Temperature-Dependent Alterations in Sugar Transport in Cells Infected by a Temperature-Sensitive Mutant of Rous Sarcoma Virus

(2-deoxyglucose uptake/transformation/cytosine arabinoside)

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ABSTRACT Cells transformed by Rous sarcoma virus take up 2-deoxyglucose at a faster rate than uninfected cells, under conditions where transformed and nontransformed cells grow at the same rate. In cells infected by a temperature-sensitive mutant, the stimulation of 2-deoxyglucose uptake is temperature dependent: the increase is observed at the permissive (36°C), but not at the nonpermissive (41.5°) temperature. When infected cells are shifted from the nonpermissive temperature to the permissive temperature, the uptake of 2-deoxyglucose increases from a rate equal to that of uninfected cells to a rate equal to that of cells infected by the wild-type Schmidt-Ruppin Rous sarcoma virus. The reverse change occurs when the infected cells are shifted from the permissive to the nonpermissive temperature. By the use of cytosine arabinoside, an inhibitor of DNA synthesis, it was possible to show that DNA synthesis is neither required for the transformation, which occurs when the infected cells are shifted from the nonpermissive to the permissive temperature, nor for the phenotypic reversion, which occurs in the reverse shift.

Chicken-embryo fibroblasts transformed by Rous sarcoma virus (RSV) show characteristic morphological alterations and are able to form colonies when suspended in agar (1, 2). Temperature-sensitive mutants of the Schmidt-Ruppin strain of Rous sarcoma virus (3, 4) that are able to grow at the same rate as the wild type at the nonpermissive temperature, but are unable to transform the infected cells, have been isolated. Cells infected by these mutants at the permissive temperature become transformed, but when shifted to the nonpermissive temperature revert to a normal morphology and lose the ability to grow in agar suspension. These results indicate that the continued expression of a viral function is required to maintain these properties.

It has recently been shown (5) that cells transformed by RSV take up glucose and glucose analogs, such as 2-deoxyglucose, at a faster rate than uninfected cells. It was suggested, therefore, that this difference could be used as a measure of transformation. However, studies in this laboratory (ref. 6;

Sefton and Rubin, manuscript in preparation) have shown that the rate of uptake of 2-deoxyglucose by normal, uninfected cells depends upon their physiological state. Sparse, rapidly-growing cells take up 2-deoxyglucose at a faster rate than dense, slowly growing cells (6); furthermore, the uptake by density-inhibited monolayers is stimulated by the addition of serum or trypsin to the medium (Sefton and Rubin, manuscript in preparation). The present experiments make clear, however, that even under conditions where transformed and normal cells grow at the same rate, the transformed cells take up 2-deoxyglucose at a faster rate than normal cells. Thus, under these conditions, the rate of uptake can be used as a measure of transformation. We have used this property of transformed cells to study the effect of temperature shifts on cells infected by the temperature-sensitive mutant, T5 (4) of the Schmidt–Ruppin strain of RSV, subgroup A (SR-RSV-A).

MATERIALS AND METHODS

Virus infection

The methods used for the preparation of chicken embryo fibroblasts and propagation of virus stocks have been described (3, 7). Freshly cloned virus was used, in order to minimize the number of temperature-resistant revertants and of nontransforming variants (Martin and Duesberg, manuscript in preparation) in the virus stocks. Cells for infection were plated at 3×10^6 per 100-mm plastic dish in medium A (medium 199, supplemented with 2% tryptose phosphate broth, 1% calf serum, and 1% chicken serum). 4 hr later, the medium was removed and the cells were infected with 1 ml of virus (titer 5×10^5 to 1×10^6 focus-forming units/ml). After 1 hr, medium B (medium 199, supplemented with 10% tryptose phosphate broth, 4% calf serum, and 1% chicken serum) was added. The cells were grown for 4 days at 41°C, with daily medium change, then treated with trypsin and plated at 1.2 to 1.5×10^6 cells per 35-mm dish in 3 ml of medium B supplemented with 0.1% glucose. Plates were incubated at 36 or 41.5°C as indicated, and the medium was changed every 12 or 18 hr. Experiments were started 24 hr after plating.

Measurement of 2-deoxyglucose uptake

Cells were washed twice with warm, glucose-free Hanks solution. They were then incubated for 10 min at 39°C with the same solution, containing 0.25 μCi/ml of 2-deoxy-[³H]glucose (New England Nuclear Corp.; specific activity
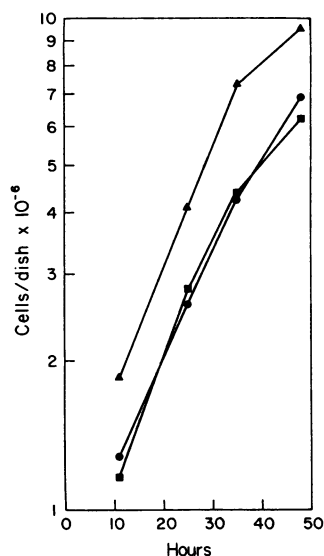
FIG. 1. Growth of SR-RSV-A-infected, T5-infected, and uninfected cells at 41.5°C. Cells were removed from the dish with trypsin and counted in a Coulter counter. ●———● SR-RSV-A-infected; ▲———▲ T5-infected; ■———■ uninfected.

7.2 Ci/mmol). The cells were then washed four times with ice-cold Hanks solution, drained, dried, and taken up directly into 2 ml of Lowry's C-reagent (8). Part of the sample was used for protein determination by the method of Lowry *et al.* (8), while 1 ml was neutralized with 0.15 ml of 100% trichloroacetic acid mixed with 12 ml of a toluene–triton scintillation mixture, and counted in a liquid scintillation counter.

Measurement of DNA synthesis

1. Fraction of Cells Synthesizing DNA. Cells were labeled for 1 hr with [³H]thymidine (New England Nuclear Corp.; 5 μCi was added to each plate). Cells in dense cultures overlap considerably, particularly when transformed; they were therefore dispersed before processing for autoradiography as follows. The cells were washed, treated with trypsin, and pelleted by low-speed centrifugation. They were then resuspended in Tris–saline and an equal volume of methanol-acetic acid 3:1 was added. After 10 min, the cells were again pelleted, washed in methanol–acetic acid, repelleted, and resuspended in methanol–acetic acid, at a concentration of 5 × 10⁵/ml. A drop was then dried onto a clean glass slide and processed for autoradiography as described in ref. 9.

2. Rate of [³H]Thymidine Incorporation into DNA. Cells were labeled for 1 hr with [³H]thymidine (1.5 μCi/3 ml of medium). The cells were then washed three times with ice-cold 5% Cl₃CCOOH and once with cold Hanks solution. They were then taken up in Lowry's C-reagent and the incorporated radioactivity was determined as described above.

RESULTS

Cells were infected with SR-RSV-A (wild-type) or T5, the infected and uninfected cells were grown for 4 days at 41°C, treated with trypsin, and plated in medium containing 5% serum (medium B) at 41.5°C. The medium was changed

every 12 hr. Under these conditions, normal and transformed cells grew at the same rate for at least 36 hr (Fig. 1). 24 hr after plating, the rate of uptake of 2-deoxyglucose and the fraction of cells synthesizing DNA were determined (Table 1). The rate of uptake of 2-deoxyglucose was 3-fold higher in SR-RSV-A-infected than in uninfected cells, while the fraction of cells synthesizing DNA was about the same. Thus, the difference in 2-deoxyglucose uptake observed between transformed and normal cells is not a result of differences in growth rates. Cells infected with the temperature-sensitive strain T5 at 41.5°C took up 2-deoxyglucose at the same rate as uninfected cells, indicating that the mutation also affects the ability of the virus to induce this change in 2-deoxyglucose uptake.

The effects of temperature shifts on T5-infected cells were investigated in the following way. SR-RSV-A-infected, T5-infected, and uninfected cells were grown at 41°C, and then transferred and incubated at 36 or 41.5°C. The next day, the T5-infected cells that were incubated at 41.5°C appeared normal, whereas those incubated at 36°C appeared morphologically transformed. 24 hr after plating, some plates from each set were shifted from one temperature to the other, and the rate of 2-deoxyglucose uptake was determined at intervals. The results of this experiment are shown in Fig. 2. The rate of uptake into uninfected or SR-RSV-A-infected cells was not significantly affected by the temperature shifts. T5-infected cells at 36°C took up 2-deoxyglucose at the same rate as SR-RSV-A-infected cells; when they were shifted to 41.5°C, the rate of uptake dropped (without any apparent lag) and reached a level equal to that of uninfected cells by 12–24 hr. Similarly, when T5-infected cells incubated at 41.5°C were shifted to 36°C, the rate of uptake rose to the same level as that of SR-RSV-A-infected cells within 12–24 hr. Thus, the T5 function is required not only to induce, but also to maintain, the enhanced rate of 2-deoxyglucose uptake in transformed cells.

Similar temperature shifts were also performed in the presence of 0.3 mM cytosine arabinoside to determine if DNA synthesis is necessary for these alterations to occur. This concentration of cytosine arabinoside was found to inhibit [³H]thymidine incorporation into material insoluble in cold Cl₃CCOOH by 95% within 15 min. The alterations in the rate of 2-deoxyglucose uptake in T5-infected cells were again observed (Fig. 3) when the cells were shifted from 41.5 to 36°C or from 36 to 41.5°C. Thus, DNA synthesis is not required for these changes to occur.

DISCUSSION

The results reported here show that transformed cells take up 2-deoxyglucose more rapidly than normal cells growing at the same rate, and confirm that under appropriate condi-

TABLE 1. *Effect of infection by SR-RSV-A or T5 at 41.5°C on the rate of uptake of 2-deoxyglucose*

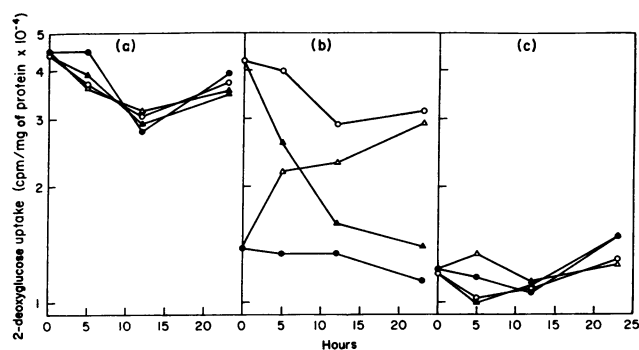|	Uninfected cells	SR-RSV-A-infected cells	T5-infected cells
2-Deoxyglucose uptake (cpm/mg of protein)	12,200	44,500	13,800
Fraction of cells synthesizing DNA	102/304 (34%)	107/341 (31%)	109/413 (26%)

FIG. 2. Effect of temperature shifts on 2-deoxyglucose uptake by (*a*) SR-RSV-A-infected (*b*) T5-infected and (*c*) uninfected cells. O——O cultures maintained at 36°C; ▲——▲ cultures shifted from 36 to 41.5°C; ●——● cultures maintained at 41.5°C; △——△ cultures shifted from 41.5 to 36°C.
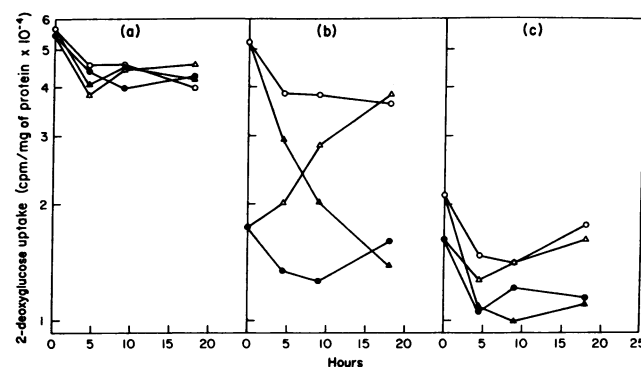

FIG. 3. Effect of temperature shifts on 2-deoxyglucose uptake by cells incubated with 0.3 mM cytosine arabinoside. The cytosine arabinoside was added 30 min before the temperature shifts. Symbols are the same as those of Fig. 2.

tions, this rate of uptake can be used as a measure of transformation. It may be that this alteration results from virus-specific membrane changes, as suggested by Hatanaka and Hanafusa (5). However, as mentioned above, the rate of uptake of 2-deoxyglucose is higher in sparse, rapidly growing cells than in dense, slowly growing cells, and the addition of serum or trypsin to density-inhibited monolayers results in a rapid stimulation of uptake (ref. 6; Sefton and Rubin, manuscript in preparation). Thus, the mechanisms involved in controlling the rate of uptake of 2-deoxyglucose in normal cells may also be involved in the stimulation of uptake resulting from transformation by RSV.

The morphological alterations shown by the cells infected by T5 virus at the permissive temperature are usually less apparent than those in cells infected by the wild type, although the rate of uptake of 2-deoxyglucose is the same. Thus, it would appear that, at least under the conditions used in these experiments, the extent of transformation required to produce alterations in 2-deoxyglucose uptake is less than that required to produce visible morphological alterations. The behavior of T5-infected cells due to temperature shifts is consistent with this idea. When shifted from 41.5 to 36°C, morphological alterations were only apparent by 12–18 hr, while a stimulation of uptake was observed by 6 hr after the shift. Furthermore, when T5-infected cells were shifted from 36 to 41.5°C, they appeared completely normal after 4 hr, whereas the rate of uptake reached the level characteristic of uninfected cells only 12–18 hr after the temperature-shift. These observations may be related to those of Goldé and Villaudy (10) and of Weiss (11), who have shown that under certain conditions, release from density-dependent inhibition of growth after infection by RSV can occur without any apparent morphological transformation.

If host-cell DNA synthesis is inhibited in cells freshly infected by RSV, the growth of the virus is inhibited (12, 13), and transformation does not occur. The experiments described here, however, show that DNA synthesis is not required for the initiation of transformation in cells in which infection is established. Thus, the dependence of transformation on

cellular DNA synthesis after infection is probably due to a requirement for DNA synthesis for the early stages of viral replication. It has been suggested (12, 14) that the cell must pass through mitosis for transformation to occur. However, the results described above indicate that the extent of the changes in 2-deoxyglucose uptake is not significantly affected by the inhibition of DNA synthesis. Since inhibition of DNA synthesis would be expected to prevent mitosis in the majority of the cells, these results argue against the idea that these changes are dependent upon mitosis.

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