Virus genome integration as a function of the host cell genome replicative cycle

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Mouse 3T3 cells were grown and synchronized in monolayer with the double thymidine block. Their infection with SV40 took place continuously during the cellular cycle. However, integration of viral DNA into host cell DNA occurred preferentially during the S phase. Phase G_1 appeared to be necessary for virus-cell DNA recombination in S phase. Phase G_2 did not alter the stability of the integrated viral genome.

Integration *SV40 DNA* *Cell cycle* *3T3 cell*

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 are always initiated during the HeLa cell life cycle, the biosynthesis of plus strand RNA and hence the production of progeny virions take place only during S phase [1–3]. The importance of this phase for development of lytic virus infection was confirmed later for both RNA and DNA viruses [4–12]. This investigation shows that the oncogenic virus infection is also initiate continuously during the cell cycle, while 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 us [13,14] and others [15–19].

2. MATERIALS AND METHODS

Mouse 3T3 cells were synchronized in monolayer with the double thymidine block [20,21], while the length of each phase of their cell cycle was measured as in [21]. Infection of synchronized 3T3 cells was made with SV40 [22] the DNA of which was previously labelled with 20 Ci/mmol [^{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 monolayer [20]. Detection of labelled integrated SV40 DNA of 3T3 cells was performed through DNA/DNA hybridization in solution [24]. For this purpose, DNA from the infected synchronized cells was extracted [25], purified on a neutral CsCl gradient at high M_r [22], then fragmented by sonication, deionized on Sephadex-G25, and concentrated [26]. To increase the hybridization efficiency, the unlabelled non-fragmented SV40 DNA (4.5 kbp) was employed at saturating concentrations with respect to the input of labelled and fragmented transformed DNA (1.5 kbp). For a biological control of integration, the 'rescue' of SV40 containing labelled DNA was made using UV-irradiated Sendia virus in the system of transformed 3T3 and permissive CV-1 cells [27]. The radioactive DNA was then extracted from rescued virions as in [22].

3. RESULTS AND DISCUSSION

In the first experiment, virus infection occurred

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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 phase (fig.1b). In this case, in S phase (namely, in the course of the same

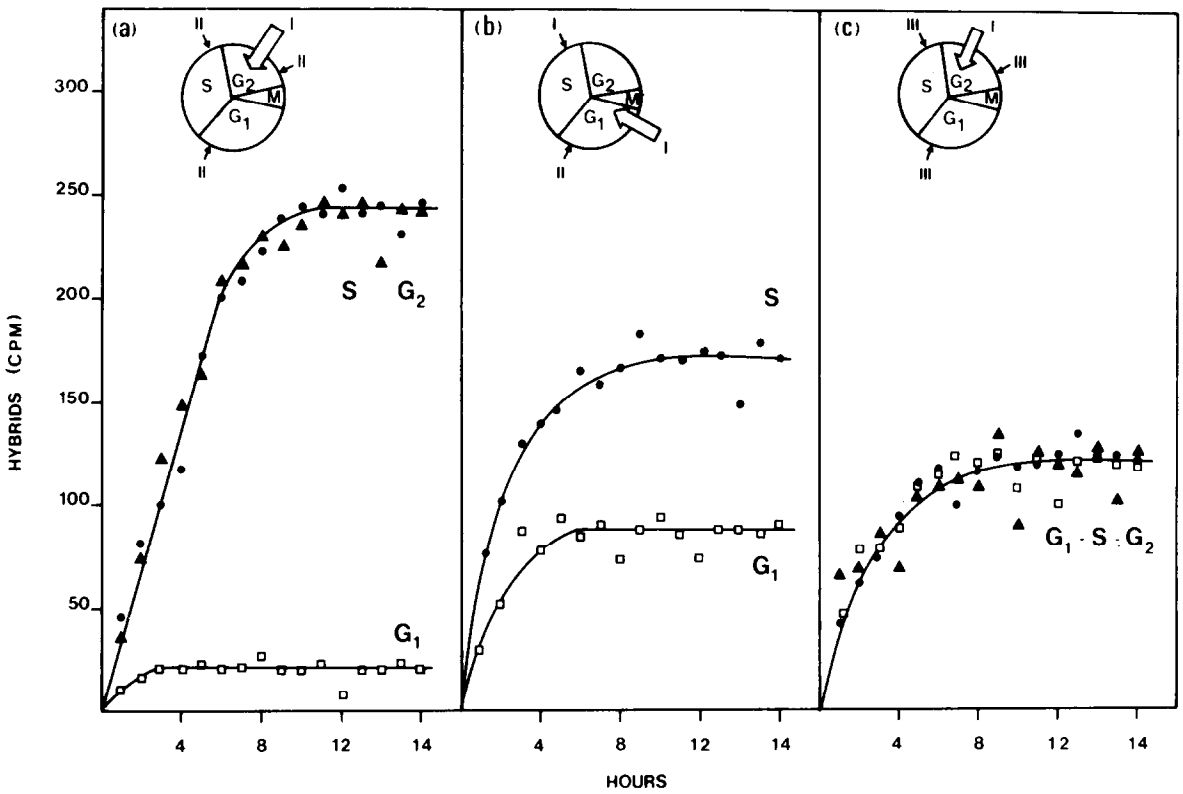

Fig.1. Kinetics of SV40/SV40-3T3 DNA hybridization. At the times of an initial cell cycle indicated by the large arrows, 1×10^9 3T3 cells were suspended in 9 ml of Joklik modified minimum essential medium (with 1% foetal calf serum) and mixed with 1 ml Hanks salt solution containing [^{3}H]DNA SV40 (multiplicity of infection 1000:1). The adsorption lasted 1 h 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, desalting and heat denaturation. The annealing of the unlabelled SV40 DNA with labelled SV40-3T3 DNA pieces was performed at 60°C in 0.12 M potassium phosphate buffer (pH 6.8) [24]. The hybrids were purified with 0.40 M potassium phosphate on HAP columns [28] of 5 ml at 30 min intervals during the course of 16 h. 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 trichloroacetic acid-precipitable radioactivity of the hybridized samples was measured in Inste-gel, using a computerized 460 CD Packard scintillation radiospectrometer. (a) The infection was made in mid-G_2 phase of cell cycle I, while the hybrids were checked at the end of phases G_1 and G_2 of cell cycle II. (b) The infection was made in early-G_1 phase of cell cycle I, while the hybrids were checked at the end of S phase of the same cell cycle and in late G_1 phase of cell cycle II. (c) The infection was made in mid-G_2 phase of cell cycle I, while the hybrids were checked at the end of G_1, S, and G_2 phases of cell cycle III. Hybrids detected in G_1 (□—□), S (●--●), and G_2 (▲—▲).

cell cycle), the volume of the SV40/3T3 DNA detectable hybrids was appreciably reduced in comparison with that observed in fig.1a. In the new G_1 phase (i.e., in the course of the successive cell cycle), the volume of the SV40/3T3 DNA hybrids became, as expected, half of that observed in the preceding S phase. 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 phases G_1, S and G_2 of the third cell cycle, all showed the same volume, becoming, however, half of those found during phase S of the second cell cycle (fig.1a).

Similar infections with SV40, containing [^{3}H]thymidine-labelled DNA, were carried out using CV-1 cells. In all cases, however, plots as in fig.1 were not observed. Such controls excluded, of course, that in 3T3 cells the integrated tritium might represent some nucleotide turnover. By contrast, the rescue experiments (table 1) 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 labelled virions rescued in the G_2 phase was the same as that in S phase.

With respect to the background information, 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 appear 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 the S phase 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 question whether integration indeed generates small duplications of cellular DNA at the point of entry [17,18] or, vice versa, if it occurs only in the host DNA that has replicated after infection [19]. We therefore used the cell cycle technology [21,29] employed in [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 phase – we thought – since integration had not yet occurred. However, during the next S phase these hybrids were detectable because

Table 1

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	1050
		end of S of cell cycle II	8634
		end of G_2 of cell cycle II	8831
2nd	Early-G_1 of cell cycle I	end of S of cell cycle I	6418
		end of G_1 of cell cycle II	2944
3rd	Mid-G_2 of cell cycle I	end of G_1 of cell cycle III	3841
		end of S of cell cycle III	4188
		end of G_2 of cell cycle III	3740

* 1×10^9 synchronized 3T3 cells were infected with [^{3}H]DNA SV40 at a multiplicity of infection of 1000:1. Every value was normalized per 1×10^9 cell samples plus 1×10^9 CV-1 cells used for rescue experiments

integration took place sharply. The hybrids were found in the G_2 phase as well as in S phase since new integrations should not occur. In addition, when infection was made in early G_1 phase (fig.1b), the SV40/SV40-3T3 DNA hybrids were detectable to a reduced extent, while in the next G_1 phase these hybrids were half of those found in S phase. This experiment would signify that the G_1 phase is helpful for integration in S phase and that integrated oncogenomes are not lost. Finally, when infection was made in G_2 phase (fig.1c), in G_1, S, and G_2 phases of the third cell cycle the SV40/SV40-3T3 DNA hybrids were half of those in S phase of the second cell cycle (fig.1a), because integration should be stable, as is known [30].

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