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Synchronous replication of SV 40 DNA in virus infected TC 7 cells induced by transient hypoxia

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We transiently exposed SV 40 infected TC 7 cell cultures to a reduced O_2 tension (4–8 h, about 200 ppm relative to 10^5 Pa total pressure). Under the hypoxic conditions, 'working' viral replication forks were greatly retarded or stopped, and initiation of daughter strand synthesis in further SV 40 DNA molecules was suppressed. Reoxygenation released an immediate burst of SV 40 replication which mainly consisted of a synchronous viral replication round. This synchronous in vivo replication began at the known origin of replication and proceeded at normal rates to the known termination region. Viral replicons seemed to accumulate under hypoxia in a state fully prepared to begin replication immediately after recovery of a normal pO_2. The shut-down and sudden reactivation of DNA synthesis under hypoxia and reoxygenation, respectively, were not accompanied by changes of the phosphorylation state of large T antigen. The described synchronization procedure can be applied to optionally large SV 40 infected cell cultures.

DNA replication; Replicon initiation; Synchronisation of replication; Hypoxia; Simian virus 40

1. INTRODUCTION

In previous communications we demonstrated that DNA replication in Ehrlich ascites cells is subject to a regulation which depends on the O_2 tension in the cellular environment [1–4]. This regulation operates at O_2 tensions distinctly above the minimum required to support mitochondrial respiration [5] and to establish a normal adenylate energy charge [3]. Furthermore, we have shown that this regulation takes place during ascites tumor growth in vivo [3,4]. There, and possibly in other tumors, it may represent a major determinant of tumor cell propagation by adapting the latter to the supply of nutrients (not only of O_2). Repeatedly we pointed to the possible significance of the phenomenon in critical situations during embryonic development of mammals before placentation and in certain stages of wound healing [6]. Work with cultured cells under controlled O_2 tension [3,4,7] revealed the responses of the cellular replication machinery of Ehrlich ascites cells: when the pO_2 is reduced to values between 200 and 2000 ppm (relative to 10^5 Pa total pressure) scheduled replicon initiations are specifically, reversibly and coordinately suppressed, whereas DNA chain growth and maturation in replicons initiated before reduction of the pO_2 continue essentially normally. Re-elevating the pO_2 triggers a burst of initiations within a few min. When the pO_2 is reduced to values below 150 ppm, cell damage occurs and the reversibility is lost [3,4].

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A prominent feature of this regulation is the short span of time between reoxygenation (after several h hypoxia) and the emergence of the replicon initiation burst. It was demonstrated that a 'proliferative' state of expression of 'growth related' genes is retained when growing cells are subjected to hypoxia [7]. Therefore, we supposed that the O_2-dependent replication control acts very directly on the replication apparatus of the Ehrlich ascites cells. The ease and the quickness of releasing the hypoxic block of replicon initiation by simply adding an appropriate quantity of O_2 saturated medium to the hypoxic cells provided a basis for the selective and synchronous activation of early-S-phase replicons in large cultures of Ehrlich ascites cells [6].

Recently, we extended our studies to further cell lines and to simian virus 40 (SV 40) which is a commonly used model system of mammalian DNA replication [8]. In the present communication, we provide evidence that replication of the SV 40 genome in vivo depends, like that of Ehrlich ascites cells [3] and of further mammalian cell lines (unpublished), on a critical O_2 tension. Replication of viral DNA in infected cell cultures almost completely ceases in the course of 4–6 h hypoxia. Reoxygenating hypoxic cultures triggers, within < 2 min, a synchronous burst of SV 40 replication which starts in the ori region of the viral genome.

Initiation of SV 40 DNA replication depends on the viral large T antigen which binds and unwinds the replication origin [9,10]. Moreover, some activities of the large T antigen during (pre-) initiation appear to be regulated by reversible phosphorylation (reviewed in

[10]). Thus, it was conceivable that the observed rapid changes in replicating activity were mediated by changes of the phosphorylation state of the large T antigen. We observed, however, no changes of the phosphorylation pattern of large T antigen.

2. MATERIALS AND METHODS

2.1. *Cells and virus*

The TC 7 subline of monkey CV 1 cells (American Type Culture Collection CCL 70) was grown in plastic flasks in DMEM medium containig 5% fetal calf serum under standard tissue culture conditions. For a typical transient hypoxia experiment usually 6–15 glass dishes (95 mm) were seeded with identical cell inocula yielding $4–6 \times 10^6$ cells per dish at the planned time of infection with SV 40 according to [11] (usually 1 or 2 days thereafter). Virus stocks had been plaque purified according to [12]. Hypoxia experiments were started 38–40 h after infection.

2.2. *Transient hypoxia and radioactive labeling*

For the use with monolayer cultures, the original hypoxia procedure developed for suspension cultures of Ehrlich ascites cells, was modified with respect to some technical details described below (for unmodified details see [3]). Hypoxic gassing of cultures is performed in uncovered glass dishes inside of flat metal pots covered by a glass lid sealed by an O-ring made from acrylnitril-butadiene rubber. The pots are equipped with a gas inlet at the bottom and an outlet near the lid conducting escaping gas 2–3 cm below the surface of water in a small 'bubble tube' mounted on the outside for controlling gas flow and maintaining a slight overpressure. Moreover, a special construction permits to plunge ends of small glass spatulas, carrying substances dried up from solutions on their surface ([^{3}H]dThd, $^{32}PO_4$), into the culture medium without opening the pot. Up to 18 pots (cultures) can be operated at the same time in a room thermostatized at 37°C. Cultures were pre-equilibrated by gassing with artificial air containing 5% CO_2 for 1 h. Hypoxia was started by a quick stream (1 pot volume per 2–3 min) of hypoxic gas which was maintained for 20–30 min and then reduced to about 1/5 of this flow rate. For reoxygenation, 1/4 vol. of medium equilibrated with 95% O_2, 5% CO_2 was added to cultures and gassing was continued with artificial air. Aerated control cultures were gassed only with artificial air. For DNA labeling with [^{3}H]dThd (Me-^{3}H, Amersham, 80 Ci/mmol) 10–40 μCi per ml culture medium were added either directly or, in the case of hypoxic labeling, by spatula plunging as described above. To stop incubations, dishes were rinsed extensively with ice cold Hanks solution and then processed at 0–40°C to prepare Hirt supernatants according to [11] or T antigen according to [13].

2.3. *Alkaline sedimentation analysis of nascent daughter strands, estimation of radioactivity of StyI fragments of SV 40 DNA*

5–40% (w/w) linear sucrose gradients in 0.25 M NaOH, 0.6 M NaCl, 1 mM EDTA, 0.1% sarcosyl, built up over a 1.5 ml cushion in centrifuge tubes for the Beckman SW40 Ti rotor were overlaid with 150 μl of Hirt supernatants redissolved after ethanol precipitation in 50 mM EDTA, 0.2 M NaOH and then centrifuged at 23°C, 32,000 rpm for 15 h. 0.6 ml fractions were collected from the top and processed for determination of acid insoluble radioactivity as described [14]. Calibration of this procedure using appropriate restriction fragments of plasmids labeled by growth in [^{3}H]dThd containing medium revealed sufficiently isokinetic sedimentation at 1.75 S per fraction (after deducting the first 1/2 fraction).

For determination of radioactivity incorporated from [^{3}H]dThd pulses into individual *Sty*I fragments of SV 40 DNA, ethanol precipitated Hirt supernatants (from 95 mm dishes) were redissolved in 50 μl of 10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0 containing additionally 10 μg/ml RNase A and then adjusted to 10 mM $MgCl_2$, 100 mM NaCl, 1 mM dithiotreitol, pH 7.5 and digested, after adding 60 U *Sty*I

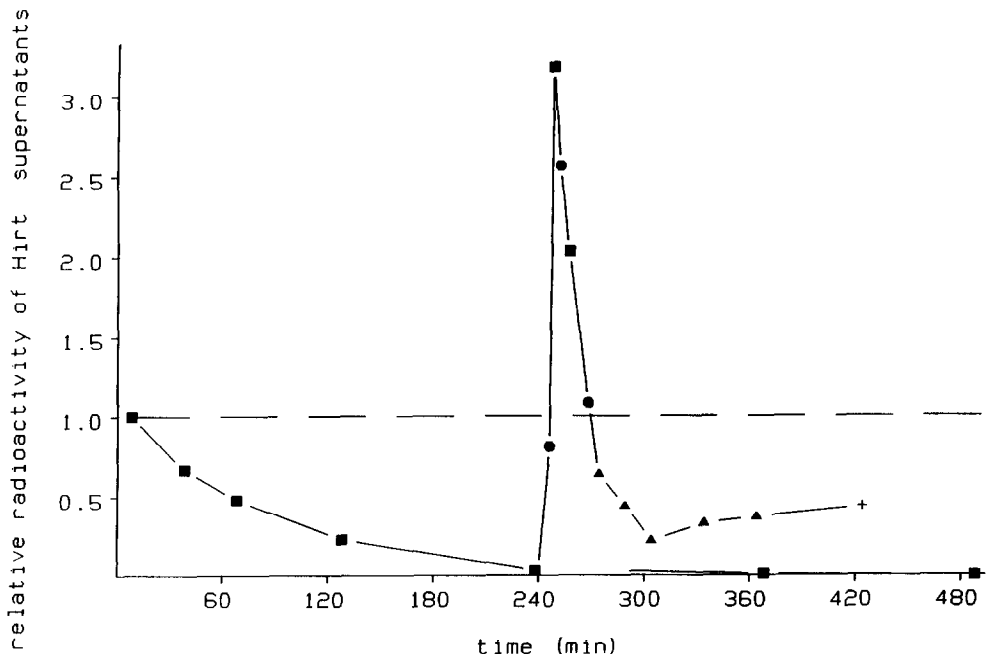

Fig. 1. Temporal course of the rate of incorporation of radioactivity from [^{3}H]dThd pulses into SV 40 DNA (Hirt supernatants) of virus infected cells in transient hypoxia experiments. The graph was compiled from data obtained in 4 independent experiments. Reoxygenation was performed in all 4 experiments at 240 min after the start of hypoxic gassing (time 0). We included only data of hypoxia treated samples for which an appropriate untreated control existed (i.e. the control sample originated from the same batch of identically prepared virus infected cell culture dishes and was pulse-labeled within ± 30 min in relation to the respective hypoxia treated sample(s)). Points indicate the quotients of total ^{3}H cpm in samples and controls. ■, experiment A, (8 min pulses employing 10 μCi/ml [^{3}H]dThd, total cpm of controls were between 10,000 and 11,000, 2 samples remained hypoxic in this experiment beyond 240 min until hypoxic labeling at 250 or 370 min, respectively). ●, ▲, +, experiments B–D (4 min pulses employing 40 μCi/ml [^{3}H]dThd, total cpm of controls were 4,800–5,700).

(Boehringer), overnight at 37 °C. Total digests were loaded to single large wells of 3% agarose gels (12 × 11 × 0.5 cm) in 40 mM Tris, 5 mM Na-acetate, 1 mM EDTA, pH 7.8 and electrophoresed for 15 h at 2.5 V/cm and 25°C. Bands were visualized by ethidium bromide staining, excised and hydrolyzed in scintillation vials after addition of 750 μl 0.5 M HCl at 80°C for 2 h and then counted in 7 ml Ultima Gold scintillation cocktail (Packard).

2.4. *Fingerprint analysis of T antigen phosphopeptides*

Hypoxic, reoxygenated and aerated (control) cultures were incubated for 2 h with 2 mCi/2 ml $^{32}PO_4$ (Amersham, carrier free) in phosphate free medium and then processed for fingerprint analysis of large T antigen phosphopeptides by immunoprecipitation, electrophoresis, electroblotting to nitrocellulose membranes, digestion directly from membranes and electrophoresis/chromatography as described in [13,15,16].

3. RESULTS

3.1. *Rate of [^{3}H]thymidine incorporation into viral DNA in transiently hypoxic cells*

When virus infected cell cultures were exposed to controlled hypoxia, incorporation of [^{3}H]dThd into viral DNA (Hirt supernatant) decreased within 4–6 h to nearly zero. When 4–8 h hypoxic cultures were reoxygenated, an immediate burst of [^{3}H]dThd incorporation could be reproducibly triggered. This burst normally peaked between 8 and 14 min after O_2 recovery and the maximum rate of [^{3}H]dThd incorporation exceeded that of parallel control cultures not treated by hypoxia 3- to 4-fold. About 30 min after reoxygenation, the incorporation rate fell again below that of controls. A minimum in the range of 20–30% of controls usually occurred at about 60 min and was then followed by a relatively slow re-increase. The incorporation rate of controls was not re-attained during the following 2 h. The course of [^{3}H]dThd incorporation shown in Fig. 1 is composed of data from 4 independent experiments using 4 h hypoxia. The relatively good fit of values originating from different experiments into a common curve demonstrates reproducibility of the reaction of virus replication to transient hypoxia. The general observations described above are valid for hypoxic periods between 4 and 8 h which were used in our experiments performed so far.

3.2. *Growth of daughter chains of SV 40 DNA in transient hypoxia experiments*

To estimate sizes of growing daughter strand DNA in the course of transient hypoxia experiments, we analyzed [^{3}H]dThd labeled DNA of Hirt supernatants by alkaline sucrose gradient centrifugation. Under the conditions of sedimentation used, nascent SV 40 daughter chains (up to about 16 S [8]) covered gradient fractions 1–10, covalently closed full length single stranded circles (about 18 S) reached fractions 11–12, and covalently

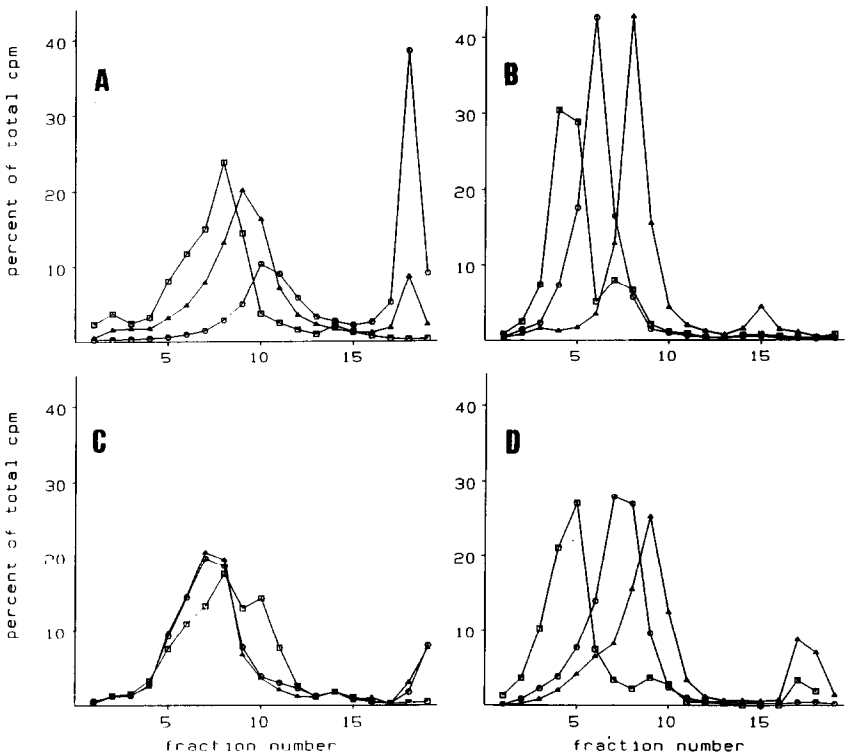

Fig. 2. Alkaline sedimentation profiles of radioactivity of Hirt supernatants prepared from SV 40 infected cell cultures after labeling with [^{3}H]dThd. (A) Aerated (control) cultures (□, 4 min pulse; △, 11 min pulse; ○, 6 min pulse/20 min chase). (B,C) Series of 4 min pulses started at different times after reversal of a 4 h hypoxia block (B: □, ○, △, pulses started at 2, 8, 24 min after reoxygenation; C: □, ○, △, pulses started at 45, 90, 120 min after reoxygenation). (D) Series of pulses of different lengths started concurrently with reoxygenation after 8 h hypoxia (□, 4 min pulse; △, 15 min pulse; ○, 30 min pulse). The total cpm recovered from the gradients varied between 5,000 and 18,000. The direction of sedimentation was from left to right.

closed circular duplexes (form I, > 50 S) sedimented to the bottom of the centrifuge tubes. Fig. 2 shows a selection of sedimentation profiles of radioactivity obtained with Hirt supernatants prepared from untreated (controls, A) and reoxygenated (B–D) virus infected cells labeled by [^{3}H]dThd using different labeling schedules.

Fig. 2A fits well the present knowledge of SV40 replication in vivo [8]: In untreated cells, a short pulse (4 min, □) mainly labeled a broad spectrum of daughter chains producing a peak in the 13 S region (about 3 kb). Extension of the pulse to 11 min shifted the accentuation of the profile towards full length daughter strands, and a significant portion of radioactivity sedimented like covalently closed single stranded circles and form I DNA (△). After pulse/chase labeling (6/20 min, ○), most of the radioactivity was recovered from the bottom of the tubes, supposedly as form I DNA.

If a period of hypoxia of 4–8 h preceeded short pulses (4 min) performed within the first 30 min after O_2 recovery, we generally obtained distinctly narrower profiles than with untreated control cells, indicating significantly increased uniformity in length of labeled chains (Fig. 2B). A narrow peak appeared in the 6–8 S region (< 1kb) when the start of the pulse coincided with reoxygenation (Fig. 2D, □), or was performed shortly thereafter (Fig. 2B, □). However, we reproducibly observed a small accessory peak or a shoulder representing significantly faster sedimenting chains of labeled DNA. This indicates the additional presence of a minor fraction of relatively long growing chains within the first 4–6 min after reoxygenation. The relative height of the accessory peak usually declined, and its position was shifted towards longer chains as the duration of the preceding hypoxic period increased (examined in the range from 4 to 8 h hypoxia). Note the respective difference of the corresponding profiles shown in Fig. 2B (□, 4 h hypoxia) and Fig. 2D (□, 8 h hypoxia). Supposedly, this accessory peak represents SV 40 DNA molecules hit by hypoxia while engaged in replication (see below). When the time span between reoxygenation and pulse was increased up to about 30 min the position of the narrow peak was increasingly shifted towards S values of full length viral DNA (Fig. 2B; ○, 8 min; △, 24 min). This observation is compatible with the assumption of a synchronous start of replication in many SV 40 replicons within about 1–2 min after reoxygenation and a further synchronous chain elongation. When the temporal distance between the reoxygenation event and the pulse was extended to >30 min (Fig. 2C), an increased proportion of the incorporated radioactivity appeared again in the region of small chains, and the radioactivity profiles produced by short pulses adopted the shape of controls not treated by hypoxia. Thus, at later times after O_2 recovery, the synchronous surge of viral replication released directly after reoxygenation seems to be followed by asynchronous replication as in untreated cells. In the profile obtained by the pulse started at 45 min after reoxygenation (Fig. 2C, □), a shoulder in the 16–18 S region supposedly reflects a leftover of the synchronous surge discussed above.

Fig. 2D shows profiles produced by a series of pulses of different lengths which were started concurrently with reoxygenation and extended to 4, 15 and 30 min thereafter. The significant 'tailing' of the peaks of the 15 and the 30 min profile indicates the occurrence of a minor portion of nascent chains which supposedly ceased to be elongated after an initial period of growth in the presence of the radioactive label. Thus, most, but not all daughter chains initiated at recovery of O_2 seem to be completed to give full-length viral genomes. A small, but significant fraction of them appears to remain in earlier stages of replication for a significant period of time. It cannot be excluded that, at least a part of these viral replicons rested abortive at all.

Short pulses performed during the decline of radioactivity incorporation within the first 2 h of the hypoxic period produced profiles (not shown) the shapes of which were indistinguishable from that of untreated controls. Possibly, this indicates that a working viral replication machinery hit by threshold hypoxic conditions either simply stops in whatever state it may be, or greatly slows down fork progress without leaving the replicative state. The shift of the accessory peak produced by pulses at or shortly after reoxygenation (see above) argues for the latter possibility.

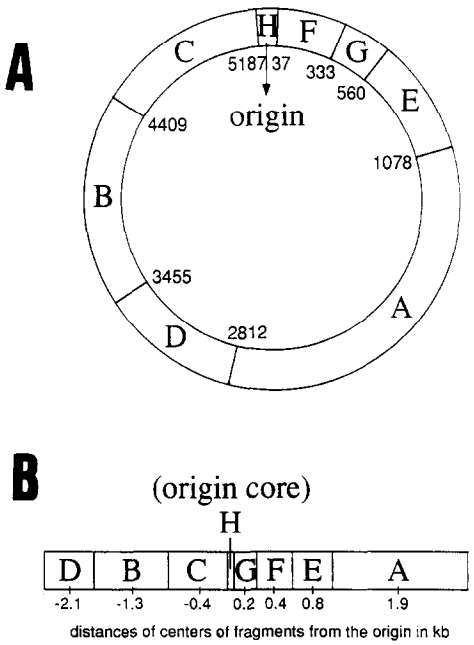

Fig. 3. *Sty*I restriction map of the SV 40 genome. (A) Circular map, numbering of bases according to [30]. (B) Same map linearized by a cut at position 2812 (between fragments A and D). This linearized structure serves as abscissa in Fig. 5. Fragment H, including the core of the origin of replication, lies about in the center. Both ends correspond to the termination region.

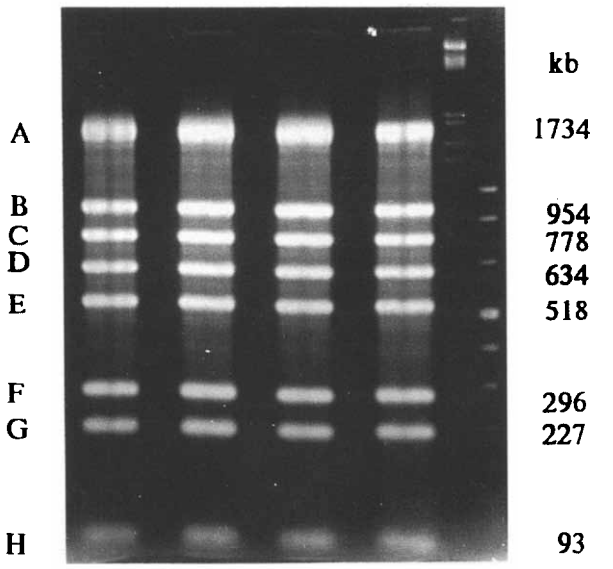

Fig. 4. Separation of *Sty*I fragments of SV 40 DNA by preparative agarose gel electrophoresis in the course of the experiment yielding the data presented in Fig. 5. 8 of 10 identical virus infected cultures were rendered hypoxic and reoxygenated after 5 h. The reoxygenated cultures were labeled by 4 min [^{3}H]dThd pulses staggered at 5 min intervals from 0 to 35 min after O_2 readmission. Two controls which remained aerated were labeled at the beginning and the end of this period. *Sty*I digests of the Hirth supernatant DNA's were separated on two identical preparative agarose gels run in parallel (4 or 6 samples per gel, respectively). The 4-sample gel after ethidium bromide staining is shown. The 4 broad lanes correspond to the last 3 reoxygenated samples and to one of the control samples. The two narrow lanes at the right were loaded with Boehringer length standards VI and VIII. The visible bands, termed A–H according to Fig. 3, were excised and processed for measuring the ^{3}H radioactivity.

3.3. *Reoxygenation releases synchronous bidirectional SV 40 replication starting in the ori region*

*Sty*I cuts SV 40 DNA into 8 fragments (Fig. 3A) which can be well separated by gel electrophoresis (Fig. 4). The smallest fragment (termed H in Fig. 3) is almost completely occupied by the 'core' of the origin of replication [8] whereas termination occurs in a broad region (about 700 bp [8]) opposite to the origin. This region includes the *Sty*I site at position 2812, thus covering the adjacent ends of fragments A and D. An imagined cut at the last mentioned *Sty*I site would bring the origin into the center and the termination site(s) to the ends of the resulting linear structure (Fig. 3B). In a normally scheduled, but synchronous, round of in vivo replication incorporation of radioactivity from short [^{3}H]dThd pulses should first be focused around fragment H and later divide into two foci proceeding towards the ends as replication proceeds. Exactly this was found in the experiment the data of which are presented in Figs. 4 and 5.

The labeling schedule used in this experiment is analogous to that used for the alkaline gradients presented in Fig. 2B, i.e. staggered [^{3}H]dThd pulses were performed at different times after reoxygenation. The results of the analysis of the distribution of incorporated radioactivity to the *Sty*I fragments are shown in Fig. 5. The chosen presentation compares the relative radioactivities of the fragments with that measured using DNA from untreated controls which were assumed to represent normal asynchronous virus replication (broken line at 1.0 in Fig. 5). Fig. 5 clearly demostrates that the synchronous growth of the daughter strand molecules detected by sedimentation analysis begins at the origin of replication and proceeds towards the normal termination region. However, although distinctly centered around the origin, the radioactivity incorporated during the pulse started concurrently with reoxygenation is not restricted to this region (Fig. 5A). The rather peripheral fragments D, B, E and A carried, even in this stage, about 1/2 of the relative radioactivity incorporated by the respective control fragments. This confirms the presence of a minor portion of functional replicons representing later stages of SV40 replication which we postulated above on the basis of the alkaline sedimentation analyses and which obviously continue to elongate their daughter strands upon reoxygenation. The curve produced by the last pulse which was performed from 35 to 39 min after reoxygenation (Fig. 5E) deviates only little from the broken line at 1.0. This indicates that mainly asynchronous replication again followed the synchronous replication round released by the reoxygenation event. The same conclusion was drawn from the sedimentation profiles shown in Fig. 2E.

3.4. *The phosphorylation state of large T antigen is not significantly altered by transient hypoxia*

Changes of the phosphorylation status of the large T antigen represent a possible means by which initiation of SV 40 replication in vivo is suppressed or activated, respectively [9,10]. Therefore, we isolated T-antigen from hypoxic, reoxygenated and untreated control cells after labeling with $^{32}PO_4$. However, two-dimensional phosphopeptide analyses revealed no significant differences of the phosphorylation pattern in 3 independent experiments. This result diminishes, but does not exclude, the possibility that the O_2 dependendent activation/suppression of the SV 40 replication occurs by changing the phosphorylation status of the large T antigen. It is conceivable that only a minor fraction of total cellular T antigen is competent to initiate replication of SV 40 DNA [10,17], and therefore could have been obscured by a large excess of initiation-incompetent molecules in our experiments.

4. DISCUSSION

The influence of transient controlled hypoxia on replication of mammalian cellular replicons was detected in our laboratory [1]. The phenomenon was analyzed in detail in Ehrlich ascites cells, mainly using DNA fiber autoradiography and ultracentrifuge sedimentation

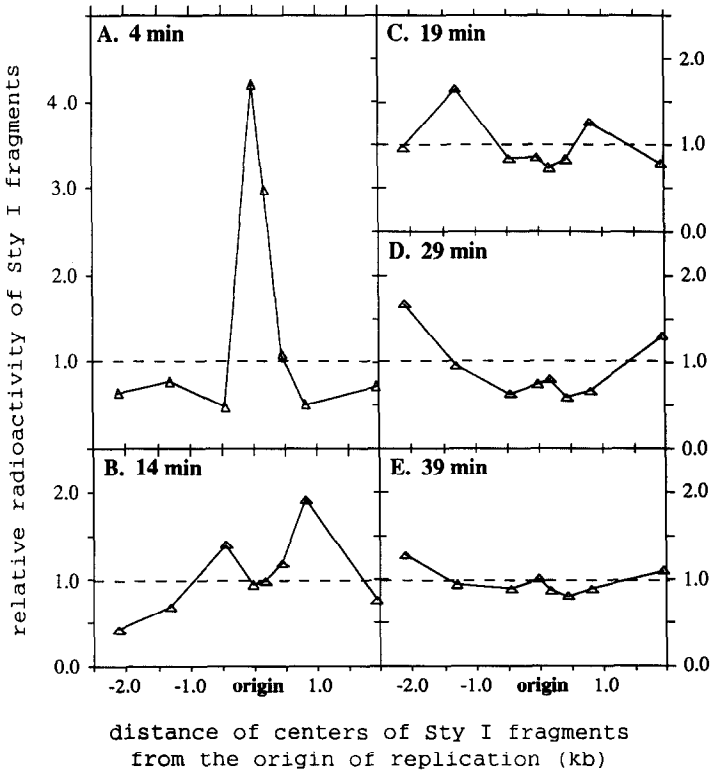

Fig. 5. Relative radioactivity of SV 40 *Sty*I fragments after pulse labeling at different times after reoxygenation (details see legend of Fig. 4). Abscissas indicate distances from the origin of replication according to Fig. 3B. Ordinates indicate radioactivities recovered from excised gel bands (Fig. 4), relative to all 8 bands of the same lane, and then normalized to the corresponding relative radioactivities obtained from the untreated controls (average). The times indicated within the partial figures reflect ends of the [^{3}H]dThd pulses relative to the time of reoxygenation, i.e. the (presumed) time between start of the SV 40 replication released by the reoxygenation event and the stop at the pulse end. Sum of cpm recovered in excised *Sty*I fragments: A, 9,059; B, 18,608; C, 18,095; D, 7,976; E, 5,524. The plots representing the pulses ending at 9, 24 and 34 min were omitted for the sake of clarity. They fit well into the presented series, but provide no additional information.

techniques [2,3,6,7]. Comparable data were obtained by both techniques for HeLa and TC 7 cells and will be published elsewhere. In summary, data collected so far indicate that the O_2 dependent replication control is a widespread phenomenon among mammalian cells. However, distinct qualitative and quantitative differences concerning the responsiveness of replicon initiation and/or the rate of propagation of replication forks in different cell lines were found; e.g. HeLa and TC 7 cells reversibly suppress replicon initiation and greatly retard fork progression under hypoxic conditions. In contrast, the reversible hypoxic response of Ehrlich ascites cells [2,4,6,7] is restricted to a suppression of replicon initiation. A common feature of Ehrlich, HeLa and TC 7 cells is the hypoxic accumulation of considerable amounts of replicons which obviously are completely prepared to initiate immediately after recovery of O_2.

In this report, we have shown that replication of SV 40 DNA in cultured infected cells is also subject to the O_2 dependent replication control. Daughter strand elongation in already active viral replicons (R I molecules [8]) stopped or was greatly retarded, and initiation of DNA synthesis in further viral DNA molecules was prevented at low pO_2. Like cellular replicons, SV 40 replicons also accumulated under hypoxia in a state ready to begin DNA synthesis immediately upon reoxygenation. The hypoxic response of the SV 40 replication thus resembles that of cellular replication of Hela and TC 7 cells (primate cells) rather than that of Ehrlich ascites cells (mouse cells). However, the homogeneity of the SV 40 molecules greatly facilitated to demonstrate that reoxygenation triggers a synchronous start of hypoxically accumulated replicons, followed by a normal replication round. The suitability of transient hypoxia for synchronizing mammalian cellular replication at the level of replicons has been demonstrated by the example of Ehrlich ascites cells [6].

The molecular mechanisms mediating the response of the cellular replication to transient hypoxia are still largely unknown. First attempts to identify a possible cellular O_2 sensor and a related signal transduction system are reported in [18]. However, the relevant alteration(s) in the replication machinery under hypoxia were not identified so far. One possibility to achieve rapid changes in enzymatic activities is reversible phosphorylation. Large T antigen and cellular replication factors (i.e. RF-A) are known to be regulated by phosphorylation [19–23]. In addition, recent data [24] suggested that

an indispensable protein phosphorylation immediately precedes initiation of cellular replicons in cultured mammalian cells. However, our phosphopeptide analyses revealed no significant differences in the phosphorylation patterns of large T antigen under the different O_2 conditions. The possibility remains that changes occur only in that portion of large T antigen that is actively engaged in replication, whereas the major (soluble) fraction is unaffected. Moreover, to our present knowledge, phosphorylation affects mainly activities of large T antigen during the pre-initiation phase of replication [10]. This phase, observed in cell free systems of SV 40 replication [25–28], has a characteristic duration of 10–15 min and comprises assembly of components of the pre-initiation complex (including T antigen) at the origin and unwinding of the origin region. It remains to be demonstrated that initiation in vivo, which is organized on nuclear structures [29], follows the same schedule. The strikingly fast reconstitution of replicon activity upon reoxygenation suggests that hypoxia possibly affects preformed initiation/replication complexes. In this case, it could be speculated that probably not T antigen, but rather a cellular factor involved in later stages of initiation (and elongation) reactions may be a target of protein modifications perhaps mediating the phenomena reported here.

The detection of the responsiveness of the SV 40 replication in vivo to transient hypoxia described in the present communication may provide a helpful model system for further investigation of the O_2 dependent regulation of mammalian DNA replication. In addition, the convenient O_2 dependent switch of replicon initiation, which seems to be operating in cellular replication and the SV 40 model system as well, may be useful in future studies about the still largely obcure mechanisms of mammalian replicon initiation as such.

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