Mutant Chinese Hamster Ovary Cells Pleiotropically Defective in Receptor-mediated Endocytosis

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ABSTRACT Populations of Chinese hamster ovary cells selected for resistance to diphtheria toxin were found to be highly enriched for mutants deficient in the uptake of lysosomal hydrolases via the mannose 6-phosphate receptor. One doubly defective mutant, DTF 1-5-1, exhibited increased resistance to Sindbis virus, although it was able to bind and internalize virus normally. Normal production of virus was obtained when, subsequent to virus binding, the mutant was exposed for 2 min to acidic pH. Similarly, a shift to acidic pH increased the sensitivity of DTF 1-5-1 to diphtheria toxin 12-fold. Decreased uptake of lysosomal hydrolases by the mutant correlated with decreased mannose 6-phosphate receptor activity at the cell surface; results of lactoperoxidase-catalyzed iodination indicated that the surface-associated receptor was present but inactive on DTF 1-5-1. Total mannose 6-phosphate receptor activity was also decreased in the mutant and this decrease was reflected by increased secretion of lysosomal hydrolases. The phenotype of DTF 1-5-1 resembles in many ways that of cells treated with ammonia. We suggest that the defect in DTF 1-5-1 stems from an inability to deliver virus, diphtheria toxin, and lysosomal hydrolases to an acidic compartment. Other ligands may be endocytosed through a different pathway since the defect of DTF 1-5-1 did not decrease the endocytosis of ricin, modeccin, or Pseudomonas toxin and had minimal effects on uptake and degradation of low density lipoprotein.

A variety of macromolecular ligands bind to specific receptors on the cell surface and are subsequently internalized via endocytosis (for reviews see references 1–4). Results of morphological studies suggest that there is a common pathway followed by receptor-bound ligands en route to lysosomes (5–9). To dissect the complex sequence of events culminating in appearance of ligand in lysosomes, it would be helpful to have mutants defective in various steps of this pathway. Mutants deficient in the uptake of lysosomal hydrolases (10), low density lipoprotein (LDL) (11), insulin (12), diphtheria toxin (13, 14), ricin (15), and other ligands have been isolated, but in general these mutants were found to be defective in the initial step of receptor-mediated endocytosis, i.e., ligand binding.

One approach for obtaining mutants altered in steps subsequent to ligand binding is to isolate mutants defective in the uptake of two ligands, entry of which is dependent on unrelated receptors (16). In this paper we present the isolation and characterization of Chinese hamster ovary (CHO) cell mutants resistant to diphtheria toxin and deficient in uptake of lysosomal hydrolases through the mannose 6-phosphate receptor. A preliminary report of this work has been presented (17).

MATERIALS AND METHODS

Materials: Ethyl methanesulfonate was purchased from Eastman Laboratory and Specialty Chemicals, Eastman Kodak Co., (Rochester, NY). Diphtheria toxin was provided by Dr. William H. Habig (Bureau of Biologics, Bethesda, MD), Pseudomonas exotoxin by Dr. Stephen Leppia (U.S. Army Medical Research Institute of Infectious Diseases, Frederick MD), and ricin by Dr. Richard J. Youle (National Institutes of Health, Bethesda, MD); modeccin was purchased from Pierce Chemical Co. (Rockford, IL). Iodinated bovine testicular β-galactosidase and rabbit antisera directed against bovine mannose 6-phosphate receptor were provided by Dr. G. Gary Sahagian (National Institutes of Health). Immuno-precipitin (formalin-fixed Staphylococcus A cells, 10% (wt/vol) was purchased from Bethesda Research Laboratories (Rockville, MD). [35S]Methionine (1,000-1,500 Ci/m mole) was from Amersham Corp. (Arlington Heights, IL), proteinase K from Boehringer Mannheim Biochemicals (Indianapolis, IN), SDS (L-5750) from Sigma Chemical Co. (St. Louis, MO), bovine serum albumin (A grade) and lactoperoxidase (B grade, 15 U/mg) were from Calbiochem, (4-Methylumbelliferyl a-L-iduronide was supplied by Dr. Bernard Weissmann (University of Illinois, Chicago, IL), other 4-methylumbelliferyl substrates were purchased from Research Products International Corp. (Mt Prospect, IL); Percoll was from Pharmacia Inc. (Piscataway, NJ). Human lipoprotein-deficient serum, LDL, and [3H]-Putrescine (39 Ci/m mole) and [13C]-carrier-free, ~17 Ci/mg) were from New England Nuclear (Boston, MA), N-N'-dimethylated casein was from Calbi-
ochsen-Behring Corp. and methylamine was from Aldrich Chemical Co. (Milwaukee, WI).

**Cells and Cell Culture:** The isolation of the parental CHO cell line has been previously described (18). All media for cell culture were prepared in the Media Supply Unit of the National Institutes of Health. Growth media (19) and labeling media (20) contained 5% fetal bovine serum or 5% dialyzed fetal bovine serum (Gibco Laboratories, Grand Island, NY), respectively. Procedures of cell culture have been previously described (21). Unless otherwise specified, cells were kept at 34°C.

**Preparation of Labeled Secretions:** Medium conditioned by CHO cells grown in the presence of ammonia is enriched in newly synthesized lysosomal enzymes that are ligands of the mannose 6-phosphate receptor. Thus, for an easily prepared, albeit crude, source of radioactive ligand, WTB cells was used. CHO cells and of that 95% appeared to be dependent on the man 6-P receptor (20). Secretions from human diploid fibroblasts were unsatisfactory as only 2-3% of the radioactive protein was internalized by CHO cells and more than half of this uptake appeared to be independent of the man 6-P receptor.

**Isolation of Mutants:** A stock of WTB was distributed into three T-75 flask/dishes. "E," "F," and "G". These cultures were grown to about 1 x 10^7 cells/T-150 flasks were washed three times with medium lacking methionine and labeled for 12-16 h with 10 ml of medium containing 1 MCl^35S)methionine, 10 μg of nonradioactive methionine and NACl, 10 Mm. The secretions were concentrated, dialyzed, and centrifuged as previously described (20). One flask of cells yielded 1 ml of final material containing 3 x 10^6 cpm; 5-8% of this radioactivity could be taken up by recipient CHO cells and of that 95% appeared to be dependent on the man 6-P receptor (22). Secretions from human diploid fibroblasts were unsatisfactory as only 2-3% of the radioactive protein was internalized by CHO cells and more than half of this uptake appeared to be independent of the man 6-P receptor.

**Radio labeling of the Mannose 6-Phosphate Receptor:** For metabolic labeling with [35S]methionine cells grown in 100-mm dishes were washed three times with medium lacking methionine then incubated for 1 h in 4 ml of medium containing 0.4 MCl^35S)methionine and 4 μg of nonradioactive methionine. Radioactivity was chased by changing the medium to normal growth medium.

**RESULTS**

Three independently mutagenized populations of wild type CHO cells (WTB) were treated with diphertheria toxin (100 ng/ml) for 24 h, survivors were replicated, and the replicating colonies were screened for the ability to take up lysosomal hydrolases via the mannose 6-phosphate receptor (see Materials and Methods). From a total of 6.3 x 10^4 cells treated with toxin, 2,100 colonies were obtained. Of 1,800 colonies tested for hydrolase uptake, 130 appeared to be uptake-deficient. In contrast, no uptake-deficient colonies were observed among 3,200 colonies of mutagenized cells not previously treated with toxin. Putative mutants were picked and cloned.

Two independent mutants were tested for their response to a variety of agents that enter the cell via receptor-mediated endocytosis. As expected, both mutants exhibited increased resistance to diphertheria toxin (Table I). No increase was ob-
served in the resistance of the mutants to modeccin, Pseudomonas toxin, or ricin; instead, both mutants showed an increase in sensitivity to ricin and mutant DTE 1-6-4 exhibited increased sensitivity to Pseudomonas toxin.

Uptake mediated by the mannose 6-phosphate receptor was reduced in DTE 1-6-4 and DTF 1-5-1 (Table II). Using as ligand either purified bovine testicular β-galactosidase, labeled with 125I, or crude [35S]secretions we found that uptake into DTF 1-5-1 was 4% of that measured with parental cells (Table II).

Increased resistance to diphtheria toxin and decreased endocytosis through the mannose 6-phosphate receptor would be expected in the mutants since these were the characteristics employed in their isolation. In addition, the mutants exhibited increased resistance to Sindbis virus and vesicular stomatitis virus. Resistance was manifested in two ways: first, a two- to six-fold reduction in the number of plaques formed on monolayers of mutant versus parental cells; second, a uniform decrease in the size of plaques formed on the mutants (Table III). That plaques were obtained at fairly high frequency on the mutants does not appear to reflect the presence of revertants; identical results were obtained using freshly cloned mutants; moreover, in a test of 10⁷ colonies of DTF 1-5-1 no revertants were detected.

**Mechanism**

The sensitivity of DTF 1-5-1 to diphtheria toxin was increased if, following incubation with toxin, the mutant was shifted to medium of pH 4.5 (Fig. 1). This procedure has

| Agent | WTB | DTE 1-6-4 | DTF 1-5-1 |
|-------|-----|-----------|-----------|
| Diphtheria toxin | 30  | 400  | 2,000 |
| Ricin  | 100 | 30   | 33   |
| Modeccin | 2   | 2    | 2    |
| Pseudomonas toxin | 550 | 250  | 530  |

Cells grown in 24-well dishes to a density of ~1.5 x 10⁶ cells/well were incubated with diphtheria toxin (2 h, growth medium), ricin (3 h, medium + 2 mg/ml BSA without serum), modeccin (2 h, medium + 2 mg/ml BSA without serum) or Pseudomonas toxin (3 h, growth medium). Protein synthesis was assayed in duplicate samples as described in Materials and Methods; EC₅₀ is the dose required to inhibit protein synthesis to 50% of that measured in parallel samples of untreated cells.

**TABLE II**

| Cell | [35S]Secretions 125I-β-Galactosidase |
|------|-------------------------------------|
| WTB  | 19,000 35,000                       |
| DTE 1-6-4 | 3,400 Not tested                   |
| DTF 1-5-1 | 800 1,300                            |

Cells grown in 60-mm dishes to ~1.5 x 10⁶ cells/dish were incubated with 2 ml of growth medium containing 1.7 x 10⁶ cpm of ammonia-induced secretions (see Materials and Methods) or 1 x 10⁶ cpm of [35S]secretions for 4 h. Uptake into WTB cells proceeded linearly during this period. Following uptake the recipient cells were washed and solubilized in 0.1 M NaOH; lysates were neutralized with 0.1 M HCl, and radioactivity and protein content were determined. Results presented have been corrected for uptake in the presence of 2 mM man 6-P; values for nonspecific uptake were similar in WTB and mutant cells ([35S]secretions, 900 cpm/mg; [35S]β-galactosidase, 3,500 cpm/mg).

**TABLE III**

| Fraction of WTB Response |
|-------------------------|
| DTE 1-6-4 | DTF 1-5-1 |
| Sindbis virus |
| Size of plaques | 0.38 | 0.25 |
| Number of plaques | 0.46 | 0.17 |
| Vesicular stomatitis virus |
| Size of plaques | 0.40 | 0.33 |
| Number of plaques | 0.50 | 0.18 |

More than 40 plaques were measured for each virus on each cell type. It should be noted that the size of the plaques increased with increasing time (e.g. Sindbis plaques on monolayers of DTF 1-5-1 increased from 1 to 3 mm from 40 to 72 h), but the number of plaques did not change.

**FIGURE 1** Effect of pH on diphtheria toxin sensitivity of WTB and DTF 1-5-1. Cells (grown in 24-well dishes to ~1.8 x 10⁵ cells/well) were incubated with the indicated amounts of toxin for 2 h at 34°C, then washed three times and incubated for 30 min at 34°C in growth medium plus 20 mM HEPES, pH 4.5 or pH 7.2. The medium was replaced with standard growth medium and after 1 h protein synthesis was measured in duplicate samples (see Materials and Methods); controls were parallel samples taken through the incubations without added toxin. (C) WTB, pH 7.2; (□) WTB, pH 4.5; (●) DTF 1-5-1, pH 7.2; (■) DTF 1-5-1, pH 4.5; (△) DTF 1-5-1 incubated with toxin plus 5 mM ATP, pH 4.5.
previously been shown to overcome the block in toxin sensitivity observed in the presence of ammonia (25, 33). The pH-induced sensitivity of DTF 1-5-1 to toxin appeared dependent on binding of toxin to receptors, since the presence of ATP, an inhibitor of toxin binding (34), during incubation with toxin, abolished the subsequent effect of the shift to acidic pH (Fig. 1). These results suggest that resistance of DTF 1-5-1 to toxin reflects a block occurring subsequent to toxin binding, but before the appearance of toxin in the cytoplasm.

In similar fashion, a brief shift (2 min) to pH 5.0 following incubation of DTF 1-5-1 with Sindbis virus increased subsequent production of virus sixfold (Table IV). Thus, the mutant appeared able to bind virus and to support viral replication. Resistance to virus, like resistance to diphtheria toxin, seems to result from either a failure to internalize bound virions or to move virus from within the endocytic apparatus to the cytoplasm.

To distinguish between these possibilities, DTF 1-5-1 and WTB cells were infected with Sindbis labeled with [35S]methionine. Internalization of viral protein was determined by treating the cells with proteinase K to remove surface-bound virions (35), then measuring the remaining cell-associated radioactivity. Mutant and parent cells appeared similar with respect to both the rates of virus binding and internalization (Fig. 2). After 90 min degradation of virus, determined by measuring TCA-soluble radioactivity, represented only 15% and 25% of the levels of virus internalized by DTF 1-5-1 and WTB, respectively. Binding of virus to mutant and parent at 4°C was similar; 30% of the radioactive virus added was bound to the cells in 1 h. No differences were observed between DTF 1-5-1 and WTB with respect to the rate of internalization of bound virus following a shift of the cells from 4°C to 34°C. Thus, DTF 1-5-1 appeared to be blocked in the translocation of internalized virus to the cytoplasm.

While binding of both diphtheria toxin and Sindbis virus was normal in DTF 1-5-1, binding of ligand to surface-associated mannose 6-phosphate receptors was only 5% of that measured with parental cells (Table V). Lack of surface binding appears to reflect mainly decreased receptor activity, rather than decreased receptor number. Lactoperoxidase-catalyzed iodination followed by immunoprecipitation revealed that the level of surface receptor in DTF 1-5-1 was much greater than that predicted from binding studies (Fig. 3). We observed the same phenomenon in WTB cells that had been preincubated with NH4Cl; no binding of ligand to man 6-P receptors at the cell surface was detected (Table V), yet on iodination significant levels of receptor were found on amine-treated cells (Fig. 3).

In three experiments the level of iodinated man 6-P receptor, ranged from 30-60% of that measured in WTB cells. Lack of surface binding appears to reflect mainly decreased receptor activity, rather than decreased receptor number. Lactoperoxidase-catalyzed iodination followed by immunoprecipitation revealed that the level of surface receptor in DTF 1-5-1 was much greater than that predicted from binding studies (Fig. 3). We observed the same phenomenon in WTB cells that had been preincubated with NH4Cl; no binding of ligand to man 6-P receptors at the cell surface was detected (Table V), yet on iodination significant levels of receptor were found on amine-treated cells (Fig. 3).

![FIGURE 2 Binding and internalization of Sindbis virus by WTB and DTF 1-5-1. Cells (grown to a density of 1 x 10^6 cells/dish in 60-mm dishes) were incubated with [35S]Sindbis, 2.9 x 10^10 cpm, 10 pfu/cell, at 34°C for the treatment. Infected cells were washed twice with cold PBS, then incubated at 4°C with 1 ml of PBS containing 0.5 mg/ml bovine serum albumin, or 0.5 mg/ml proteinase K, for determination of total cell-associated virions or internalized virions, respectively (35). After 45 min 1 ml PBS containing 30 mg bovine serum albumin and 1 mM phenylmethylsulfonyl fluoride was added to each sample, cells were harvested and washed as described (35) then solubilized in 0.1 M NaOH. All assays were done in triplicate; in control samples incubated with virus at 4°C, subsequent treatment with proteinase K removed >95% of cell-associated radioactivity. Values for surface-associated virus were calculated as total cell-associated virus minus internalized (proteinase K-resistant) virus; (W) WTB, surface-associated virus; (□) WTB, internalized virus; (■) DTFT 1-5-1, surface-associated virus; (■) DTF 1-5-1, internalized virus.

**TABLE V**

|                | Binding | cpm/mg cell protein |
|----------------|---------|---------------------|
| **WTB** - Cells | 5,400   |
| **DTF 1-5-1**  - Cells | 200     |
| **WTB** + NH4 - Cells | 20      |
| **WTB** - Membranes | 41,000  |
| **DTF 1-5-1** - Membranes | 8,300   |

Binding to the receptor was determined by displacement of radioactivity bound to intact cells or membranes with 5 mM man 6-P (see Materials and Methods). Values given have been corrected for radioactivity displaced from cells or membranes when binding was conducted in the presence of 5 mM man 6-P (intact cells 250, membranes 300 cpm/mg cell protein). No inhibition of binding to intact cells or membranes was observed when binding was conducted in the presence of 5 mM man 6-P.

*WTB cells were grown with 10 mM NH4Cl for 12 h before the experiment; NH4Cl was also present during the course of the binding assay.

**TABLE IV**

| Cell          | Virus produced (pfu x 10^-3) |
|---------------|-----------------------------|
|               | pH 7.2 | pH 5.5 |
| WTB           | 6.0    | 9.0    |
| DTF 1-5-1     | 0.9    | 5.8    |
| WTB + NH4     | 0.8    | 4.6    |

Cells in 24-well dishes (1.0 x 10^6 cells/well) were chilled to 4°C, then incubated for 1 h at 4°C with Sindbis virus at 10 pfu/cell. Cells were washed three times (at 4°C) then incubated for 2 min at 34°C in medium containing 2-[morpholino]ethane sulfonic acid, 10 mM, and BSA, 2 mg/ml, at pH 7.2 or pH 5.5 (35). This medium was replaced with growth medium and incubation was continued at 34°C. (In samples treated with NH4Cl, 10 mM, the agent was present during the entire course of the experiment). After 5 h, aliquots of the medium were diluted and plated on monolayers of WTB for determination of plaque-forming units (see Materials and Methods).
phate receptors indicated that the mutant had 20% of the receptor activity of parental cells (Table V). Examination of biosynthesis and degradation of receptor by metabolic labeling indicated equal rates of receptor biosynthesis in DTF 1-5-1 and WTB; the half-life of the receptor was found to be 9.5 h in the mutant and 18 h in the parent (Fig. 4). Addition of NH₄Cl to WTB cells after a radioactive pulse also resulted in increased turnover of the receptor (data not shown).

Consistent with its decreased level of mannose 6-phosphate receptor activity (20), DTF 1-5-1 exhibited reduced intracellular levels of many acid hydrolases and secreted those enzymes in correspondingly elevated amounts (Table VI). Similar results were obtained by metabolically labeling the cells and immunoprecipitating α-L-iduronidase and β-hexosaminidase from cell extracts and media (data not shown). When cell-free extracts of mutant and parent were fractionated on 27% Percoll gradients (37), two peaks of acid hydrolase activity were obtained, consistent with previous reports (21, 38). Extracts of DTF 1-5-1 showed a diminution of enzyme activity only in the denser of the two peaks, i.e., in the region corresponding to lysosomes (Fig. 5).

Fig. 6 shows the uptake and degradation of 125I-low density lipoprotein by mutant and wild type cells. At 5 μg/ml of LDL the rate of uptake into DTF 1-5-1 was 75% of that measured for WTB; degradation was 50% that of WTB. This reduction in the rate of degradation of LDL may reflect the mutant's deficiency in acid hydrolases. The kinetic parameters of LDL uptake differ slightly between DTF 1-5-1 and WTB at concentrations of LDL < 50 μg/ml; at concentrations of ligand ≥50 μg/ml the kinetic parameters were identical for the two cell types (Fig. 7).

The phenotype of DTF 1-5-1 is similar in many ways to that of amine-treated cells. One of the proposed targets in inhibition of endocytosis by the amines is transglutaminase (39), but measurements of the activity of this enzyme in crude extracts of mutant and parent cells revealed no significant differences either in the Kₘ or rate of transfer of putrescine, or in the inhibition of enzyme activity by methylamine.

**DISCUSSION**

The initial goal of this study was the isolation of mutants pleiotropically defective in receptor-mediated endocytosis. The mutants described here, DTE 1-6-4 and DTF 1-5-1, were found to be altered in the uptake of diptheria toxin, lysosomal enzymes, and two lytic RNA viruses, Sindbis virus and VSV. Preliminary studies indicate that DTF 1-5-1 also is unable to accumulate iron from complexes of transferrin-iron (Klausner,
R. D., and A. R. Robbins, unpublished data). To ensure that these multiple defects result from a single pleiotropic mutation, rather than multiple mutations, it will be necessary to isolate revertants of the mutants. The high frequency at which mutants deficient in lysosomal enzyme uptake were found among the diphtheria-toxin-resistant cells, especially in the absence of selective pressure, argues against the possibility of multiple mutations. Interestingly, cross-resistance to lytic RNA viruses was found in a number of KB cell mutants that had been selected only for resistance to diphtheria toxin (40).

Although temperature-shifts were employed in both selection and screening, neither resistance to diphtheria toxin nor the deficiency in lysosomal enzyme uptake were found to be temperature-sensitive in any of seven mutants tested to date (data not shown). Using our procedure it may not be possible to isolate mutants with temperature-sensitive defects in steps subsequent to internalization. Toxin accumulated within the cell at the nonpermissive temperature may result in cell death on return to the permissive temperature.

The defect in DTE 1-6-4 and DTF 1-5-1 impairs endocytosis of some ligands but not of others. Resistance to ricin, modeccin, and Pseudomonas toxin was not increased in the mutants, and only marginal effects were observed on uptake of low density lipoprotein in DTF 1-5-1. Thus, while morphologic studies indicate that diphtheria toxin (9), Pseudomonas toxin (41), LDL (5), and lysosomal enzymes (8) share a common uptake pathway, endocytosis of these ligands must differ at some stage subsequent to ligand binding. CHO cell mutants defective in uptake, but not in binding, of both Pseudomonas toxin and ricin have recently been reported (16). No change was found in sensitivity of these cells to diphtheria toxin. These results, like our own, suggest different structural or functional requirements for endocytosis of different ligands.

Our results indicate that the block in Sindbis infectivity in DTF 1-5-1 occurs at penetration into the cytosol. Our findings with diphtheria toxin are consistent with this localization of the defect. Entry of Sindbis into the cytoplasm, like that of Semliki Forest virus (35, 42), appears to be induced by acidic pH; this is also the case for diphtheria toxin (25, 33). One explanation for the phenotype of DTF 1-5-1 is that internalized virus and toxin never encounter an acidic environment, due either to failure of the endocytic vesicle to fuse with an acidic compartment or to a defect in acidification of the endosome (43) and/or lysosome (44). Alteration in lysosomal pH seems least likely since DTF 1-5-1 was able to degrade LDL.

Is this postulated inability to deliver ligand to an acidic compartment consistent with our observations regarding mannose 6-phosphate receptor mediated uptake in DTF 1-5-1? Dissociation of ligand from the mannose 6-phosphate receptor has been proposed to require an acidic milieu (45); thus lack of surface-binding activity in DTF 1-5-1 and ammonia-treated normal cells (reference 45 and this paper) could result from intracellular accumulation of ligand-receptor complexes. However, iodination at 4°C revealed near-normal numbers of surface-associated receptor molecules in both the mutant and ammonia-treated cells. It is unlikely that these inactive recep-

**Figure 5** Profile of β-hexosaminidase activity from WTB and DTF 1-5-1 on Percoll gradients. Cells (grown to 1.5 x 10⁷ cells/flask in T-150 flasks) were harvested and disrupted as previously described (21, 38). Cell extracts were layered on 27% Percoll suspensions (37) over cushions of 60% sucrose (wt/vol) and centrifuged for 1 h at 18,500 rpm in a Sorvall SV 288 rotor. Fractions (one ml) are numbered in the order of decreasing density. Enzyme activity is expressed as nmol substrate hydrolized per h. (A) DTF 1-5-1; (B) WTB.

**Figure 6** Uptake of ¹²⁵I-LDL into WTB and DTF 1-5-1. Cells (grown to 8 x 10⁶ cells/dish on 60-mm dishes) were incubated for 24 h in medium plus 5% human lipoprotein-deficient serum. Medium (1.5 ml) containing 7.5 µg ¹²⁵I-LDL (3.3 x 10⁶ cpm) was added. At the times indicated medium was removed and chilled, the cells were washed four times with cold medium (without serum) and twice with PBS. Cells were solubilized in 0.1 N NaOH for determination of cell-associated radioactivity and protein (32). Aliquots of the medium were precipitated with trichloroacetic acid (15%) for determination of degraded LDL; <2% of the starting material was soluble in trichloroacetic acid. Values presented are from duplicate dishes and have been corrected for nonspecific uptake and degradation (measured in the presence of 300 µg/ml LDL); (○) WTB, cell-associated LDL; (■) WTB, degraded LDL; (●) DTF 1-5-1, cell-associated LDL; (■) DTF 1-5-1, degraded LDL.

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The similarities between mutant DTF 1-5-1 and amine-treated parental cells have been emphasized, but the resemblance is not without exception. Amines have been shown to inhibit toxicity of modeccin (47) and to toxic effects of monensin (46). Total mannose 6-phosphate receptor activity without loss of surface receptor molecules (46). Total mannose 6-phosphate receptor activity was also decreased in DTF 1-5-1 albeit to a lesser degree than surface receptor. We have at present no explanation for these observations.

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