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Abstract

Previous investigation showed that mouse ascites sarcoma cells permeabilized with appropriate concentrations of detergents (Triton X-100, Nonidet P-40 and Brij 58) had high replicative DNA synthesis in the presence of the four deoxyribonucleoside triphosphates, ATP, Mg2+ and proper ionic environment. The present study showed the optimum detergent concentration for DNA synthesis coincided closely with the minimum detergent concentration for inducing cell swelling. Phase contrast microscopy and electron microscopy of Triton-permeabilized cells showed the characteristic swollen cytoplasms and nucleus. Autoradiographic study showed that the DNA synthesis in permeable cells was confined to the nucleus. Cell viability and [3H] deoxythymidine uptake were impaired at much lower concentrations of Triton X-100 than the optimum concentration for in vitro DNA synthesis. In Triton-permeabilized cells, the minimum Triton concentration that produced cell swelling also seemed to produce high repliative DNA synthesis, which reflects the in vivo state of DNA synthesis.

KEYWORDS: cell swelling, DNA synthesis, permeable cells

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SWELLING AND REPLICATIVE DNA SYNTHESIS OF DETERGENT-TREATED MOUSE ASCITES SARCOMA CELLS

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Abstract. Previous investigation showed that mouse ascites sarcoma cells permeabilized with appropriate concentrations of detergents (Triton X-100, Nonidet P-40 and Brij 58) had high replicative DNA synthesis in the presence of the four deoxyribonucleoside triphosphates, ATP, Mg^{2+} and proper ionic environment. The present study showed the optimum detergent concentration for DNA synthesis coincided closely with the minimum detergent concentration for inducing cell swelling. Phase contrast microscopy and electron microscopy of Triton-permeabilized cells showed the characteristic swollen cytoplasm and nucleus. Autoradiographic study showed that the DNA synthesis in permeable cells was confined to the nucleus. Cell viability and [^{3}H] deoxythymidine uptake were impaired at much lower concentrations of Triton X-100 than the optimum concentration for *in vitro* DNA synthesis. In Triton-permeabilized cells, the minimum Triton concentration that produced cell swelling also seemed to produce high replicative DNA synthesis, which reflects the *in vivo* state of DNA synthesis.

Key words: cell swelling, DNA synthesis, permeable cells

Nucleotide-permeable cell systems have been established for studying DNA replication *in vitro* in eukaryotic cells (1–7), as well as in prokaryotic cells (8–11). We have been working with the permeable cell system of eukaryotic cells (2, 5, 6, 12–14), and in the present paper we morphologically characterize detergent-permeabilized mouse ascites sarcoma cells.

MATERIALS AND METHODS

Materials. The reagents used in these experiments were the same as those described in a previous paper (6). Mouse ascites sarcoma (SR-C3H/He) cells (15) were obtained and maintained as reported previously (5).

Preparation of permeable cells. Permeable cells were prepared from SR-C3H/He cells by an almost isotonic detergent procedure (6). Ascites cells were chilled in an ice-water bath and precipitated by centrifugation at 800xg for 5 min. Cells were suspended at $1 \cdot 10^6$–$2 \cdot 10^6$ cells/ml in buffer B (0.25 M sucrose,

0.01 M Tris-HCl, 1 mM EDTA, 4 mM $MgCl_2$ and 6 mM 2-mercaptoethanol, pH 8.0) supplemented with various concentrations of non-ionic detergent (detergent-buffer B). The cells were distributed in test tubes at $1 \cdot 10^6$-$2 \cdot 10^6$ cells each. After centrifugation the permeable cells were suspended in 0.4 ml of the same permeabilizing buffer.

Assay of deoxyribonucleotide incorporation in vitro. DNA replicase activity (12) was measured as described previously (6). Permeable cells suspended in 0.4 ml of permeabilizing buffer were mixed with 0.2 ml of a DNA replicase substrate mixture consisting of 0.1 M Tris-HCl buffer, 7 mM $MgCl_2$, 0.24 M NaCl, 7.5 mM ATP, 0.15 mM dATP, 0.15 mM dCTP, 0.15 mM dGTP and 7.5 μM [^{3}H] dTTP (0.5 Ci/mmol), adjusted to pH 8.0 at 25°C. Incubation was conducted at 37°C for 10 min. [^{3}H] dTTP incorporated into the acid-insoluble fraction was measured by the glass fiber disc method as described previously (5).

Other methods. The detergent effect on cell swelling was measured essentially according to the method of Kraemer and Coffey (16). Autoradiography was conducted as described previously (5), except that the specific activity of [^{3}H] dTTP was increased twenty times (10 Ci/mmol). Electron microscopy was carried out according to the method described previously (5). Cell viability was determined by the trypan blue exclusion test.

RESULTS

Effects of Triton X-100 on cell viability and [^{3}H] deoxythymidine ([^{3}H] dT) and [^{3}H] deoxythymidine triphosphate ([^{3}H] dTTP) incorporation in cellular DNA. As shown in Fig. 1 maximal incorporation of [^{3}H]dTTP was observed in SR-C3H/He cells treated with Triton X-100 at 0.015–0.020%. [^{3}H]dTTP incorporation was very low in cells treated with buffer B or at Triton-concentration lower than 0.005%. Cell viability and [^{3}H]dT-uptake, which were high in non-treated cells, were sharply decreased by increasing the Triton-concentration of permeabilizing buffer, and reached very low levels at 0.01% Triton X-100. The dye exclusion test showed that cells treated with Triton X-100 at 0.005% or higher were permeable to trypan blue whose molecular weight (960.83) is higher than the molecular weights of deoxyribonucleoside triphosphates. The clear difference of Triton X-100 concentrations required for permeability to trypan blue and for obtaining high DNA replicase activity suggests that the high DNA replicase activity obtained by treating SR-C3H/He cells with 0.015–0.020% Triton X-100-buffer B was not simply due to free permeability of nucleotides, but also due to an other unclarified effect of Triton X-100 on DNA synthesis (6). Cells treated with 0.015–0.020% Triton X-100-buffer B were permeable not only to nucleotides but to higher molecules, such as histones, heparin, DNAase I and staphylococcal nuclease (5, 13).

Effect of detergents on cell swelling. SR-C3H/He cells were markedly swollen

by treatment with the permeabilizing buffer containing detergent (Fig. 2). The minimum concentration of the detergent inducing cell swelling corresponded closely with the optimum concentration of the detergent for DNA synthesis, *e.g.*, 0.015–0.020% with Triton X-100, 0.020% with Nonidet P-40 and about 0.0025% with Brij 58 (Fig. 1) (6). Cell swelling was also revealed by phase contrast microscopy and electron microscopy. Cell organelles in SR-C3H/He cells treated with buffer B were rarely visible under the phase contrast microscope, because the cells were still compact and maintained high density cytoplasms (Fig. 3A). By treating cells with 0.015–0.020% Triton X-100-buffer B, the swollen nuclei, mitochondria and nucleoli became clearly visible, because of marked cell swelling and part of the protein being released from cells during treatment (Fig.

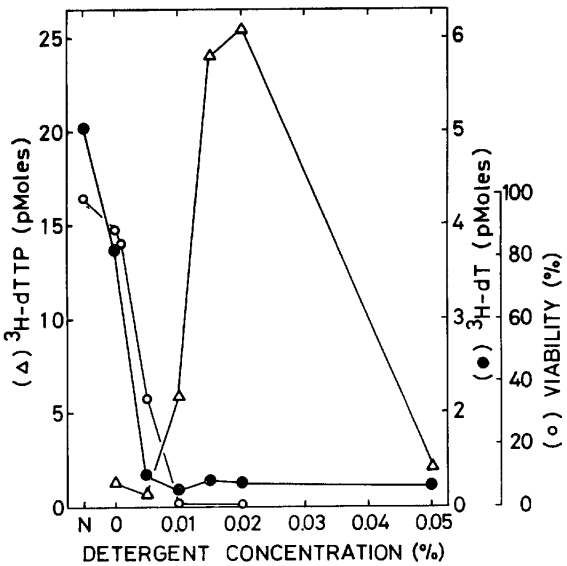


Fig. 1. Effects of various concentrations of Triton X-100 on cell viability and [^{3}H]dT and [^{3}H]dTTP incorporation in cellular DNA. SR-C3H/He cells taken 3 days after intraperitoneal ascites injection (3-day-old ascites) were suspended in buffer B and distributed in test tubes at $1 \cdot 10^6$ cells each. After centrifugation precipitated cells were suspended in 1 ml of buffer B containing Triton X-100 at the concentrations indicated. The Triton-treated cells at $1 \cdot 10^6$ were suspended in 0.6 ml of the same detergent solution containing [^{3}H]dT (2.0 μM, 1 Ci/mmol) for [^{3}H]dT uptake. The suspension was incubated at 37°C for 10 min. Radioactivity in acid-insoluble fraction was measured. [^{3}H]dTTP incorporation (DNA replicase activity) into Triton-treated cells and the viability of Triton-treated cells were measured as described in Methods. N, no treatment; △, pmoles of [^{3}H]dTTP incorporated per 10^6 cells per 10 min; ●, pmoles of [^{3}H]dT incorporated per 10^6 cells per 10 min; ○, per cent viability.

3B) (6). Similar morphological cell changes were observed by treatment with buffer B containing Nonidet P-40 or Brij 58 at the optimum concentrations for DNA synthesis. Fig. 4B shows an electron micrograph of SR-C3H/He cells swollen by treatment with 0.0175% Triton X-100-buffer B. Nuclear swelling was prominent, though the double nuclear membranes were still well preserved. Cells treated with 0.1% Triton X-100-buffer B were shrunk and had low density cytoplasm and nucleus (Fig. 4C). The outer nuclear membrane was largely stripped off. Chromatin-like electron dense materials appeared in the cytoplasm close to the nuclear membrane, suggesting the partial release of nuclear chromatin from the nucleus. The partial release of protein and RNA but not DNA from Triton-treated cells was reported in a previous paper (6). These results suggested that the absorbance decrease in cell suspension treated with Triton X-100 at the high concentration (as 0.1%) was not due to cell swelling but due to solubilization of a large part of the cellular constituents.

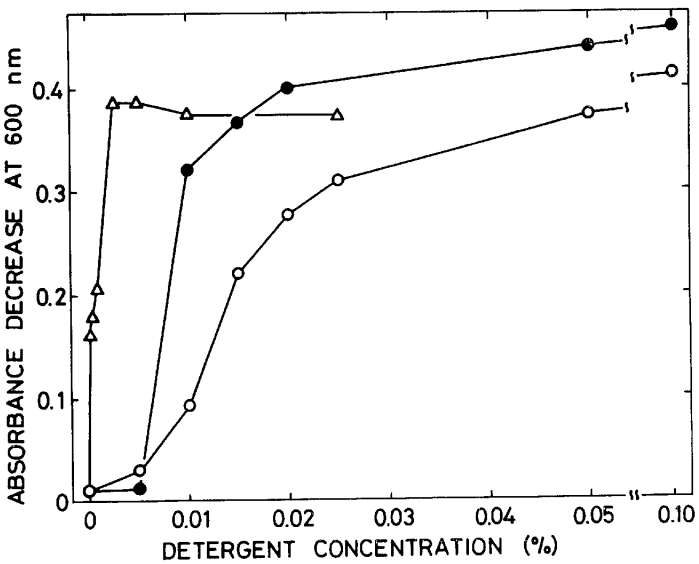


Fig. 2. Effect of different detergents on cell swelling. SR-C3H/He cells were collected from 6-day-old ascites, washed once with buffer B, and then treated with buffer B containing the detergent at the concentration indicated. The detergent treated cells (3.8×10^6 cells/tube) were suspended in 2 ml of detergent buffer B and 1 ml of the replicase substrate mixture with no radioisotope. Absorbance of the suspension was measured at 600 nm after 10 min incubation at 37°C. SR-C3H/He cells (3.8×10^6 cells/tube) suspended in 2 ml of buffer B and 1 ml of substrate mixture and kept at 0°C were used as a standard. The absorbance decrease from the standard was calculated. △, Brij 58; ●, Triton X-100; ○, Nonidet P-40.

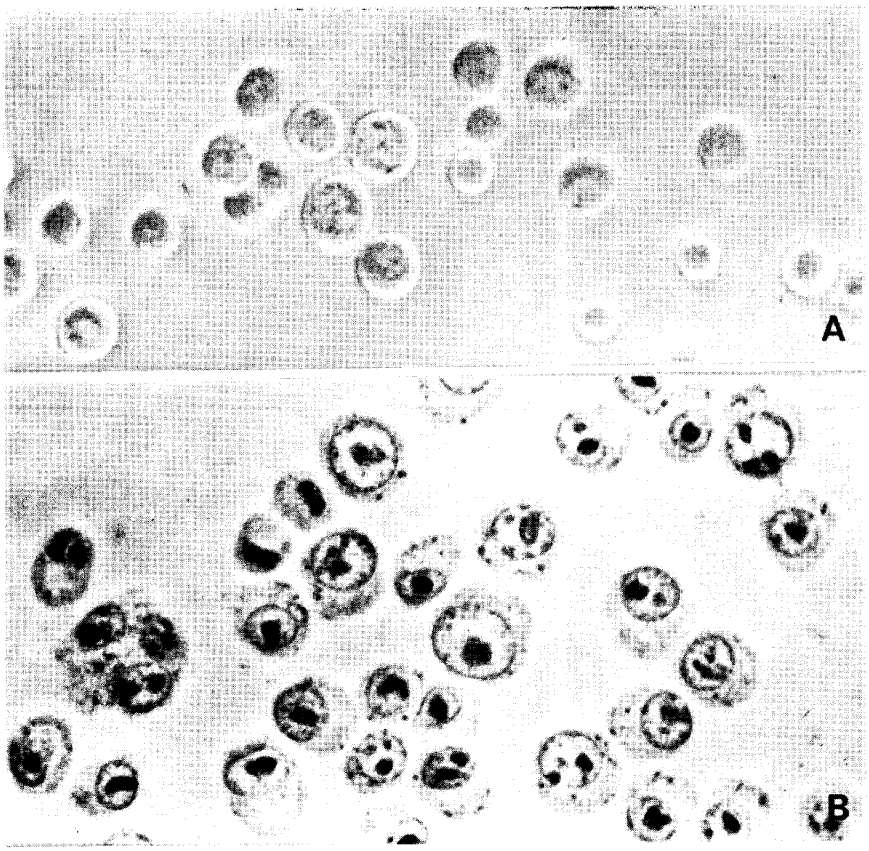
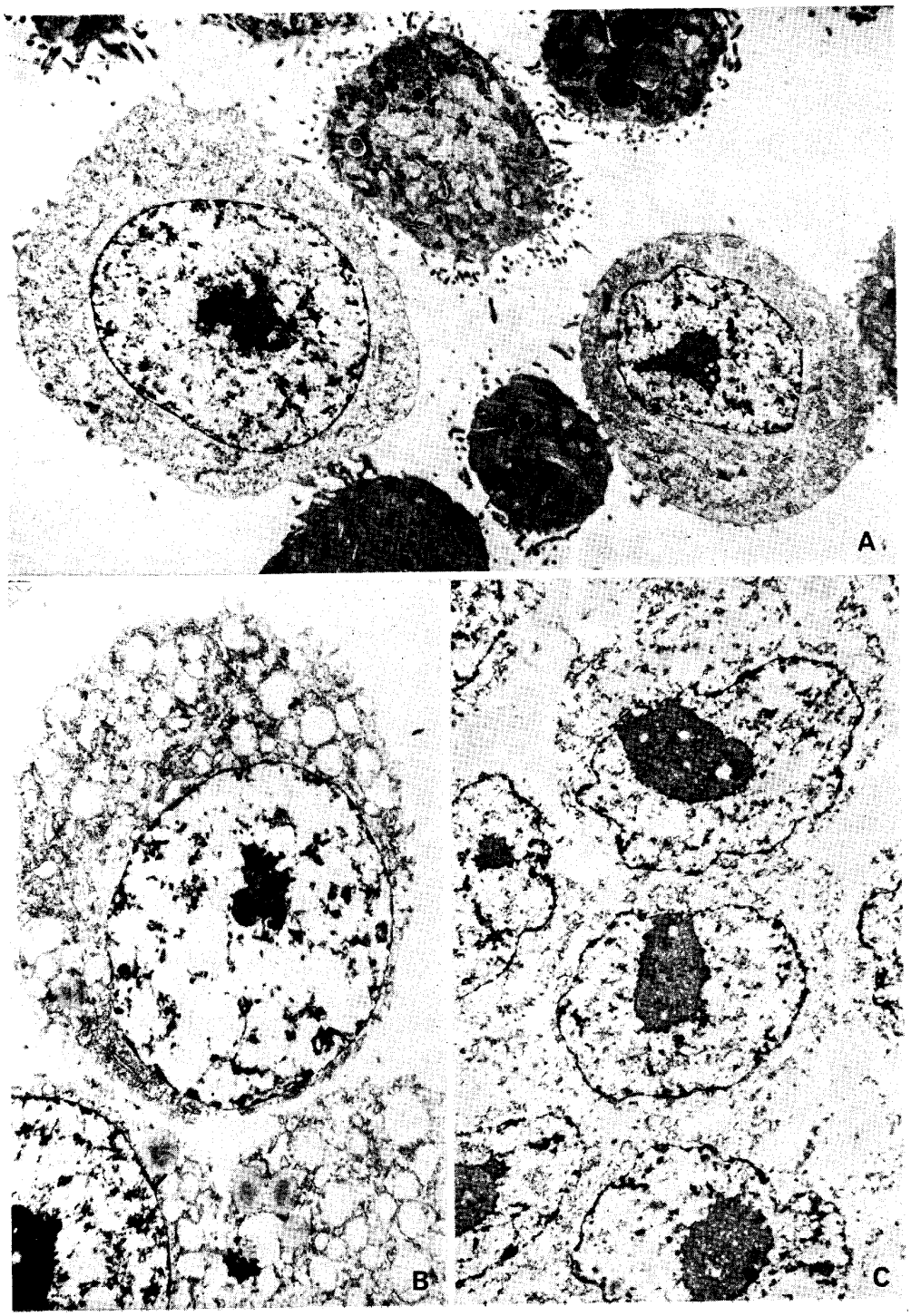


Fig. 3. Interference phase contrast micrographs. A, SR-C3H/He cells treated with buffer B (control); B, SR-C3H/He cells treated with 0.0175% Triton X-100-buffer B. ×1,400.

Site and character of DNA synthesis. DNA synthesis in permeable cells depended highly on ATP, suggesting the replicative nature of DNA synthesis. In permeable cells incubated with the ATP-free assay mixture, the permeable cells were labeled lightly with [^{3}H]dTTP, indicating that almost no DNA synthesis occurred without ATP (Fig. 5A). ATP-dependent, replicative DNA synthesis occurred in nuclei of some permeable cells (Fig. 5B). The labeling index with [^{3}H]dTTP of about 40% in logarithmically growing cells was almost equal to the labeling index with [^{3}H]dT of intact cells.


A
B
C

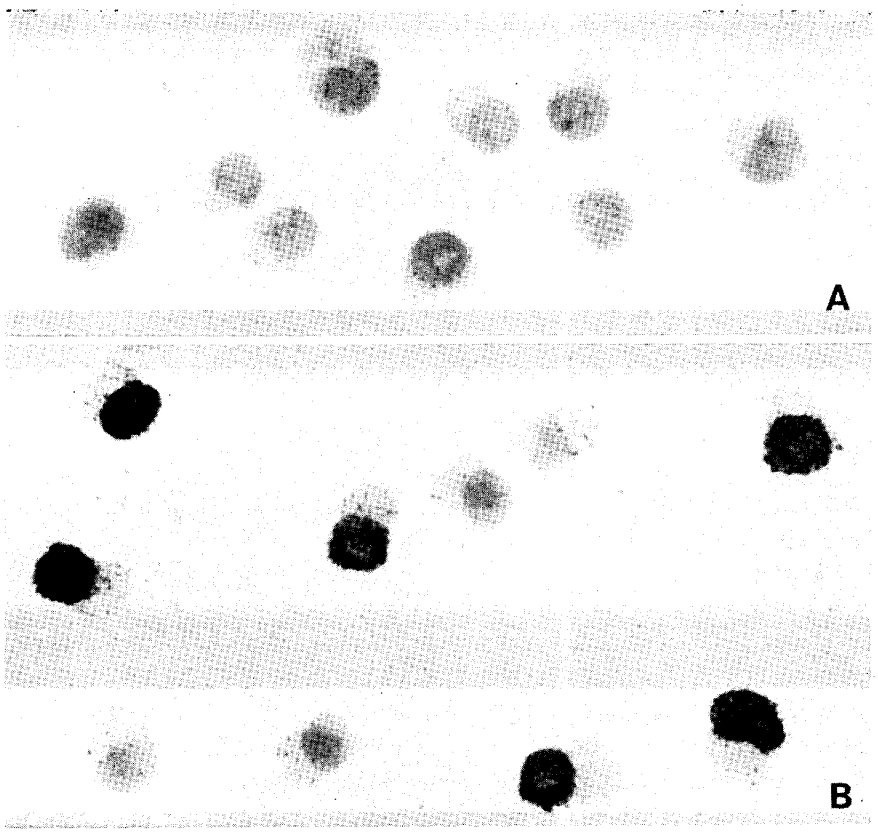


Fig. 5. Autoradiograms of [^{3}H]dTTP incorporation into Triton-permeabilized SR-C3H/He cells. Permeable cells were incubated with DNA replicase substrate mixture ([^{3}H]dTTP, 10 Ci/mmol) with ATP or without ATP. The cells were smeared on microscope slides and fixed with methanol. After three extractions with 2% perchloric acid, the specimens were dipped in photographic emulsion. After exposure for 2 months in a light-proof box at 4°C, the autoradiographs were developed and fixed. The specimens were stained with Giemsa. ×1,400. A, without ATP; B, with ATP.

DISCUSSION

In vitro replicative DNA synthesis of mammalian cells has been confirmed to date in only isolated nuclei and permeable cells. Reconstitution of replicative

Fig. 4. Electron micrographs of Triton-treated cells. SR-C3H/He cells were washed once with buffer B, and then treated twice with buffer B, 0.0175% Triton X-100-buffer B or 0.1% Triton X-100-buffer B. After treatment the cells were fixed in 0.1 M cacodylate-buffered 2.5% glutaraldehyde (pH 7.2), and then fixed with 0.1 M cacodylate-buffered 1% OsO_4. The thin sectioned specimens were stained with uranyl acetate and lead citrate. ×4,170. A, cells treated with buffer B (control); B, permeable cells prepared by treatment with 0.0175% Triton X-100-buffer B; C, cells treated with 0.1% Triton X-100-buffer B.

DNA synthesis from subnuclear components is still very difficult (17). In test so far, the DNA replicase activity of isolated nuclei was lower than that of permeable cells. These results indicate that the replicating machinery is very labile and is easily destroyed by preparative procedures. We do not know at present whether the replicating machinery is dependent on morphological structure or whether structural damage indirectly affects the replicating machinery by extracting some essential component(s) from the machinery. In considering these possibilities, it might be worthwhile to study the morphology of permeable cells which show high replicating activity.

In the present communication we studied the correlation between morphology and DNA synthesis of Triton-treated cells. In addition to free permeability of low molecular weight substances, cell swelling seemed to be a prerequisite for high replicative DNA synthesis in Triton-permeabilized cells. Seki *et al.* (2, 5) showed that hypotonic permeable cells were swollen. Berger and Johnson (3) reported that their permeable L cells swelled during incubation for DNA synthesis. Billen and Olson (4) reported that their permeable cells were viable and that the scanning electron micrographs showed no gross morphological differences between Tween 80-permeabilized Chinese hamster ovary cells and control cells. In their report DNA synthesis of permeable cells showed about a 10 min lag period before rapid DNA synthesis, and the viability test and morphological examination were conducted before and probably not after incubation. Therefore, it is not clear whether the rapidly DNA synthesizing permeable cells were still viable or had the same morphology as intact cells. Further studies are required to determine what kinds of changes in permeable cells are essential for high replicative DNA synthesis.

This and previous studies (6, 13) on Triton-permeabilized SR-C3H/He cells showed that almost no unscheduled DNA synthesis was present in cells and that the permeable cell system was useful for studying replicative DNA synthesis.

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REFERENCES

1. Burgoyne, L. A.: Deoxyribonucleic acid synthesis in mammalian systems. Permealyzed Ehrlich ascites cells *in vitro* first label Okazaki-type low-molecular-weight deoxyribonucleic acid. *Biochem. J.* **130**, 959-964, 1972.
2. Seki, S., LeMahieu, M. and Mueller, G. C.: A permeable cell system for studying DNA replication in synchronized HeLa cells. *Biochim. Biophys. Acta* **378**, 333-343, 1975.
3. Berger, N. A. and Johnson, E. S.: DNA synthesis in permeabilized mouse L cells. *Biochim. Biophys. Acta* **425**, 1-17, 1976.
4. Billen, D. and Olson, A. C.: DNA replication in Chinese hamster ovary cells made permeable to nucleotides by Tween-80 treatment. *J. Cell Biol.* **69**, 732-736, 1976.

5. Seki, S. and Oda, T.: DNA synthesis in permeable mouse ascites sarcoma cells. *Cancer Res.* **37**, 137-144, 1977.
6. Seki, S. and Oda, T.: DNA synthesis in detergent-treated mouse ascites sarcoma cells. *Biochim. Biophys. Acta* **476**, 24-31, 1977.
7. Umeda, T. and Koga, M.: Synthesis of deoxyribonucleic acid in liver cells made permeable to nucleotides. *Biochim. Biophys. Acta* **478**, 115-127, 1977.
8. Mordoh, J., Hirota, Y. and Jacob, F.: On the process of cellular division in *Escherichia coli*. V. Incorporation of deoxynucleoside triphosphates by DNA thermosensitive mutants of *Escherichia coli* also lacking DNA polymerase activity. *Proc. Natl. Acad. Sci. U. S. A.* **67**, 773-778, 1970.
9. Moses, R. E. and Richardson, C. C.: Replication and repair of DNA in cells of *Escherichia coli* treated with toluene. *Proc. Natl. Acad. Sci. U. S. A.* **67**, 674-681, 1970.
10. Kohiyama, M. and Kolber, A. R.: Temperature sensitive mutant of the DNA replication system in *Escherichia coli*. *Nature* **228**, 1157-1160, 1970.
11. Vosberg, H. and Hoffmann-Berling, H.: DNA synthesis in nucleotide-permeable *Escherichia coli* cells. I. Preparation and properties of ether-treated cells. *J. Mol. Biol.* **58**, 739-753, 1971.
12. Seki, S. and Mueller, G. C.: A requirement for RNA, protein and DNA synthesis in the establishment of DNA replicase activity in synchronized HeLa cells. *Biochim. Biophys. Acta* **378**, 354-362, 1975.
13. Seki, S. and Oda, T.: Differential effects of heparin on replicative DNA synthesis and unscheduled DNA synthesis. *Biochim. Biophys. Acta* **479**, 391-399, 1977.
14. Seki, S., Tsutsui, K. and Oda, T.: Effect of aurintricarboxylic acid on DNA synthesis of permeable mouse ascites sarcoma cells. *Biochem. Biophys. Res. Commun.* **79**, 179-185, 1977.
15. Yamamoto, T. and Takeuchi, M.: Studies on Rous sarcoma virus in mice. I. Establishment of an ascites sarcoma induced by Schmidt-Ruppin strain of Rous sarcoma virus in C3H/He mouse. *Jpn. J. Exp. Med.* **37**, 37-50, 1967.
16. Kraemer, R. J. and Coffey, D. S.: The interaction of natural and synthetic polyanions with mammalian nuclei. II. Nuclear swelling. *Biochim. Biophys. Acta* **224**, 568-578, 1970.
17. Seki, S. and Mueller, G. C.: Dissociation and reconstitution of the DNA replicase system of HeLa cell nuclei. *Biochim. Biophys. Acta* **435**, 236-250, 1976.
18. Kaufman, D. G., Grisham, J. W. and Stenstrom, M. L.: Unscheduled incorporation of [^{3}H]TTP into DNA of isolated rat liver nuclei. *Biochim. Biophys. Acta* **272**, 212-219, 1972.