Selection of Mutant Chinese Hamster Ovary Cells Altered in Glycoproteins by Means of Tritiated Fucose Suicide

CARLOS B. HIRSCHBERG,^{1*} RAYMOND M. BAKER,^{2†} MARY PEREZ,¹ LYNETTE A. SPENCER,²
AND DOUGLAS WATSON¹

E. A. Doisy Department of Biochemistry, St. Louis University School of Medicine, St. Louis, Missouri 63104¹; and Department of Biology and Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139²

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Mutant Chinese hamster ovary cells altered in glycoproteins have been isolated by selecting for ability to survive exposure to [6-³H]fucose. Mutagenized wild-type cells were permitted to incorporate [³H]fucose to approximately 1 cpm of trichloroacetic acid-insoluble radioactivity per cell and then frozen for several days to accumulate radiation damage. The overall viability of the population was reduced by 5- to 50-fold. Four consecutive selection cycles were carried out. The surviving cells were screened by replica plating-fluorography for clones showing decreased incorporation of fucose into trichloroacetic acid-insoluble macromolecules. Considerable enrichment for cells deficient in fucose uptake or incorporation into proteins (or both) was found in populations surviving the later selection cycles. Two mutant clones isolated after the fourth selection cycle had the same doubling time as the wild type, but contained only 30 to 40% as much fucose bound to proteins as the wild type. Sialic acid contents of the mutants and the wild type were similar. The mutants differed quantitatively and qualitatively from the wild type and from each other with respect to total glycoprotein profiles as visualized by sodium dodecyl sulfate gel electrophoresis. Differences were also found in resistances to cytotoxicity of lectins such as concanavalin A and wheat germ agglutinin.

Glycoprotein components of mammalian cell surfaces are thought to play vital roles in such processes as regulation of cell growth, intercellular recognition, and cellular adhesion (8, 31, 32). A powerful approach for investigating relationships between glycoprotein structure and functions and for elucidating the biosynthesis of the oligosaccharide moieties of these molecules is to examine the phenotypes of somatic cell mutants with altered glycoprotein synthesis (5, 10, 14, 16, 18, 20, 22). One notably successful tactic for the isolation of such glycoprotein mutants has been the selection of cells resistant to cytotoxic lectins (4, 6, 9, 11, 23-25, 30, 33). Such resistance presumably reflects alterations in cell surface glycoproteins, particularly those which are part of or adjacent to the lectin receptors. Recent studies have suggested that more than one sugar may be involved in the surface recognition system for some lectins (2).

We have initiated studies based on an alternative method for selection of glycoprotein mutants with altered oligosaccharide moieties. Our

approach was to search directly for mutants showing reduced overall incorporation of a particular carbohydrate, in contrast to selecting for phenotypic alterations at the cell surface as with lectins or immunoselection. To enrich for mutant cells, we employed a procedure for suicide (21, 28, 29) of normally metabolizing cells with ³H-sugar, where the ³H label was not significantly metabolized to other compounds. The selected populations were then screened by replica plating-fluorography for putative mutants which do not incorporate radiolabeled sugars into glycoproteins. In this paper we report the isolation of two different mutants of Chinese hamster ovary (CHO) cells on the basis of their increased resistance to [6-³H]fucose suicide. These mutants show substantial reductions in fucose incorporation and marked differences from the wild-type cells and from each other in fucose-containing glycoproteins.

MATERIALS AND METHODS

Cells, culture conditions, and mutagenesis. The Chinese hamster ovary cell line 51-11, which is auxotrophic for glycine and proline (15), was obtained

† Present address: Roswell Park Memorial Institute, Buffalo, NY 14263.

from L. Chasin. A subclone of this line, 51-11B, was utilized as the parental wild-type stock for selection of fucose mutants. Cells were grown in alpha MEM medium (KC Biological, Lenexa, Kans.) supplemented with 5 or 10% fetal calf serum (Reheis Chemical Co., Kankakee, Ill., or KC Biological), penicillin (0.1 mg/ml), streptomycin (0.1 mg/ml), and, on occasion, amphotericin B (1 μ g/ml). General cell culture procedures were as previously described (29). Cell concentrations were determined with a Coulter electronic cell counter model ZBI or a Celloscope particle counter.

The wild-type cell population subjected to [^{3} H]fucose selections was first mutagenized with ethyl methane sulfonate; 3.5×10^6 cells in a 150-cm ^{2} flask were treated with 250 μ g of ethyl methane sulfonate in culture medium for 18 h at 37°C. At the end of the mutagen exposure, cell survival (plating efficiency relative to an untreated control) was 0.2. The mutagenized cells were then cultured at $\geq 5 \times 10^6$ cells per flask for 8 days at 34°C to permit expression of induced recessive mutations. The frequency of mutants resistant to 3 mM ouabain (1) in this treated culture was determined to be 1.5×10^{-5} per cell compared with $<1 \times 10^{-6}$ for an unmutagenized sister culture.

[^{3} H]fucose selection cycles. Approximately 5×10^6 cells were seeded in a 150-cm ^{2} flask. After about 6 h of incubation at 34°C, the medium was removed and replaced with 10 ml of medium containing 2 mCi of [^{6} - ^{3} H]fucose (13.1 Ci/mmol; New England Nuclear Corp.). The culture was incubated at 39°C for a further 24 h; then, the ^{3} H-labeled medium was removed, and the cell monolayer was rinsed six times with fresh growth medium. The cells were harvested by trypsinization, suspended in 5 ml of growth medium containing 10% fetal calf serum and 10% dimethylsulfoxide, and frozen and stored at -90°C to accumulate radiation damage. Immediately before freezing, in order to determine the amount of macromolecular radioactivity per cell, a 0.1-ml sample of the cell resuspension was taken to measure cell number, and another 0.1-ml sample was added to cold phosphotungstic acid (PTA) (1.9 ml; 1% in 0.5 N HCl) for assay of radioactivity incorporated into acid-insoluble macromolecules (12). After 4 to 11 days of storage at -90°C, the selection cultures were thawed. At this point, samples of the cell suspension were plated on 60-mm dishes and incubated for colony formation to determine survival in terms of plating efficiency relative to control cells subjected to parallel treatment in the absence of radiolabeled fucose. The bulk of the selection culture was cultivated at 34°C to allow recovery from radiation damage and regrowth to confluence. A sample was frozen to preserve for later screening of fucose incorporation by replica plating. The remainder of the culture was channeled through further fucose suicide selection cycles by the protocol described above.

Replica plating-fluorography. Replica plating was performed essentially as described by Esko and Raetz (7), with minor modifications. Cultures were plated at 50 to 200 cells per 100-mm dish, overlaid with Whatman no. 50 filter paper and glass beads, and grown for approximately 12 days at 34°C. To screen for colonies deficient in incorporation of fucose into macromolecules, the paper was removed, rinsed twice with phosphate-buffered saline (140 mM NaCl, 2.68

mM KCl, 0.9 mM CaCl $_2$, 0.5 mM MgCl $_2$, 1.47 mM KH $_2$ PO $_4$, 8.1 mM Na $_2$ HPO $_4$, pH 7.4), and incubated in a 100-mm dish with 1.25 ml of growth medium containing 100 μ Ci of [^{6} - ^{3} H]fucose for 24 h at 39°C. The paper was subsequently rinsed, treated with cold 20% trichloroacetic acid to precipitate macromolecules, washed free of acid-soluble counts, and stained with Coomassie blue to visualize colonies as described elsewhere (7). The dry papers were immersed briefly in 8% 2,5-diphenyloxazole in acetone, and radioactive colonies were visualized by exposure to Kodak X-Omat film at -90°C for 4 to 8 days. For storage, the master plate was rinsed with phosphate-buffered saline, covered with 10 ml of growth medium containing 10% fetal calf serum and 10% dimethyl sulfoxide, and immediately frozen at -90°C. After evaluation of results from fluorographs of the replicas, colonies desired for further study could be recovered by rapidly thawing the original dishes, incubating for a few days with fresh medium, and removing the appropriate cells with the aid of a cloning cylinder. Isolates obtained in this manner were then cloned at limiting dilution in multiwell Linbro dishes to obtain pure mutant lines for further characterization.

Sodium dodecyl sulfate-gel electrophoresis. For sodium dodecyl sulfate-gel electrophoresis of glycoproteins, cells (2.5×10^5 per 35-mm plate) were labeled for 36 h at 37°C in 0.8 ml of complete medium containing radiolabeled sugar as follows. Wild-type 51-11B cell cultures contained [^{6} - ^{3} H]fucose (50 μ Ci; 13 Ci/mmol), [^{6} - ^{3} H]glucosamine (5 μ Ci/19 Ci/mmol), or [^{2} - ^{3} H]mannose (50 μ Ci, 14 Ci/mmol). Fuc $^-$ 687 cell cultures contained [^{3} H]fucose (200 μ Ci), [^{3} H]glucosamine (10 μ Ci), or [^{3} H]mannose (100 μ Ci). Fuc $^-$ 699 cell cultures contained [^{3} H]fucose (200 μ Ci), [^{3} H]glucosamine (10 μ Ci), or [^{3} H]mannose (75 μ Ci). After the removal of radiolabeled medium, the plates were washed seven times with 3 ml of cold solution A (140 mM NaCl, 2.68 mM KCl, 1.47 mM KH $_2$ PO $_4$, 8.1 mM Na $_2$ HPO $_4$, pH 7.4), and 1.5 ml of cold 10% trichloroacetic acid was added to each plate. The cells were scraped into a centrifuge tube, and the suspension was centrifuged for 5 min at maximum speed in a clinical centrifuge. The pellet was washed three times with 3 ml of cold 10% trichloroacetic acid and then with 50% acetone and redissolved in 2% sodium dodecyl sulfate-8 M urea-10 mM tris(hydroxymethyl)aminomethane-hydrochloride-5% 2-mercaptoethanol (pH 7.5) by boiling for 2 to 5 min. For Coomassie blue profiles, samples containing approximately 2×10^5 cpm of [^{3} H]fucose, 5×10^5 cpm of [^{3} H]glucosamine, 3.5×10^5 cpm of [^{3} H]mannose, and 300 μ g of protein were electrophoresed in 10% slab gels by using the buffer system of Neville (19). Gels were processed for fluorography by the method of Bonner and Laskey (3) and exposed at -70°C to Kodak RP Royal X-Omat film which had been preflashed to increase sensitivity (17).

RESULTS

The principal steps in the mutant selection scheme employed in this work can be summarized as follows. (i) A large population of mutagenized wild-type (51-11B) cells was permitted to incorporate [^{3} H]fucose for 24 h under stand-

ard growth conditions. (ii) The treated cells were frozen and stored at -90°C for 4 to 11 days to allow accumulation of radiation damage in proportion to the amount of incorporated radioisotope. (iii) The samples were thawed, cultured to recover survivors from the radiation damage, and then recycled through another round of [^3H]fucose selection. (iv) The populations obtained after each selection cycle were assayed for the fucose incorporation phenotypes of constituent clones by the replica plating procedure of Esko and Raetz (7). The replicas of colonies on a filter paper were assayed for incorporation of [^3H]fucose into acid-insoluble macromolecules by fluorographic techniques.

The basic premise of this selection scheme is that, compared with the wild type, cells with reduced ability to incorporate [^3H]fucose should exhibit increased resistance to radiation damage and preferential survival after storage at -90°C . In order that our selected cell populations might include any mutants temperature sensitive (*ts*) for fucose incorporation, the cultures were cultivated at 34°C , except for the 24-h incubation with [^3H]fucose itself, during which the temperature was 39°C .

Table 1 shows ^3H incorporation, storage time, and survival of cell viability for consecutive cycles of [^3H]fucose selection. These data are consistent with our expectation that cytotoxicity would vary with the amount of macromolecule-bound radioactivity per cell and with the length of radiation exposure at -90°C , as we have observed in more detail for comparable suicide selections with [$2\text{-}^3\text{H}$]mannose (R. M. Baker, W. A. O'Brien III, C. Hirschberg, L. A. Spencer, T. Awerbuch, and T. O. Weisman, *J. Cell Biol.* **83**: 453a, 1979).

Successive [^3H]fucose selection cycles were indeed enriching for a population of cells with reduced ability to incorporate radiolabeled fucose into fucoproteins at 39°C . Figure 1 shows the results of replica plating-fluorography assays to screen the cultures of survivors after each cycle for the presence of clones showing reduced

incorporation of radiolabeled fucose into macromolecules. Most colonies from the populations obtained after the first and second selection cycles were able to incorporate radiolabeled fucose into fucoproteins in amounts comparable to those in wild-type colonies from the unselected culture (compare samples c-c' and d-d' to the control a-a' in Fig. 1). After the third and fourth selection cycles, however, an increasing frequency of colonies showed reduced incorporation of fucose (see samples e-e' and f-f'). After four selection cycles, well over half of the colonies examined gave a substantially reduced or undetectable response in the fluorographic assay for acid-insoluble [^3H]fucose.

From the population obtained after four cycles of selection, we isolated two colonies that exhibited reduced fucose incorporation in the replica plating-fluorography assay. These isolates were recloned and designated Fuc⁻687 and Fuc⁻699 respectively. As expected, replica assays for fucose incorporation using these clonal isolates yielded uniformly negative readings for all colonies compared with wild-type control colonies. Figure 2 shows the results obtained when cultures of the wild-type and mutant clones were assayed for time courses of [^3H]fucose incorporation into PTA-insoluble macromolecules at 37°C . Whereas fucose incorporation was linear in all cases, the rates of incorporation were 2.3-fold and 3.5-fold lower in the Fuc⁻687 and Fuc⁻699 lines, respectively, than in the wild-type parent. By contrast, assays performed on five sister clones or subclones of wild-type cells showed the same fucose incorporation rates within $\pm 7\%$ (data not shown), confirming that the fucose phenotype is not substantially affected by adventitious clonal variation. The above results show that the Fuc⁻ isolates are, indeed, defective in fucose incorporation, and the specificity of the [^3H]fucose suicide selection is verified.

Although the protocols used for the suicide selections and replica screening were designed to allow recovery of *ts* mutants, neither the Fuc⁻687 nor the Fuc⁻699 clones showed a temperature-sensitive phenotype. With respect to growth, at both 34 and 39°C each mutant clone showed the same high plating efficiency and the same doubling time (21.5 and 19.5 h, respectively) as did wild-type cells. With respect to fucose incorporation, results analogous to those displayed in Fig. 2 were also obtained at both 34 and 39°C (data not shown); the Fuc⁻ phenotype was exhibited at each temperature.

Reduced incorporation of fucose by the Fuc⁻687 and Fuc⁻699 mutant clones was not due to reduced fucose transport into the cells. Table 2 shows comparative data for 24-h incorporation

TABLE 1. Fucose suicide survival after each selection cycle^a

Cycle	PTA-insoluble radioactivity (cpm/cell)	Days at -90°C	Fraction of cells surviving
1	2.0	4	0.21
2	2.0	11	0.018
3	1.5	11	0.24
4	0.8	11	0.22
Cumulative			2×10^{-4}

^a Data are given relative to control cells exposed to the same treatments, but without [^3H]fucose.

FIG. 1. Screening by replica plating-fluorography for mutants with reduced fucose incorporation into macromolecules. Unselected wild-type cells and the cell populations obtained from consecutive suicide selection cycles were plated and assayed as described in the text. The left panel shows Coomassie-stained colonies on replica filter papers, as follows: unselected wild-type cells (a and b) and the surviving populations after the first (c), second (d), third (e), and fourth (f) selection cycles. The right panel shows the corresponding fluorograms of these samples, in the same orientation, indicating [^{3} H]fucose incorporation into trichloroacetic acid-insoluble radioactivity by colonies shown in the left panel. Only 20% as much radiolabeled fucose was used for the incorporation assay of the unselected wild-type sample shown in b and b' as for the other samples, to provide a reference for estimating amount of [^{3} H]fucose incorporation in relation to intensity of spots on the fluorogram.

of various ^{3} H-sugars into the acid-insoluble and acid-soluble fractions of the wild type and the Fuc $^-$ cells. With respect to fucose in particular, the total (acid-insoluble plus soluble) radioactivity taken into Fuc $^-$ 687 cells was similar to that for the wild type, whereas the total taken into Fuc $^-$ 699 cells was only 17% less. Furthermore, uptakes by the mutants and wild-type cells during 3- to 9-min incubations were not significantly different (data not shown). Direct chemical analyses of fucose bound to macromolecules in wild-type, Fuc $^-$ 687, and Fuc $^-$ 699 cells gave the following values, respectively (nanomoles of fucose per 10^6 cells): 0.455, 0.189, and 0.140. This indicates that the reduced levels in radioactivity of fucoproteins reflected in Table 2 are, indeed, a reflection of reduced steady-state content of fucose and not of altered turnover rates.

Table 2 shows that the two mutant clones also differed from the wild type in utilization of other radiolabeled sugars involved in glycoproteins. Thus, although incorporation of radioactive glu-

cosamine into the acid-insoluble fraction was similar in the mutants and the wild type, uptake into the acid-soluble fraction was 60 to 80% greater in the mutants. Compared with the wild type or Fuc $^-$ 699, Fuc $^-$ 687 had 50 to 65% more radioactive sialic acid in the acid-insoluble fraction. Since direct chemical analyses showed that there was no difference in macromolecular sialic acid content in these lines (data not shown), Fuc $^-$ 687 may have altered pools of sialic acid, cytidine monophosphate-sialic acid, or acceptor proteins or increased sialoglycoprotein turnover. Fuc $^-$ 699 had 60% more radioactive mannose in the acid-insoluble fraction than did the wild type or Fuc $^-$ 687.

We also examined the patterns of total cellular glycoprotein in mutant and wild-type cells, as displayed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Figure 3 shows that the mutants differed quantitatively and qualitatively from the wild type and from each other in the glycoprotein profiles revealed by fucose,

FIG. 2. Kinetics of fucose incorporation into glycoproteins by the wild type and *Fuc*⁻ mutants. Cells were labeled with [³H]fucose at 37°C for different time intervals. Conditions for incubation and subsequent determination of PTA-insoluble radioactivity are described in the legend of Table 2. Each time point is the mean for duplicate cultures; a control value for zero time incubation has been subtracted.

glucosamine, or mannose labeling, although no differences were visible in total cellular proteins stained by Coomassie blue. The main fucose-labeled proteins of the wild-type had molecular weights of approximately 200,000 (200K); *Fuc*⁻699 showed bands of equal intensity in the 200 and 70K regions, whereas *Fuc*⁻687 showed bands between the 200 and 60K regions. The predominant glucosamine-labeled proteins of the wild type had a molecular weight of approximately 200K; *Fuc*⁻699 showed a strongly labeled band in the 200K region and two bands in the 70K region, whereas *Fuc*⁻687 showed strongly labeled bands in the 200 to 80K region. The most prominent mannose-labeled proteins of the wild type had molecular weights of 100 and 200K; *Fuc*⁻699 showed two very intense bands in the 70K region, whereas *Fuc*⁻687 showed a series of bands in the 90 to 60K region.

To ascertain that these differences in glycoprotein composition were also manifested in cell

TABLE 2. Incorporation of radiolabeled sugars into wild-type and mutant CHO cells^a

Cells	Fucose			Glucosamine			Sialic acid			Mannose		
	PTA insoluble	PTA soluble	Total	PTA insoluble	PTA soluble	Total	PTA insoluble	PTA soluble	Total	PTA insoluble	PTA soluble	Total
	cpm/10 ⁶ cells/24 h	cpm/10 ⁶ cells/24 h	cpm/10 ⁶ cells/24 h	cpm/10 ⁶ cells/24 h	cpm/10 ⁶ cells/24 h	cpm/10 ⁶ cells/24 h	cpm/10 ⁶ cells/24 h	cpm/10 ⁶ cells/24 h	cpm/10 ⁶ cells/24 h	cpm/10 ⁶ cells/24 h	cpm/10 ⁶ cells/24 h	cpm/10 ⁶ cells/24 h
Wild type 51-11B	55,370	12,350	67,720	291,500	146,500	438,000	7,900	1,030	8,930	45,400	20,600	66,000
<i>Fuc</i> ⁻ 699	16,740	39,500	56,240	278,000	270,000	548,000	8,700	1,630	10,330	71,900	38,000	109,900
<i>Fuc</i> ⁻ 687	23,600	45,000	68,600	259,500	234,500	494,000	13,030	2,860	15,890	48,050	31,600	79,650

^a Cells were labeled for 24 h at 37°C on 60-mm dishes. The numbers shown represent the mean of measurements on two dishes; the average range of the measurements was ±3% of the mean. Complete growth medium (1.50 ml) containing [6-³H]fucose (5 μCi), [6-³H]glucosamine (2 μCi), [2-³H]mannose (10 μCi), or [9-³H]N-acetylneuraminic acid (11 μCi; 19 Ci/mmol) was used. PTA-soluble and -insoluble radioactivity was determined after washing and fractionation of the cells directly in the dish, without trypsinization, as previously described (12). Cell numbers per plate after 0 and 24 h of labeling were: wild type 51-11B, 5.5 × 10⁶ to 1.4 × 10⁶; *Fuc*⁻687, 7.9 × 10⁶ to 1.4 × 10⁶; *Fuc*⁻699, 4.4 × 10⁶ to 1.2 × 10⁶.

FIG. 3. Composite sodium dodecyl sulfate electrophotogram of wild-type, *Fuc*⁻687, and *Fuc*⁻699 glycoproteins. Glycoproteins from cells labeled with different sugars were separated on sodium dodecyl sulfate-gel electrophoresis as described in the text. The following molecular weight markers were used as indicated by the short lines, from top to bottom: phosphorylase A (92,500), transferrin (80,000), catalase (60,000), and ovalbumin (43,000).

surface properties, the responses of the different cell lines to lectin cytotoxicity were surveyed (Table 3). The *Fuc*⁻ mutants differed from the wild type by increased sensitivity to concanavalin A and decreased sensitivities to wheat germ agglutinin, phytohemagglutinin, ricin I, and ricin II. Differences between *Fuc*⁻687 and *Fuc*⁻699 were evident in sensitivities to concanavalin A, wheat germ agglutinin, and ricin I.

DISCUSSION

The experiments described in this paper show that somatic cell mutants with substantial alterations in the glycose moiety of glycoproteins can be obtained by a ³H-carbohydrate suicide selection protocol. This approach differs from previous selections for glycoprotein mutants based on resistance to lectins in that it depends directly upon reduced content of a particular sugar in cellular macromolecules as opposed to an altered reactive configuration at the cell surface. Therefore, one might expect to obtain by the suicide selection procedure a different (though presumably overlapping) spectrum of mutants affecting glycoprotein biosynthesis.

In evaluating the selection method described here, one can be reasonably certain that most radiation damage originated from radioactive fucose, since fucose is not metabolized to a substantial extent to another metabolite. Furthermore, it seems fairly clear that a principal source of radiation damage was fucose bound to mac-

TABLE 3. Cytotoxicity of lectins for wild-type and *Fuc*⁻ mutant cells

Lectin	Effective dose ^a (μg/ml) on cells:		
	Wild type	<i>Fuc</i> ⁻ 687	<i>Fuc</i> ⁻ 699
Concanavalin A	40	20	<10
Wheat germ agglutinin	10	40	>40 ^b
Phytohemagglutinin	5	>40 ^b	>40 ^b
Ricin I	0.02	0.08	>0.20 ^b
Ricin II	0.10	0.30	>0.40 ^b

^a The effective dose shown was the lowest lectin concentration that sufficed to reduce plating efficiency by 100-fold or more, as determined in the following type of screening assays. Cultures were seeded in 16-mm wells of Linbro multiwell dishes with 10² and 10⁴ cells and 2 ml of medium containing the indicated final concentrations of lectin: concanavalin A (Boehringer Mannheim Corp.) at 0, 10, 20, 40, 60, or 80 μg/ml; wheat germ agglutinin (Boehringer Mannheim) or phytohemagglutinin (Pharmacia Fine Chemicals) at 0, 2, 5, 10, 20, or 40 μg/ml; ricin I (Sigma Chemical Co.) at 0, 0.02, 0.05, 0.08, 0.10, or 0.20 μg/ml; or ricin II (Sigma) at 0, 0.05, 0.10, 0.20, 0.30, or 0.40 μg/ml. The dishes were incubated at 34°C for approximately 1 week to allow colony formation and then stained with methylene blue and scored.

^b Highest dose tested.

romolecules (i.e., in glycoproteins). The data in Table 2 show that two *Fuc*⁻ clones which survived this selection exhibited reduced incorporation of [³H]fucose into acid-insoluble macro-

molecules but no significant reduction in total fucose uptake. Apparent failure of soluble [^3H]-fucose taken up within the cells to cause substantial killing may be explained by leakage of this compound from the cells when they were trypsinized from the culture surface before freezing during the selection. Evidence for leakage of soluble metabolites under comparable conditions has been observed in another study (13). In other suicide selections employing [$2\text{-}^3\text{H}$]-mannose as the labeled carbohydrate (Baker et al., J. Cell Biol. 83:453a, 1979), we found that cytotoxicity was correlated with macromolecule-bound radioactivity per cell as well as with increasing length of radiation exposure at -90°C . The results shown for [^3H]fucose in Table 1 are consistent with that experience. (The reduced average incorporation of [^3H]fucose to acid-soluble macromolecules in the later selection cycles probably reflects enrichment for Fuc $^-$ variant cells.)

The selection design that we have employed should enrich for all variant cells that are unable to incorporate radiolabeled sugar into glycoproteins at the nonpermissive temperature (39°C). Cells which are defective in fucose transport, fucose kinase, fucosyltransferases, guanosine diphosphate-fucose pyrophosphorylase, or oligosaccharides which are acceptors for fucosyltransferase and cells which are temperature sensitive for protein synthesis at 39°C will all probably survive the selection.

The initial characterizations of the Fuc $^-$ 687 and Fuc $^-$ 699 clones presented above verify the selection of different lines with the anticipated deficiencies in fucose incorporation to macromolecules. These mutants are not deficient in fucose transport, but do display substantial changes in carbohydrate compositions of their glycoproteins. Moreover, these two Fuc $^-$ isolates appear to represent two new classes of glycoprotein mutants. The profile of lectin cytotoxicities for Fuc $^-$ 687 does not resemble that for any previously described CHO cell line. The profile of Fuc $^-$ 699 does show increased resistance toward wheat germ agglutinin, ricin, and phytohemagglutinin and hypersensitivity toward concanavalin A similar to those in previously described lectin-resistant CHO mutants Pha $^{\text{I}}$ (24, 25) and 15 B (11). However, Fuc $^-$ 699 differs significantly from those isolates with respect to certain biochemical characteristics. While sialic acid and glucosamine are markedly reduced in the lectin-selected clones compared with the wild type (10, 22), no such reduction was detected for Fuc $^-$ 699 in labeling studies (Table 2), or in direct chemical analyses of sialic acid.

Although our experiments were designed to

allow recovery of mutants with a temperature-sensitive (*ts*) phenotype (including conditional lethals), we have not identified any such isolates to date. Analogous suicide selections using [$2\text{-}^3\text{H}$]mannose did yield Man $^-$ mutants temperature sensitive for growth (Baker et al., J. Cell Biol. 83:453a, 1979) resembling concanavalin A-resistant mutants previously described (6). A hamster cell mutant that is temperature sensitive for fucose and mannose incorporation, originally selected as a temperature-sensitive cell cycle mutant, has been described by Tenner et al. (26, 27). In the experiments reported here, we chose to carry out a sequence of relatively mild selection cycles to increase the likelihood of obtaining an assortment of different mutants in the final enriched population. Pouyssegur et al. (21) have recently described use of a strong suicide selection with 2-deoxy[^3H]glucose to obtain a hamster cell mutant deficient in phosphoglucose isomerase activity and glucose transport.

These observations indicate that suicide selection procedures with appropriate ^3H -carbohydrates will prove as effective as previous selections (28, 29) with [^3H]thymidine and ^3H -amino acids. In particular, this approach should enable assemblage of a broad variety of mutants specifically deficient in processes of glycoprotein biosynthesis. The replica plating procedure developed by Esko and Raetz (7) and employed here will certainly aid in the screening of these isolates.

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