Nuclear glycogen synthase – Fact or artifact?

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According to Oron et al. [FEBS Lett. (1980) 118, 255–258], nuclear glycogen synthase represents an artifact of preparation in rat liver nuclei. We investigated the nuclei isolated from in vitro growing HD33 ascites cells with exclusively cytoplasmic, and from in vivo growing HD33 Ehrlich–Lettré ascites tumor cells with mainly intranuclear, glycogen deposition. Biochemical and ultracytochemical analyses revealed the complete absence of any contamination of the isolated nuclei by cytoplasmic glycogen particles and associated glycogen synthase activity. The glycogen synthase residing in isolated nuclei of in vivo growing HD33 ascites tumor cells represents a truly nuclear enzyme activity

Ehrlich cell *Glycogen* *Glycogen synthase* *Nucleus* *Cytochemistry*

1. INTRODUCTION

Conflicting reports have been published on the occurrence of glycogen synthase activity in mammalian cell nuclei. On the one hand, glycogen synthase activity in damaged nuclei isolated from livers of normal and diabetic rats was characterized as an artifact of preparation and considered as a useful criterion for the degree of the damage [1]. On the other, intact nuclei isolated from HD33 Ehrlich–Lettré ascites tumor (ELAT) cells were shown to possess high autochthonous glycogen synthase activity [2]. Both reports, however, emphasize the necessity to exclude, in studies on nuclear glycogen synthase, any contamination of isolated nuclei by cytoplasmic glycogen particles and/or associated glycogen synthase activity.

Using in vitro growing HD33 ascites cells, which exhibit a pronounced but exclusively cytoplasmic glycogen storage, we examined the degree to which such contamination can be prevented. Biochemical and cytochemical data presented here indicate that, by carefully applying adequate procedures for isolating nuclei from those cells, any contamination of the nuclei by cytoplasmic glycogen particles and associated enzyme activity could be safely avoided. Furthermore, glycogen synthase present in isolated nuclei of in vivo growing HD33 ascites tumor cells was found to be unrelated to cytoplasmic glycogen deposition and represents a truly nuclear enzyme activity.

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2. MATERIALS AND METHODS

In vitro cells of the strain HD33 were kept in suspension culture as in [3] and harvested in the late exponential phase of growth. The originally existing ability of the cells to store nuclear glycogen [4] had been completely lost during several years of in vitro propagation, resulting in exclusively cytoplasmic glycogen deposition. In vivo cells of the HD33 ELAT subline were handled as in [2] but harvested on day 4 of tumor growth. At this stage, the majority of the tumor cells contains relatively small, almost exclusively nuclear glycogen deposits.

The preparation and fractionation of cells grown in vitro and in vivo, the assaying of glycogen synthase, and the determination of protein and glycogen content were performed as in [2]. The sources of chemicals, sera, reagents and radiochemicals were as in [2, 3]; osmium tetroxide was obtained from Degussa (Hanau), Epon 812 from Serva (Heidelberg) and phosphotungstic acid

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(PTA) from Merck (Darmstadt).

For electron microscopy, whole cells and isolated nuclei were fixed simultaneously with glutaraldehyde/osmium tetroxide [5] which, in our hands, gave the best conservation of glycogen. After post-fixation in 2% aqueous osmium tetroxide and dehydration in graded steps of ethanol, the material was embedded in Epon 812.

For the cytochemical demonstration of glycogen, a modification of the PTA-staining method [6] was used (in preparation). Briefly, ultrathin sections on Formvar-coated grids were immersed in 3% hydrogen peroxide for 10–30 min, and floated for 5–8 min on a 1% PTA solution made up in 1 N HCl (pH 1.0). After that staining procedure, the grids were rinsed for 10–20 s in distilled water, and air dried. Electron micrographs were obtained with a Zeiss EM 10 A electron microscope operated at 60 or 80 kV.

3. RESULTS

In vitro grown HD33 ascites cells contain large, cytoplasmic deposits of glycogen particles. The latter are completely absent from the nuclei (fig.1a). In preparations of isolated nuclei, glycogen particles could be detected neither inside, nor attached to, nor outside of the nuclei (fig.1b). Isolated nuclei with damaged nuclear envelope were free of glycogen particles, too. The only cytoplasmic structures found in preparations of isolated nuclei were cytoplasmic invaginations typical for ascites cell nuclei, and some remnants of the endoplasmic reticulum connected to the nuclear envelope without associated glycogen particles.

In in vivo grown HD33 ELAT cells from an early stage of tumor development, glycogen is accumulated almost exclusively within the nuclei (fig.2a). Cytoplasmic glycogen deposits can hardly

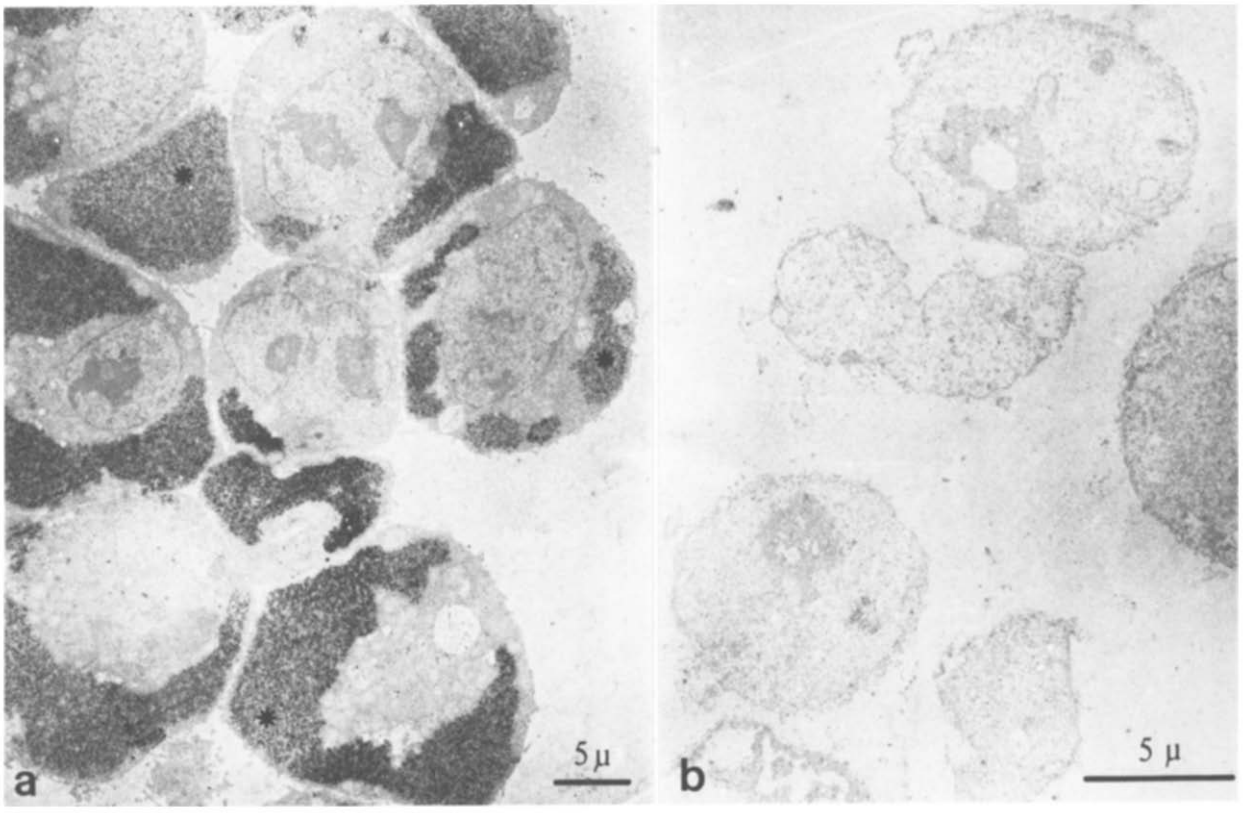

Fig.1. HD33 cells and isolated nuclei after PTA staining, resulting in a selective, intensely black staining of glycogen particles; the other cellular constituents remain unstained: (a) HD33 ascites cells grown in vitro with large cytoplasmic deposits of glycogen (some are denoted by an asterisk); (b) nuclei isolated from the cells shown in (a) (note the complete absence of heavily stained glycogen particles).

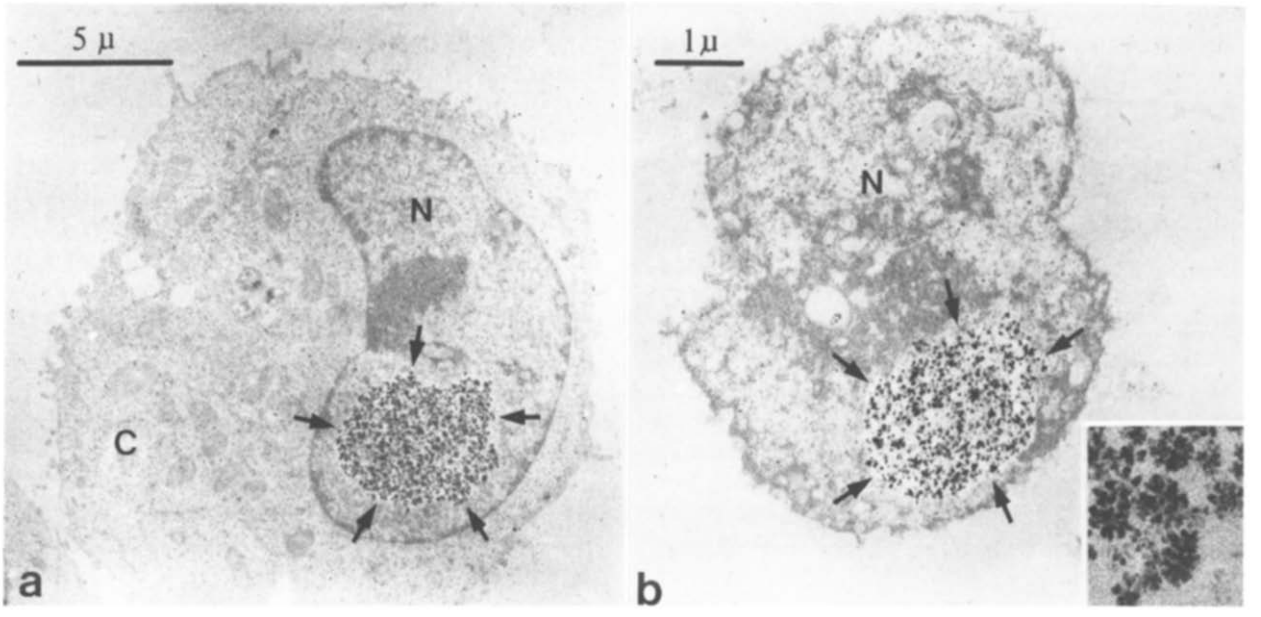

Fig.2. HD33 cells and isolated nuclei after PTA staining, resulting in a selective, intensely black staining of glycogen particles; the other cellular constituents remain unstained: (a) In vivo grown HD33 ELAT cell 4 days after transplantation with a nuclear deposit of glycogen α-particles, demarcated by arrows (C, cytoplasm; N, nucleus); (b) nucleus isolated from a cell as shown in fig.2a. Conformation and intranuclear localization of glycogen deposits remain unchanged after the isolation procedure. Insert: Glycogen particles from an isolated nucleus at high magnification (40 000 ×); N, nucleus.

be found. In situ, as well as after isolation, nuclear deposits of glycogen α-particles are surrounded by chromatin (fig.2b); the isolation procedure caused no detectable dislocation of the deposits. No glycogen particles were found attached to or outside the nuclei isolated from in vivo grown ascites tumor cells.

In summary, the ultracytochemical examination of PTA-stained whole cells and isolated nuclei reveals, in both cell types, an adequate isolation of the nuclei without detectable contamination by cytoplasmic glycogen particles, and demonstrates that the preexisting nuclear glycogen deposits of HD33 ELAT cells are not altered morphologically in the course of the isolation procedure.

The ultrastructural observations are in full agreement with biochemical data on glycogen content and glycogen synthase activity of whole cells

Table 1

Subcellular distribution of glycogen and glycogen synthase activity in HD33 cells

Growth conditions	Cell fraction	Glycogen content [µg/mg protein][a]	Glycogen synthase (a+b)-form [mU/mg protein][a]
In vitro	Isolated nuclei	0	0
	Cytoplasm[b]	1770	34.2
	Cell homogenate	967	14.4
In vivo	Isolated nuclei	41	12.7
	Cell homogenate	24	4.7

[a] Mean values of 2–6 expt; [b] Cytoplasm is defined as in [2]

and isolated nuclei (table 1). The highest total glycogen synthase activity was determined in the glycogen-rich cytoplasm of the in vitro grown cells. Preparations of nuclei isolated from these cells contained no detectable glycogen and did not incorporate UDP-[^{14}C]glucose into glycogen in the presence of 6.67 mM Glc-6-P even during prolonged incubation for up to 30 min at 30°C. Higher concentrations of Glc-6-P and of sample protein gave negative results, too. Thus, nuclei isolated from in vitro grown HD33 ascites cells contain neither glycogen nor glycogen synthase activity, in spite of the abundance of cytoplasmic glycogen particles present in the intact cells.

The amount of glycogen found in homogenates of 4 days in vivo grown HD33 ELAT cells corresponds to $<3\%$ of the values obtained from their in vitro counterpart, when compared on a mg protein basis. The essentially nuclear deposition of the polysaccharide is reflected by the increased amount of glycogen per mg protein in isolated nuclei. The specific activity of glycogen synthase in isolated nuclei of in vivo grown tumor cells is almost 3-times higher than in cell homogenates, suggesting an association of the enzyme activity with the nuclear glycogen deposits. These results conform with our earlier data on the subcellular distribution of glycogen and glycogen synthase in HD33 ELAT cells 5 days after tumor transplantation [2].

4. DISCUSSION

This investigation of HD33 ascites tumor cells from an early stage of tumor development with chiefly nuclear glycogen storage, and of their in vitro growing counterpart with excessive but exclusively cytoplasmic glycogen deposition enabled us to determine whether nuclear glycogen synthase represents a fact or an artifact of preparation. An essential requirement was to exclude any artificial contamination of the isolated nuclei by cytoplasmic glycogen particles and associated enzyme activities (cf. [1]) which may cause considerable confusion.

We used, with negative results, the most sensitive methods available to detect glycogen particles or glycogen synthase activity in preparations of nuclei isolated from in vitro grown HD33 cells. Thus, the artifacts mentioned can be safely avoided by an adequate nuclei isolation procedure, even in cells with excessive cytoplasmic glycogen deposition.

However, after applying the isolation procedure validated in this manner, high activity of glycogen synthase could be determined in nuclei of in vivo grown HD33 ELAT cells. This confirms our finding that glycogen storing nuclei of these tumor cells possess autochthonous nuclear glycogen synthase activity [2]. The presence or absence of glycogen synthase activity in the ascites cell nuclei depends, as shown here, on the growth conditions of the cells.

The association of nuclear glycogen synthase activity with nuclear glycogen deposition, as observed in in vivo grown HD33 ELAT cells, suggests the presence of glycogen synthase also in the nuclei of other mammalian cells with nuclear glycogen storage (see citations in [1,2]). However, the possibility cannot be excluded that glycogen synthase might also be present in nuclei of cells which are virtually free of glycogen particles but exhibit high levels of glycogen synthase activity [7,8].

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REFERENCES

[1] Oron, Y., Cardell, R. and Larner, J. (1980) FEBS Lett. 118, 255–258.
[2] Granzow, C., Kopun, M. and Zimmermann, H.-P. (1981) J. Cell Biol. 89, 475–484.
[3] Granzow, C. (1981) J. Cancer Res. Clin. Oncol. 102, 57–69.
[4] Zimmermann, H.-P., Granzow, V. and Granzow, C. (1976) J. Ultrastruct. Res. 57, 140–149.
[5] Franke, W.W., Krien, S. and Brown, R.M. jr (1969) Histochemie 19, 162–164.
[6] Fléchon, J.-E. (1974) J. Microsc. 19, 207–212.
[7] Baba, T. and Tsuiki, S. (1974) Biochim. Biophys. Acta 370, 419–430.
[8] Staedel, C., Castagna, M. and Beck, J.P. (1979) Cell Diff. 8, 29–38.