ULTRASTRUCTURAL LOCALIZATION OF CONCANAVALIN A RECEPTORS IN SOMATIC CELL HYBRIDS BETWEEN NORMAL MOUSE CELLS AND SIMIAN VIRUS 40-TRANSFORMED RAT CELLS

C. WALIG, J. M. M. WALBOOMERS, and J. VAN DER NOORDAA. From the Laboratorium voor de Gezondheidsleer, Universiteit van Amsterdam, Mauritskade 57, Amsterdam-O

INTRODUCTION

Genetically unstable somatic cell hybrids between normal and Simian Virus 40 (SV40)-transformed cells may be useful in studying the way of persistence of the viral genome in the host cell. Such hybrids between normal mouse and SV40-transformed human cells are described by Weiss et al. (1967, 1970). They showed a positive correlation between the loss of human chromosomes and the disappearance of the SV-40-specific

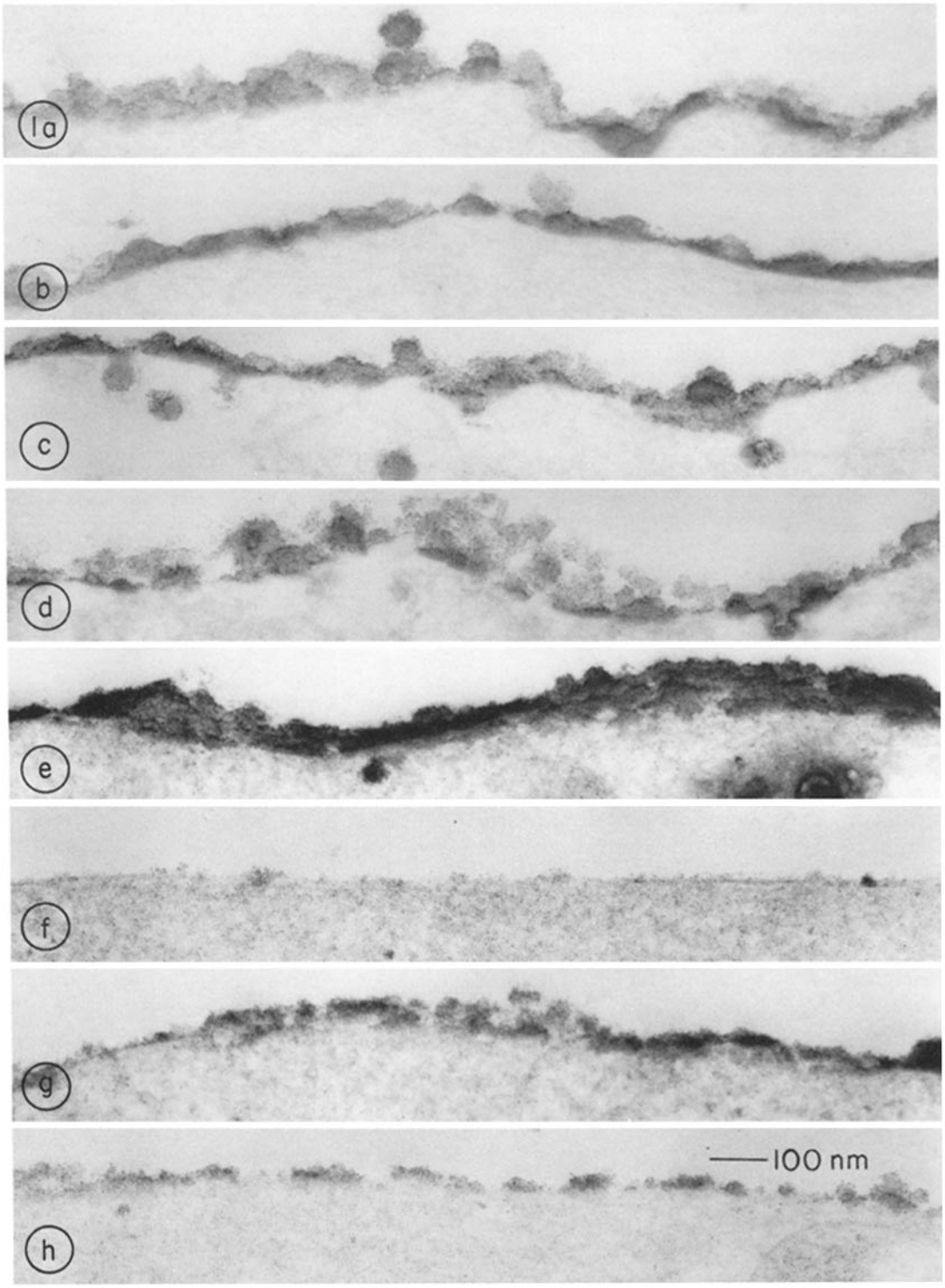

FIGURE 1. Concanavalin A-peroxidase reaction on cells from normal secondary rat brain cultures (a–d) and normal 3T3 TK-mouse cells (e–h). × 81,000.

tumor antigen (T antigen), suggesting integration of the viral genome into the chromosomes of the transformed cell. Van der Noordaa et al. (1972) have described the properties of somatic cell hybrids between SV40-transformed rat cells and normal mouse cells. In these hybrids the T antigen and the growth characteristics of the transformed parent were present. It was shown that the hybrid cells were agglutinated by concanavalin A and that the agglutination was enhanced after trypsin treatment. Thus it was assumed that in the hybrid cells both types of cell coat architecture could be detected at one cell surface. Bernhard and Avrameas (1971) have described a technique for the visualization of concanavalin binding sites on the cell membrane. By employing this technique, we have tried to localize concanavalin A receptor sites on the surface of hybrid cells and of the parent cells.

MATERIALS AND METHODS

Cells

The cells used in the experiments were normal cells from secondary cultures of brains of newborn BN/BI rats, normal mouse cells (3T3 TK-), a SV40-transformed line of rat brain cells referred to as rat-SV40 cells (at passage 20), and a hybrid cell line H3 (at passage 158). These lines and their culture conditions have been described before (van der Noordaa et al., 1972).

Methods

The method of Bernhard and Avrameas (1971) for the detection of concanavalin A sites was employed. The cells were seeded on glass slips covered with a thin layer of coal and placed in 60-mm Petri dishes (Falcon). At confluency, the cultures were washed twice with calcium- and magnesium-free phosphate-buffered saline (PBS) and incubated for 15 min with a solution of concanavalin A (50 μg/ml in PBS; Serva, Heidelberg). After washing gently, 50 μg/ml peroxidase (Boehringer and Soehne, Mannheim, Germany) in PBS was added during 15 min. The cells were washed gently again and fixed for 15 min with 1.6% glutaraldehyde in PBS. All cultures were rinsed with PBS and treated for 5 min with 3,3′-diaminobenzidine (0.5 mg/ml) in PBS containing 0.01% H_2O_2. After washing with PBS, the cultures were postfixed for 1 h with 1% osmium tetroxide in 0.1 M cacodylate buffer. Subsequently, cells were dehydrated and embedded in Epon. After polymerization of the Epon, the layer of resin containing the cultures was detached from the glass slips. Small blocks were cut and dissected perpendicular to the surface. In control experiments no concanavalin A was added: there, no precipitate was observed at the cell surface. A Philips EM300 was used for the electron microscopy. From each cell line, 15–25 cells were examined.

RESULTS

Four photoprints of each cell line are shown to reflect the most dominant aspects of their reactive surface layer.

The normal mouse 3T3 TK-cells and rat cells showed differences in staining. The reaction products on the surface of all normal rat cells formed a smooth, thick, cloudy, and rather continuous layer of 20–120 nm (Fig. 1 *a–d*). The staining pattern of the surface of normal mouse cells was more irregular and also showed variations in thickness from 20 to 100 nm (Fig. 1 *e–h*). Isolated deposits were found on both normal rat and mouse cells. The surface layer of the transformed rat cells (Fig. 2 *a–d*) differed markedly from that of the normal rat cells. The thickness of the reactive layer never exceeded 20 nm and areas with a patchy distribution were noted. It was impossible to distinguish the rather continuous layers of the transformed rat cells and the normal mouse cells, and on both cell types isolated dots were found. The staining pattern of the hybrid cells (Fig. 2 *e–h*) showed large areas with rather weak staining, while some heavy isolated dots of diaminobenzidine precipitates were found.

DISCUSSION

The cell coat reaction of the two parental cell types of our hybrids was rather similar. The rat-SV40 as well as the normal mouse cells showed a patchy distribution of the reaction product. We were not able to distinguish between the two lines. This is in agreement with recent findings of Torpier and Montagnier (1973). In their study also, a general transformation characteristic of of the surface of transformed cells could not be detected by the concanavalin A-peroxidase method. However, some areas of the surface of the normal mouse cells contained a precipitate with a thickness of 120 nm, in contrast to the reactive layer on the cell surface of the rat-SV 40 cells, which did not exceed 20 nm. On the normal rat cells we observed a rather continuous layer of electron-dense material of variable thickness. We were not able to identify a coat that was intermediate between

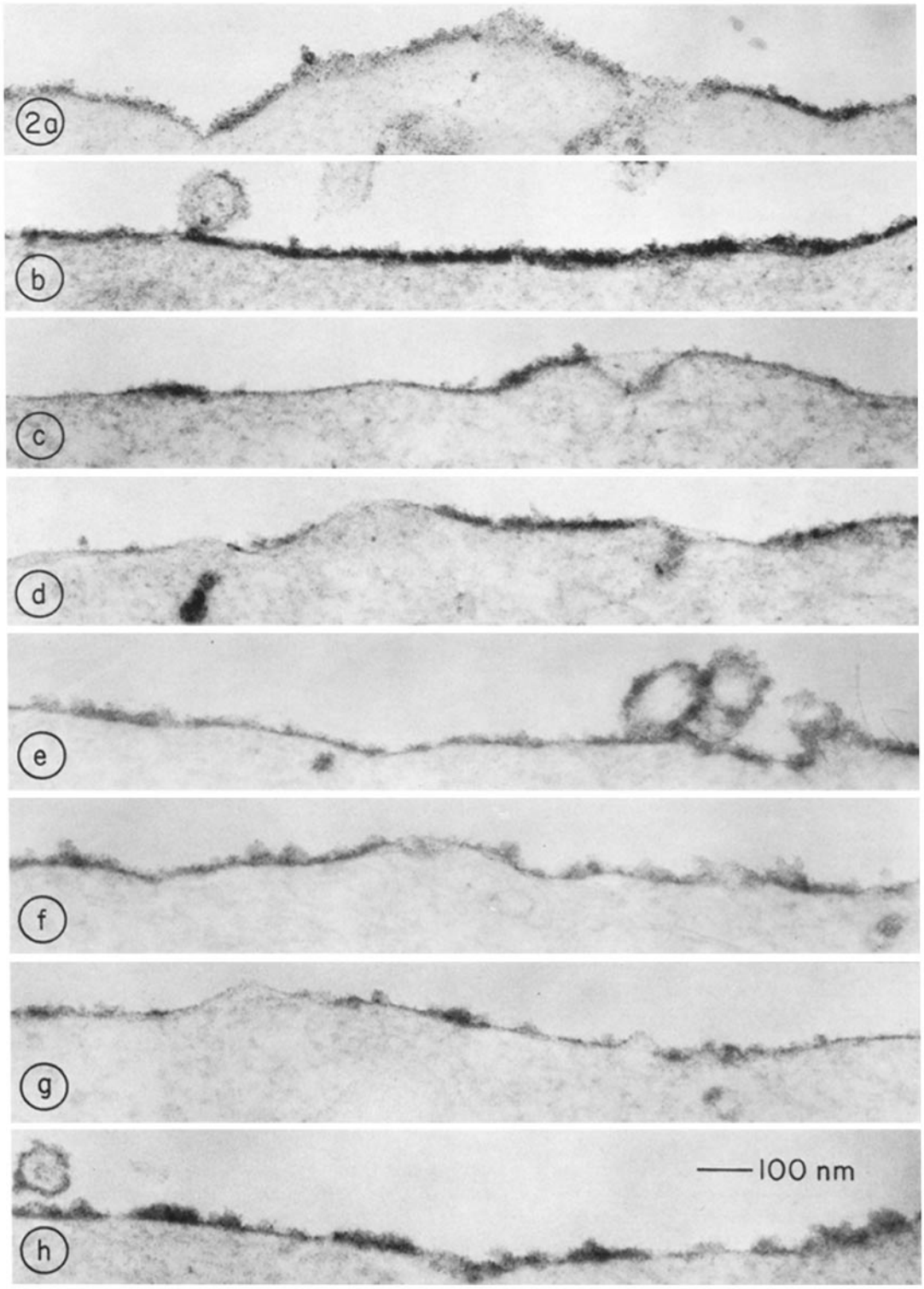

FIGURE 2 Cell surface reaction of SV40-transformed rat cells (a–d) and somatic cell hybrids between normal mouse cells and SV40-transformed rat cells (e–h). × 80,500.

those of the two parental cells nor quite specific for one of the parents. Thus, in our study of hybrid cells the application of the concanavalin A-peroxidase method does not seem suitable to recognize specific architectural aspects of each of the parental cells, and has not enabled us to study the expression and eventual disappearance of viral genes in our rat-SV40/mouse hybrids.

SUMMARY

The concanavalin A-peroxidase method was applied to the study of the ultrastructural distribution and presence of concanavalin A receptor sites on the surface of somatic cell hybrids between normal mouse cells and SV40-transformed rat cells. Since it appeared impossible to distinguish the staining pattern of both parental cells, no specific pattern for the hybrid cells could be identified.

Received for publication 6 November 1973.

REFERENCES

BERNHARD, W., and S. AVRAMEAS. 1971. Ultrastructural visualization of cellular carbohydrate components by means of concanavalin A. *Exp. Cell. Res.* **64**:232–236.

VAN DER NOORDAA, J., A. VAN HAAGEN, J. H. H. WALBOOMERS, and H. VAN SOMEREN. 1972. Properties of somatic cell hybrids between mouse cells and simian virus 40-transformed rat cells. *J. Virol.* **10**:67–72.

TORPIER, G., and L. MONTAGNIER. 1973. Etude ultrastructurale de la distribution des récepteurs pour la concanavaline A à la surface des cellules normales et transformées par des virus oncogènes. *Int. J. Cancer.* **11**:604–615.

WEISS, M. C. 1970. Further studies on loss of T-antigen from somatic hybrids between mouse cells and SV40-transformed human cells. *Proc. Natl. Acad. Sci. U. S. A.* **66**:79–86.

WEISS, M. C., and H. GREEN. 1967. Human mouse hybrid cell lines containing partial complements of human chromosomes and functioning human genes. *Proc. Natl. Acad. Sci. U. S. A.* **58**:1104–1111.