CELL-CYCLE DEPENDENT DESQUAMATION OF HEPARAN SULFATE FROM THE CELL SURFACE

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INTRODUCTION

The term heparan sulfate denotes mucopolysaccharides with a structure similar to heparin but which lack significant anticoagulant potency (1). The term also connotes a continuum or family of *N*-sulfated mucopolysaccharides of which the more highly sulfated varieties are called heparins. It appears likely that all cultured mammalian cells synthesize heparan sulfate (2, 3) and that this material becomes incorporated into a metabolically active fraction of the cell surface (4). The heparan sulfate of the cell surface is readily removed by gentle trypsin treatment under conditions where no irreversible cell damage occurs; in the case of Chinese hamster cells (line CHO), trypsin treatment removes heparan sulfate and a melange of glycopeptides from cell-surface glycoproteins (4). These two categories of cell-surface materials have independent metabolism. The ubiquity of heparan sulfate in cultured mammalian cells suggests some general role in cellular metabolism. We report here results concerning cell-cycle related fluctuations in the amount present on the cell surface as well as variations in labeling rate from supplied glucosamine-6-^{3}H. Our results encourage speculations as to the role of a generally occurring cell-surface component in the regulation of exposure of components with specific functions for a particular cell type.

MATERIALS AND METHODS

The experiments reported here utilized parasynchronous suspension cultures of line CHO Chinese hamster cells (5), prepared by first reversibly arresting the cells in G_1 by the isoleucine-deficiency method (6), followed by reversibly inhibiting DNA synthesis with thymidine (7). At the time of removal of thymidine, essentially all cells were near the G_1/S boundary. Portions of the cells were harvested at various times after release from thymidine block, in each case at the end of a 2 hr period of labeling with added glucosamine-6-^{3}H. To determine the distribution of cells among the phases of the cell cycle, a small sample of each harvest was examined for distribution of cellular DNA content using a flow microfluorometric analysis of fluorescent-Feulgen stained cells (8). The remainder of each harvest was used for analyses of the complex carbohydrates of materials removed from the cell surface as well as cell sap and internal membrane fractions. The methodology of these procedures has been described previously (4); briefly, they consist of isolations based upon molecular sieve and DEAE-cellulose chromatography. The isolated complex carbohydrate fractions were then assayed for mass (by colorimetric assays of particular sugar residues) and radioactivity.

RESULTS

An experiment of this type is illustrated in Figs. 1–4. In this experiment, cells were released from a 10 hr thymidine block which was applied 4 hr after release from a 30 hr isoleucine-deficiency block. Six portions of 1 liter each, designated A-F, were harvested 2.5, 3.5, 7.5, 8.5, 12, and 13 hr after resumption of cell-cycle traverse, in each case after a 2 hr labeling period (0.1 mCi/liter glucosamine-6-^{3}H, sp act 3.6 Ci/mM). Fig. 1 illustrates the DNA content distributions at the six harvest times; samples A and B show that the bulk of the population has a DNA content characteristic of the S period, while samples C and D show bimodal distributions due to cells which have progressed to late interphase (G_2 + M DNA content [4C]), while some cells have divided and refilled the 2C compartment with early G_1 cells.

Samples E and F consist largely of cells in G_1 and early S periods. By 13 hr, dispersion of synchrony is evident, as expected (9). A quantitative estimate of the cell-cycle phase distribution (as S, G_2 + M, or G_1) of these samples, determined from these data, together with the growth curve of the cultures is illustrated in Fig. 2. It is evident that sample C, which is coincident with the period of maximum mitotic activity, has over 30% G_2 + M cells, while sample D is highly enriched in G_1 cells.

Analyses of the surface heparan sulfate fraction

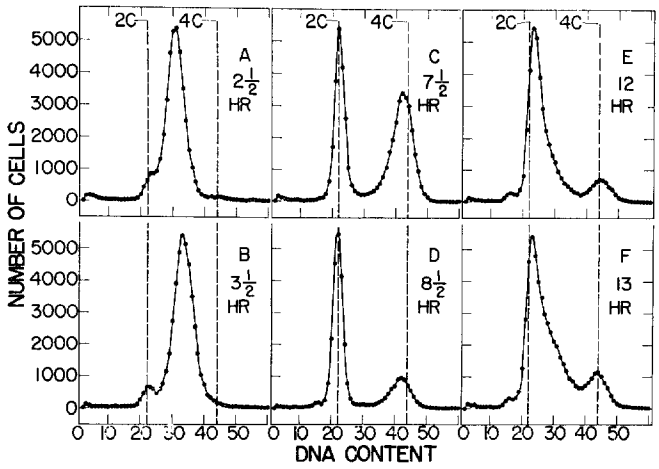

FIGURE 1 Cellular DNA mass distributions of CHO suspension cultures at various times after release from thymidine block (see text for details). The thymidine block was applied 4 hr after release from an initial isoleucine-deficiency block. The 2C DNA content corresponds to the DNA content of G_1 cells, the 4C content corresponds to that of G_2 and M cells, and S cells have intermediate values between 2C and 4C. DNA content units are arbitrary.

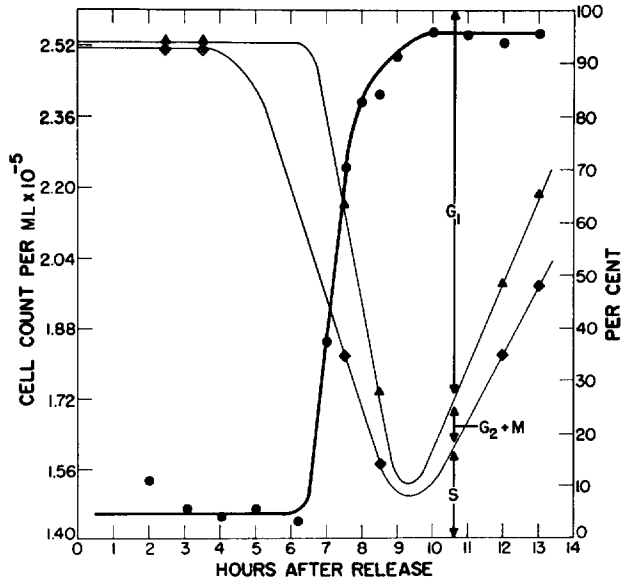

FIGURE 2 Cell growth and cell-cycle phase distribution of cultures released from thymidine block at 0 hr. Cell-cycle phase distribution values were derived from the data illustrated in Fig. 1. For instance, cells from harvest D, at 8.5 hr, according to their DNA content were distributed as follows: S = 14%, G_2 + M = 14%, and G_1 = 72%. Solid circles represent cell number per ml.

from each sample are illustrated in Fig. 3. Incorporated radioactivity was very similar in the first four samples but increased in samples E and F, suggesting an increased rate of heparan sulfate synthesis and transport to the cell surface for cells in early interphase. Since the pulse-labeling period was of 2 hr duration, the radioactivity measurement would also include a small turn-

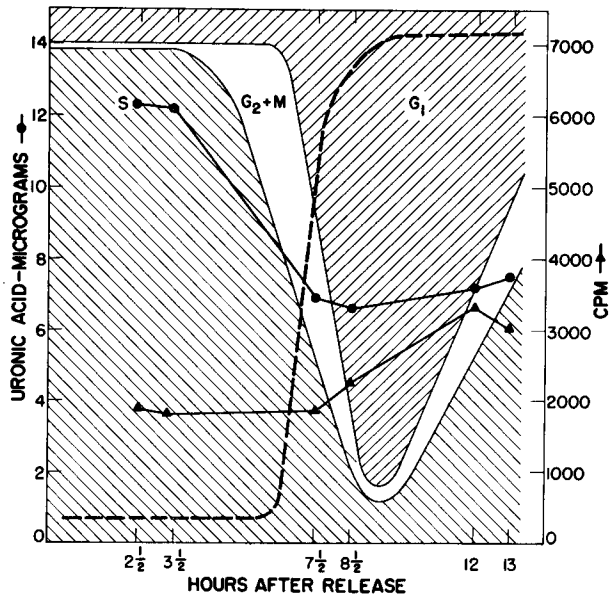

FIGURE 3 Mass and radioactivity of the surface heparan sulfate of cultures released from thymidine block at 0 hr: (●) μg uronic acid and (▲) counts per minute of ^3H. Uronic acid assays on the isolated surface heparan sulfate were done by the orcinol method (22). Each point represents 1000 ml of culture regardless of cell count.

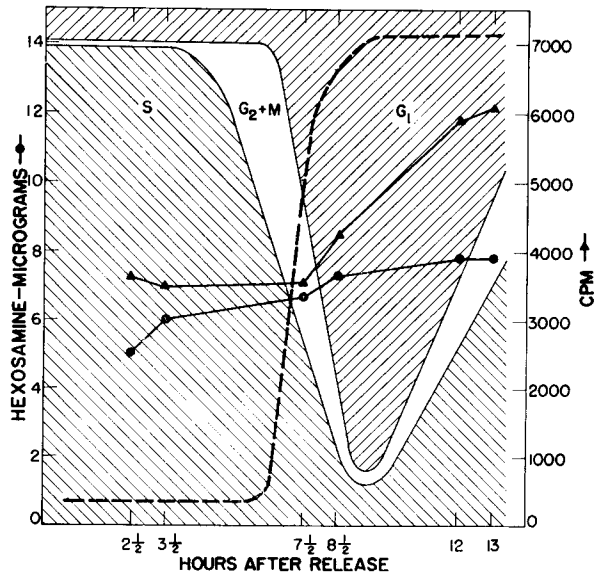

FIGURE 4 Mass and radioactivity of the surface glycopeptide fraction of cells released from thymidine block at 0 hr: (●) μg hexosamine and (▲) counts per minute of ^3H. Hexosamine assays were done by the Elson-Morgan reaction after preliminary "short-column" purification of material hydrolyzed with 4 N HCl, 100°C, 14 hr (23).

over component; however, analyses of heparan sulfate mass were clearly inconsistent with a steady state across the time span of the experiment. As illustrated in Fig. 3, there was a highly significant loss of surface heparan sulfate as the cells progressed through late interphase and divided. The similarity of the mass results for samples taken at 7.5 and 8.5 hr, an interval that includes about one-fourth of the total cell divisions, suggests that the loss of surface heparan sulfate occurred just before mitosis. Note that the data are plotted as mass or radioactivity per sample of cells rather than per cell. The results thus indicate an actual loss of heparan sulfate mass independent of any per cell decrease due to cell division.

The results illustrated in Fig. 4, which concern assays of the cell-surface glycopeptide fractions, indicate that the loss of cell-surface heparan sulfate is not merely part of a general premitotic loss of cell-surface materials but, rather, a specific loss. The apparent synthetic pattern, however, of all these cell-surface materials is evidently very similar in that both heparan sulfate and the glycopeptide fraction show increased specific activity in the late G_1 phase of the cell cycle. This result is consistent with reported fluctuations of carbohydrate content in synchronized KB cells (10). In other experiments of this type, the intracellular heparan sulfate fractions were isolated as well as the cell-surface fraction. No cell cycle-dependent losses or increases were found in either the cell sap heparan sulfate or the heparan sulfate associated with internal membranes. Hence, it seems clear that the phenomenon reported here is specific for heparan sulfate in its cell surface-associated form.

In a few experiments, the spent medium was harvested after the division wave (i.e., at 9 hr from the time of release from thymidine block into fresh medium). Because of the large volume and high serum content, it was not technically feasible to analyze the spent medium in terms of directly acid-soluble heparan sulfate. However, the acid precipitate of spent medium, digested with trypsin and processed as previously described (4), yielded 60–80% of the heparan sulfate that was calculated to have been lost from the cells during the 9 hr period.

DISCUSSION

The pronounced premitotic loss of surface heparan sulfate might suggest, alternatively, (*a*) degradation, or (*b*) return to an intracellular (or trypsin-inaccessible) location, or (*c*) release of the intact molecule to the medium. The last possibility appears to be the correct one, since (*a*) the loss of surface heparan sulfate was accompanied by loss of total cellular heparan sulfate without gain in the intracellular compartments, and (*b*) the acid-precipitable fractions of spent medium contained the bulk of heparan sulfate. Such acid-precipitable fractions, when digested with trypsin, released acid-soluble heparan sulfate with identical characteristics to that released with trypsin from the cell surface. Hence, the material released before mitosis may well represent nearly intact molecules. Warren and Glick's studies of isolated plasma membranes of L cells suggested that the membrane components, in general, underwent coupled turnover (11, 12). However, there is precedent for individualized turnover of particular membrane-associated entities in the case of microsomal enzymes (13–15).

It seems likely that certain quantitative aspects of the phenomenon reported here were the result of recovery from the perturbation of cellular metabolism induced by the synchronization method. For instance, the recovery of surface heparan sulfate after its loss, as illustrated in Fig. 3, was less than might be expected from the 2.5 and 3.5 hr values. After release from the thymidine block, the cells were larger than normal, an effect which is largely compensated for by reduced growth in cell volume during the interphase period after the first division. Hence, it might be expected that regeneration of cell-surface heparan sulfate would be less.

The phenomenon reported here is similar in cell-cycle position to a number of other reported cell-surface changes. These include increased reactivity of wheat germ agglutinin sites (16) and blood group antigen sites (17). The neuraminidase-sensitive component of cellular electrophoretic mobility is also elevated at this time (18), and there is some evidence that the mobility change is not related to a changing quantity of surface sialic acid residues but rather is due to their changing exposure at the hydrodynamic slip plane (19). Other reported cycle-dependent cell-surface changes, such as those related to reactivities of H-2 transplantation antigens and Maloney leukemia virus neoantigens, appear more closely correlated with the immediate postmitotic rather than premitotic period (20–23). The results presented here raise the possibility

that the fluctuating quantity of a generally occurring cell-surface molecular entity such as heparan sulfate may actually be a mechanism by which expression of other cell-surface entities, including cell-specific ones, is regulated.

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REFERENCES

1. MEYER, K. 1966. Introduction: Mucopolysaccharides. *Fed. Proc.* **25**:1032.
2. DIETRICH, C. P., and H. M. DEOCA. 1970. Production of heparin related mucopolysaccharides by mammalian cells in culture. *Proc. Soc. Exp. Biol. Med.* **134**:955.
3. KRAEMER, P. M. 1971. Heparan sulfates of cultured cells. II. Acid soluble and precipitable species of different cell lines. *Biochemistry.* **10**:1445.
4. KRAEMER, P. M. 1971. Heparan sulfates of cultured cells. I. Membrane associated and cell-sap species in Chinese hamster cells. *Biochemistry.* **10**:1437.
5. KRAEMER, P. M. 1968. Production of heparin related glycosaminoglycans by an established mammalian cell line. *J. Cell Physiol.* **71**:109.
6. TOBEY, R. A., and K. D. LEY. 1971. Isoleucine-mediated regulation of genome replication in various mammalian cell lines. *Cancer Res.* **31**:46.
7. PETERSEN, D. F., and E. C. ANDERSON. 1964. Quantity production of synchronized mammalian cells in suspension culture. *Nature (Lond.).* **203**:642.
8. KRAEMER, P. M., L. L. DEAVEN, H. A. CRISSMAN, and M. A. VAN DILLA. 1972. *In* Advances in Cell and Molecular Biology. E. J. DuPraw, editor. Academic Press Inc., New York. **2**: 47–108.
9. ENGELBERG, J. 1964. The decay of synchronization of cell division. *Exp. Cell Res.* **36**:647.
10. GLICK, M. C., E. W. GERNER, and L. WARREN. 1971. Changes in the carbohydrate content of the cell during the growth cycle. *J. Cell Physiol.* **77**:1.
11. WARREN, L., and M. C. GLICK. 1968. Membranes of animal cells. II. The metabolism and turnover of the surface membrane. *J. Cell Biol.* **37**:729.
12. WARREN, L. 1969. The biological significance of turnover of the surface membrane of animal cells. *Curr. Top. Dev. Biol.* **4**:197.
13. OMURA, T., P. SIEKEVITZ, P. and G. PALADE. 1967. Turnover of constituents of the endoplasmic reticulum membranes of rat hepatocytes. *J. Biol. Chem.* **242**:2389.
14. WIDNELL, C. C., and P. SIEKEVITZ. 1967. The turnover of the constituents of various rat liver membranes. *J. Cell Biol.* **35**:142A.
15. BOCK, K. W., P. SIEKEVITZ, and G. PALADE. 1971. Localization and turnover studies of membrane nicotinamide adenine dinucleotide glycohydrolase in rat liver. *J. Biol. Chem.* **246**:188.
16. FOX, T. O., J. R. SHEPPARD, and M. M. BURGER. 1971. Cyclic membrane changes in animal cells. Transformed cells permanently display a surface architecture detected in normal cells only during mitosis. *Proc. Natl. Acad. Sci. U. S. A.* **68**:244.
17. KUHNS, W. J., and S. BRAMSON. 1968. Variable behavior of blood group H on HeLa cell populations synchronized with thymidine. *Nature (Lond.).* **219**:938.
18. MAYHEW, E. 1966. Cellular electrophoretic mobility and the mitotic cycle. *J. Gen. Physiol.* **49**:717.
19. KRAEMER, P. M. 1967. Configuration change of surface sialic acid during mitosis. *J. Cell Biol.* **33**:197.
20. CIKES, M., and S. FRIBERG. 1971. Expression of H-2 and Maloney leukemia virus-determined cell surface antigens in synchronized cultures of a mouse cell line. *Proc. Natl. Acad. Sci. U. S. A.* **68**:566.
21. LERNER, R. A., M. B. A. OLDSTONE, and N. R. COOPER. 1971. Cell cycle-dependent immune lysis of Maloney virus transformed lymphocytes: Presence of viral antigen, accessibility to antibody, and complement activation. *Proc. Natl. Acad. Sci. U. S. A.* **68**:2584.
22. MEJBAUM, W. Z. 1966. *Methods Enzymol.* **8**:53.
23. BOAS, N. F. 1953. Method for the determination of hexosamines in tissues. *J. Biol. Chem.* **204**:553.