RUFFO.

RIASSUNTO. — Delle cellule di topo 3T3 venivano allevate e sincronizzate in monostrato con doppio blocco timidinico. La loro infezione con SV40 iniziava sempre durante il ciclo mitotico. L'integrazione del DNA virale in quello ospite avveniva però preferenzialmente nella fase S. La fase G_1 appariva necessaria per la ricombinazione dei DNA cellulare e virale in S. La fase G_2 non alterava la stabilità del genoma virale integrato.

1. INTRODUCTION

Several years ago, we showed that development of lytic virus infection is a function of the physiological state of the host cell [1]. In fact, in the case of poliovirus, while the early events of infection and the biosynthesis of minus strand RNA always initiate during the HeLa cell life cycle, the biosynthesis of plus strand RNA and hence the production of progeny virions take place only during S [1–3]. The importance of this phase for development of lytic virus infection was confirmed later for both RNA and DNA viruses [4–12].

The present investigation shows that the oncogenic virus infection also initiates continuously during the cell life cycle, whereas the insertion of viral DNA into the body of host cell DNA preferentially occurs during the S phase. With respect to this there is some background information available from our laboratory [13, 14] and from those of others [15, 19].

2. MATERIALS AND METHODS

2.1 – *Cell growth and synchronization.*

Mouse 3T3 cells were cultivated and synchronized in monolayer with the double thymidine block [20, 21]. The length of each phase of their cell cycle was measured as described previously [21].

2.2 – *Virus infection.*

Infection of synchronized 3T3 cells was performed in suspension with SV40 [22] the DNA of which was previously labelled with ^{3}H-thymidine in permissive CV–1 cells [23] for a full growth cycle [4]. After virus adsorption, the infected cells were washed with Hanks salt solution and replaced in mono-

(*) Faculty of Sciences, University of Naples, Naples, International Institute of Genetics and Biophysics, CNR, Naples, and Respiratory Virus Center, CNR, Rome.

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layer [20]. Their synchrony was then checked for three mitotic cycles. The first cycle proceeded with a 95–98% synchrony. The loss of synchrony of the second was less than 10–15%, while that of the third was less than 15–25%.

2.3 – *Detection of integrated viral DNA.*

Detection of labelled integrated SV40 DNA in DNA of 3T3 cells was performed through DNA/DNA hybridization in solution [24]. For this, DNA from the infected synchronized cells was extracted [25], purified on neutral CsCl gradient at high molecular weight [22], then fragmented by sonication, deionized on Sephadex–G25, and concentrated [26]. To increase the hybridization efficiency, the unlabelled and non-fragmented SV40 DNA (4.5 Kb) was employed at saturating concentrations with respect to the input of labelled and fragmented transformed DNA (1.5 Kb). For a biological control of integration, the " rescue " of labelled SV40 was made using UV-irradiated Sendai virus in the system of transformed 3T3 and permissive CV–1 cells [27]. The radioactive DNA was then extracted from rescued virions as described by Sambrook *et al.* [22].

2.4 – *Chemicals.*

Eagle minimum essential medium with Ca^{++} (MEM), Joklik-modified minimum essential medium without Ca^{++} (JM–MEM) and foetal calf serum (FCS) came from Grand Island Biological Company, New York, USA. Unlabelled SV40 DNA was furnished by Calbiochem-Behring Corporation, Lucerne, Switzerland. ^{3}H-thymidine (20 Ci/mmol) was purchased from New England Nuclear, Boston, USA. Sephadex-G25 was obtained by Pharmacia, Uppsala, Sweden. The hydroxyapatite crystals (HAP) were prepared as described by Levin [28]. TCA, trichloroacetic acid. Pb, potassium phosphate buffer at pH 6.8.

3. Results

3.1 – *Hybridization of SV40 DNA with 3T3 DNA.*

In the first experiment, virus infection was performed in the middle of the G_2 phase, this point being a reference for an initial cell cycle (Fig. 1a). During the next G_1 phase, which represented the beginning of a new cell cycle, hybrids of unlabelled SV40 DNA with nuclear DNA from virus-infected 3T3 cells were undetectable since in this phase, as a rule, host DNA was not yet labelled or insignificantly labelled. However, during the next S phase, the SV40/3T3 DNA hybrids became sharply detectable since host DNA was labelled. At the end of the new G_2 phase, the SV40/3T3 DNA hybrids maintained the same volume as that reached during the S phase (in G_2 host DNA was also highly labelled).

In the second experiment, virus infection was performed in early G_1 (Fig. 1b). In this case, in S (namely, in the course of the same cell cycle), the volume of the SV40/3T3 DNA detectable hybrids was appreciably reduced in comparison with that observed in Figure 1a. In the new G_1 (namely, in the course of the successive cell cycle), the volume of the SV40/3T3 DNA hybrids became, as expected, the half of that observed in the preceding S.

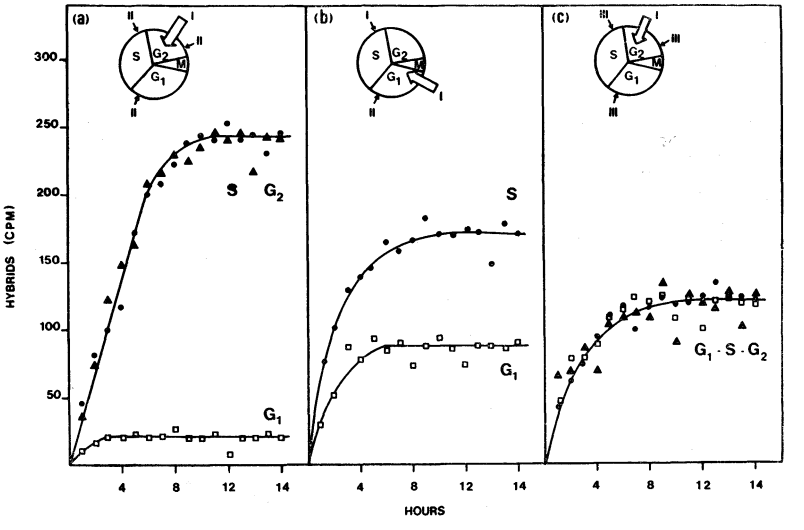

Fig. 1. – *Kinetics of SV40/SV40–3T3 DNA hybridization.* At the times of an initial cell cycle indicated by the large arrows, 1×10^9 3T3 cells were suspended in 9 ml JM–MEM (with 1 %–FCS) and mixed with 1 ml Hanks salt solution containing ^{3}H–DNA SV40 (the multiplicity of infection was 1,000 : 1). The adsorption lasted 1 hr at 37 °C under stirring and 5 % CO_2 in air. After washing, the infected cells were allowed to continue the synchronized growth in monolayer. During the successive mitotic cycles (small arrows), the cells were collected from Falcon dishes for DNA extraction, purification, desalification and heat denaturation. The annealing of the unlabelled SV40 DNA with the labelled SV40–3T3 DNA pieces was performed at 60 °C in 0.12 M Pb (24). The hybrids were purified with 0.40 M Pb on HAP columns of 5 ml at 30 min intervals during the course of 16 hrs. The amount of unlabelled SV40 DNA was 150 μg/HAP column. The input of labelled SV40–3T3 DNA was 2.5×10^5 dpm/HAP column. The TCA-precipitable radioactivity of the hybridized samples was measured in Insta-gel, using a computerized 460 CD Packard scintillation radiospectrometer. (*a*) – The infection was made in mid-G_2 of the cell cycle I, while the hybrids were checked at the end of the phases G_1 and G_2 of the cell cycle II. (*b*) – The infection was made in early-G_1 of the cell cycle I, while the hybrids were checked at the end of S of the same cell cycle and in late G_1 of the cell cycle II. (*c*) – The infection was made in mid-G_2 of the cell cycle I, while the hybrids were checked at the end of G_1, S, and G_2 of the cell cycle III. Hybrids detected in G_1 (○ – – ○), S (● – – ●), and G_2 (▲ – – ▲).

In the last experiment, as in the first, the viruses infected the cells in the middle of the G_2 phase (Fig. 1c). Hence, the SV40/3T3 DNA hybrids, detected later during the phases G_1, S and G_2 of the third cell cycle, all showed the same volume, becoming, however, half of those found during the phase S of the second cell cycle (Fig. 1a).

3.2 - *Rescue of SV40.*

The " rescue " experiments (Table I) showed that radioactive SV40 virions were released by those infected 3T3 cells which had traversed the S phase. Soon after infection, any labelled SV40 material was recovered from the G_1 cells, while the amount of the labelled virions rescued in G_2 was as that in S.

TABLE I

Radioactivity of the SV40 virions rescued from the 3T3 cells after transformation during the cell cycle.

Experiments	Timing of infection	Timing of rescue	Rescued virus radioactivity (Total dpm) *
1st	Mid-G_2 of cell cycle I	End of G_1 of cell cycle II	1,050
		End of S of cell cycle II	8,634
		End of G_2 of cell cycle II	8,831
2nd	Early-G_1 of cell cycle I	End of S of cell cycle I	6,418
		End of G_1 of cell cycle II	2,944
3rd	Mid-G_2 of cell cycle I	End of G_1 of cell cycle III	3,841
		End of S of cell cycle III	4,188
		End of G_2 of cell cycle III	3,740

* 1×10^9 synchronized 3T3 cells were infected with ^{3}H-DNA SV40 at a multiplicity of infection of 1,000 : 1. Every value was normalized per 1×10^9 cell samples plus 1×10^9 CV-1 cells used for rescue experiments.

4. DISCUSSION

The discovery of a complete insertion of SV40 DNA into 3T3 cell DNA [22] was soon followed by the observation that transformation of the cells with both RNA and DNA oncoviruses may be associated with the increased biosynthesis of their DNA [15, 16]. Concomitantly, utilization of synchronized 3T3 and NRK cells infected with SV40 and RSV, respectively, suggested that such association is likely to be manifested in S simply because in this phase cell DNA—once free from histones, despiralized or even as new fragments—might become an easy target for oncoviral input [13, 14]. In other words, the oncogenes might be recruited together with the Okazaki fragments and linked with them for errors or at specific sites [13]. This interpretation led us to ask whether integration indeed generates small duplications of cellular DNA at the point of entry [17, 18] or, vice versa, it occurs only in host DNA that has replicated after infection [19].

We used, therefore, the cell cycle technology [21, 29] employed in previous studies [1–3] to re-examine these questions more rigorously. Thus, when infection was made in mid-G_2 phase (Fig. 1a), the SV40/SV40–3T3 DNA hybrids were not detectable in the next G_1—we thought—since integration had not yet occurred. However, during the next S, these hybrids were detectable because integration took place sharply. The hybrids were found in G_2 as well as in S, since new integrations should not occur. In addition, when infection was made in early-G_1 (Fig. 1b), the SV40/SV40–3T3 DNA hybrids were detectable to a reduced extent, while in the next G_1 these hybrids were half of those found in S. This experiment would signify that G_1 is helpful for integration in S and that integrated oncogenomes are not lost. Finally, when infection was made in G_2 (Fig. 1c), in G_1, S, and G_2 of the third cell cycle the SV40/SV40–3T3 DNA hybrids were half of those in S of the second cell cycle (Fig. 1a), because integration should be stable, as known [30]. All this would suggest, in conclusion, that enzymology of replicating cellular DNA by itself is absolutely necessary for integrative recombination.

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