Disruptions in Golgi Structure and Membrane Traffic in a Conditional Lethal Mammalian Cell Mutant Are Corrected by ε-COP

Qiu Guo, Eliza Vasile, and Monty Krieger

Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Abstract. The CHO cell temperature-sensitive mutant ldlF exhibits two defects in membrane traffic at the nonpermissive temperature (39.5°C): rapid degradation of LDL receptors, possibly caused by endocytic missorting, and disruption of ER-through-Golgi transport. Here, we show that at 39.5°C, the Golgi in ldlF cells dissociated into vesicles and tubules. This dissociation was inhibited by AlF_4^-, suggesting trimeric G proteins are involved in the dissociation mechanism. This resembled the effects of brefeldin A on wild-type cells. We isolated a hamster cDNA that specifically corrected the ts defects of ldlF cells, but not those of other similar ts mutants (ldlE, ldlG, ldlH, and End4). Its predicted protein sequence is conserved in humans, rice, *Arabidopsis*, and *Caenorhabditis elegans*, and is virtually identical to that of bovine ε-COP, a component of the coatomer complex implicated in membrane transport. This provides the first genetic evidence that coatomers in animal cells can play a role both in maintaining Golgi structure and in mediating ER-through-Golgi transport, and can influence normal endocytic recycling of LDL receptors. Thus, along with biochemical and yeast genetics methods, mammalian somatic cell mutants can provide powerful tools for the elucidation of the mechanisms underlying intracellular membrane traffic.

THE past several years have seen an explosion of information about the molecular mechanisms underlying the endocytic and secretory pathways of intracellular membrane traffic (Bennett and Scheller, 1993; Rothman and Orci, 1992; Pryer et al., 1992; Warren, 1993). Two common themes that have arisen from this work are that (*a*) small (e.g., ADP ribosylation factors (ARFs)[1], rabs) and heterotrimeric GTP-binding proteins appear to participate in intracellular membrane transport; and (*b*) there are multisubunit protein complexes (e.g., coatomers, NSF/SNAPs/SNAREs) that catalyze and regulate membrane fusions and fissions. At least some of the components of these complexes participate in reactions used throughout the secretory and endocytic pathways. The isolation and characterization of yeast mutants with defects in intracellular membrane transport (reviewed in Pryer et al., 1992) and the development of numerous in vitro transport assays (reviewed in Balch, 1989; Rothman and Orci, 1992) have played critical roles in these advances.

The isolation and detailed molecular analysis of mammalian somatic cell mutants with defects in intercompartmental transport will complement these approaches and extend our understanding of transport mechanisms. To help define and analyze the gene products and functions required for intracellular membrane traffic, we have developed methods to isolate mutant CHO cells with defects in LDL receptor activity (Krieger, 1983; Krieger et al., 1981, 1983, 1985; Malmstrom and Krieger, 1991; Hobbie et al., manuscript submitted for publication[2]). These mutants define nine recessive complementation groups, designated *ldlA-ldlI* (Kingsley and Krieger, 1984; Malmstrom and Krieger, 1991; Hobbie et al., manuscript submitted for publication). Four of these groups, *ldlE-ldlH*, comprise conditional lethal mutants that express temperature-sensitive defects in the secretory pathway (Hobbie et al., manuscript submitted for publication; Malmstrom and Krieger, 1991; Guo, Q., A. Fisher, and M. Krieger, unpublished data). The *ldlE*, *ldlF*, and *ldlG* mutants also exhibit a second striking temperature-sensitive phenotype. Cell surface LDL receptors initially synthesized in these mutants at the permissive temperature (34°C) are

Address all correspondence to Monty Krieger, Room 68-483, Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.

1. *Abbreviations used in this paper*: ARF, ADP ribosylation factor; BFA, brefeldin A; COPs, coatomer proteins; DOGS, dioctadecylamidoglycylspermine; LB-amp/tet, Luria broth agar petri dishes containing 15 μg/ml ampicillin and 8 μg/ml tetracycline; P-FCS, PBS containing 10% FCS.

2. At press time, there has been an update on the status of this manuscript: Hobbie, L., A. S. Fisher, S. Lee, A. Flint, and M. Krieger. 1994. Isolation of three classes of conditional-lethal Chinese hamster ovary cell mutants with temperature-dependent defects in LDL receptor stability and intracellular membrane transport. *J. Biol. Chem.* In press.

abnormally rapidly degraded at the nonpermissive temperature (39–40.5°C), raising the possibility of temperature-dependent endocytic defects that result in missorting and receptor degradation (Hobbie et al., manuscript submitted for publication). The novel properties of ldlF and ldlG mutants have recently been exploited by Musil and Goodenough (1993) to help establish that the assembly of gap junction proteins into connexons occurs after their exit from the endoplasmic reticulum.

In the current work, we have examined the ultrastructure of the Golgi at the nonpermissive temperature in one of these mutants, ldlF, and have cloned a cDNA which, when transfected into ldlF cells, corrects all of their temperature-sensitive defects. The predicted amino acid sequence of the correcting gene is highly conserved in hamsters, humans, rice, *Arabidopsis*, and *Caenorhabditis elegans*, and is virtually identical to that of bovine ε-COP, whose sequence was very recently reported by Hara-Kuge et al. (1994). ε-COP is one of seven coat proteins (α, β, β′, γ, δ, ε, and ζ) that form a stable complex called the coatomer. Coatomers can be found in the cytoplasm and associated with the membranes of the Golgi apparatus or non-clathrin-coated (COP-coated) vesicles (Duden et al., 1991; Waters et al., 1991; Serafini et al., 1991; Stenbeck et al., 1992, 1993; Pepperkok et al., 1993; Ostermann et al., 1993). They have been shown to be required for the formation of functional Golgi transport vesicles in vitro (Ostermann et al., 1993). This work provides the first direct genetic evidence that in animal cells ε-COP, and thus the coatomer complex (Kuge et al., 1993), can play a role both in establishing or maintaining Golgi structure and in mediating ER-through-Golgi transport, and that it can influence normal endocytic recycling of LDL receptors. Thus, ldlF cells will provide a powerful tool for the genetic and biochemical analysis of ε-COP and coatomer function in intracellular membrane traffic through the secretory and endocytic pathways.

Materials and Methods

Materials

Reagents (and sources) were methionine- and cysteine-free Ham's F12 medium (Gibco Laboratories, Grand Island, NY); $Na^{125}I$ (Amersham Corp., Arlington Heights, IL); L-[^{35}S]methionine and [^{35}S]dATP-α-S(>1,000 Ci/mmol) (NEN/Du Pont, Boston, MA); polyethylene glycol 1000 (BDH Ltd., Dorset, United Kingdom); goat anti-rabbit IgG with and without FITC labeling (Cappel Research Reagents, Organon Teknika Corporation, Durham, NC); Epon 812 (Marivak Ltd., Halifax, Nova Scotia); cell culture media and supplements (Gibco Laboratories or Hazelton, Lenexa, KS). Newborn calf lipoprotein-deficient serum, LDL, and ^{125}I-LDL were prepared as previously described (Krieger, 1983). Other reagents were obtained as previously described (Krieger, 1983) or were purchased from standard commercial suppliers.

Cell Culture

Wild-type CHO and the LDL receptor–deficient and temperature-sensitive conditional lethal mutant cells (ldlE [clone 6], ldlF [clone 2], ldlG [clone 42], ldlH [clone 7], and End4 [clone V24.1]) were maintained in stock culture in medium A (Ham's F12 containing 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin), supplemented with 5% (vol/vol) fetal bovine serum (medium B), at 34°C in a 5% CO_2/95% air humidified incubator. COS-7 cells were maintained in medium C (DME [Gibco Laboratories] containing 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin). Assays of LDL receptor activity were carried out in medium D (medium A supplemented with 3% [vol/vol] newborn calf lipoprotein-deficient serum) to induce increased expression of LDL receptors (Goldstein et al., 1983; Krieger, 1983). Transfected cells were maintained in medium E (medium B supplemented with Geneticin [G418, 175 μg/ml]) at 39.5°C. The ldlE-ldlH cells were isolated as previously described (Malmstrom and Krieger, 1991; Hobbie et al., manuscript submitted for publication) and End4 clone V.24.1 was generously supplied by Dr. Rockford Draper (University of Dallas). We have used a standard complementation assay (Malmstrom and Krieger, 1991; Hobbie et al., manuscript submitted for publication) to establish that the End4 complementation group is distinct from those of ldlE, ldlF, and ldlG (not shown).

Microscopy

Immunofluorescence Microscopy. Immunofluorescence microscopy was performed as previously described (Anderson et al., 1978). Monolayers of wild-type CHO or mutant ldlF cells were grown in medium B on glass coverslips for 1–2 d at 34°C until they reached near confluence. Some of the monolayers were maintained at 34°C while others were shifted to 39.5°C for 4, 6, or 12 h. In some experiments, some of the cells were pretreated with AlF_4^- (50 μM $AlCl_3$ and 30 mM NaF) both before the temperature shift (10 min) and throughout the subsequent incubation. At the end of the incubation period, the cells were washed twice with PBS containing 1 mM $MgCl_2$ and 0.1 mM $CaCl_2$, fixed with 2% formaldehyde for 30 min, washed with PBS, permeabilized with methanol at −20°C for 5 min, rehydrated in PBS, and then incubated with PBS containing 10% fetal calf serum (10% P-FCS) for 30 min to block nonspecific protein binding. The cells on coverslips were incubated with the first antibody (rabbit polyclonal anti-mannosidase II IgG, gift from J. Donaldson and R. Klausner, National Institutes of Health, Bethesda, MD) diluted 1:500 in 10% P-FCS containing 0.2% saponin for 1 h at 37°C, washed several times with 10% P-FCS, and then incubated with the second antibody (goat anti-rabbit IgG-FITC diluted 1:1,000 in 10% P-FCS) for 30 min at room temperature. The samples were washed several times with 10% P-FCS, once with PBS, and rinsed with distilled water before mounting in Mowiol (Calbiochem-Novabiochem Corp., La Jolla, CA). The cells were viewed with an Axioplan microscope (Carl Zeiss, Inc., Thornwood, NY) using a 100× (1.3NA) objective and a fluorescein filter package, and they were photographed with Tri-X 400 film (Eastman Kodak Co., Rochester, NY).

Electron Microscopy. Wild-type CHO or mutant ldlF cells were grown on plastic culture dishes and maintained at 34°C or shifted to 39.5°C for 6 or 12 h as described above for immunofluorescence microscopy. At the end of the incubation, the cells were rapidly rinsed with PBS, fixed with 2% glutaraldehyde in 0.1 M sodium cacodylate–HCl, pH 7.2 for 60–90 min at 4°C, and postfixed with 2% osmium tetraoxide for 60 min at 4°C, followed by dehydration in graded alcohol ⩽70% and en block staining with 70% alcoholic uranyl acetate for 30 min at 4°C as previously described (Vasile et al., 1983). The samples were then processed for Epon embedding. Thin Epon sections were poststained with uranyl acetate and Reynold's lead citrate and viewed with an electron microscope (model 1200CX; JEOL USA, Cranford, NJ) operated at 80 kV.

Construction of cDNA Expression Library

Poly(A)$^+$ RNA prepared (Libermann et al., 1987) from wild-type CHO cells was used to construct a size-selected, unidirectional cDNA expression library. First-strand cDNA was synthesized from poly(A)$^+$ RNA primed with an oligo(dT) oligonucleotide containing a Not I site (Invitrogen, San Diego, CA). After synthesis of the second strand, the blunt-ended cDNA was ligated to 8-(5′ CTCTAAAG) and 12-mer (5′ CTTTAGAGCACA) phosphorylated BstXI linkers and then digested with NotI (Seed and Aruffo, 1987). The cDNAs >1 kb in length were isolated on a 1% agarose gel, purified by GeneClean II kit (BIO 101, Inc., Vista, CA), and ligated into the BstXI and NotI sites of the pcDNA I vector (Invitrogen). The ligation mixtures were electroporated into *Escherichia coli* strain MC1061/P3 (Dower et al., 1988), resulting in ~1 × 10^6 independent recombinants with an average insert size of 1.5 kb. 19 pools of 40,000–60,000 recombinant bacteria were grown on Luria broth agar petri dishes containing 15 μg/ml ampicillin and 8 μg/ml tetracycline (LB-amp/tet), and bacterial colonies were scraped from the dishes and aliquots saved in 25% glycerol stock at −150°C. Pools of recombinant bacteria from the remainder of the sample were grown for 3 h in 1 liter of LB-amp/tet, and then the plasmid DNAs were purified using Qiagen kits (Qiagen Inc., Chatsworth, CA).

DNA Transfection and Isolation of Temperature-resistant Revertants

ldlF cells were cotransfected with a pool of cDNA in pcDNA I and with

pSV2neo, and selected at the nonpermissive temperature in medium containing G418. On day 0, ldlF cells were plated at 950,000 cells/100-mm dish in medium F (medium A supplemented with 10% [vol/vol] fetal bovine serum) at 34°C. On day 2, the cells were washed three times with serum-free Ham's F12 medium. A complex of DNA and DOGS (dioctadecylamidoglycylspermine, a gift from J. R. Falck, Southwestern Medical School, Dallas, TX) was prepared by adding 150 μl of a DNA solution (12 μg of library plasmids and 1.2 μg of supercoiled pSV2neo in 300 mM NaCl) to 150 μl of solution A (0.33 mg/ml DOGS, 300 mM NaCl). The DNA/DOGS complex was added to the cells in 3.6 ml of serum-free Opti-MEM (GIBCO Laboratories) containing 100 U/ml penicillin and 100 μg/ml streptomycin (Behr et al., 1989; Loeffler et al., 1990). After a 14-h transfection period, the cells were washed three times with serum-free Ham's F12 medium and incubated in medium F for 1 d before being replated at a density of 3×10^6 cells/100-mm dish in medium F and incubated at 34°C. 1 d later, the culture medium was replaced with selection medium, medium G (medium D plus 175 μg/ml G418), and the cells were shifted to the nonpermissive temperature (39.5°C). This medium was changed every 2–3 d, and G418- and temperature-resistant colonies appeared 10–12 d after transfection. The surviving colonies were harvested and grown to mass culture for further analysis. In this experiment, only one independent colony that grew well at the nonpermissive temperature was obtained.

Recovery of Low Molecular Weight DNA after Fusion of the Surviving Colony with COS-7 Cells and Isolation of Stable Transfectants

Cell fusion was performed by modification of the methods of Dawson et al. (1991) and Naglich et al. (1992). In brief, on day 0, 2:1 mixtures of cells from the single independent colony from experiment 1 and COS-7 cells were plated in six-well dishes at a total concentration of 1.2×10^6 cells/well in medium H (1:1 mixture of medium C and medium F) at 37°C. On day 1, the cells were washed three times with PBS before fusion by exposure to 1 ml of 1:1 (vol/vol) mixture of polyethylene glycol 1000 and Ham's F12 medium for 1 min at room temperature. The cells were washed three times with 10% dimethyl sulfoxide in medium H and refed with medium H. On day 2, the hybrid cells were replated in 100-mm dishes in medium H.

On day 4, low molecular weight DNA was recovered essentially as described previously (Naglich et al., 1992). The hybrid cells were washed twice with 150 mM NaCl and 10 mM Tris-HCl, pH 7.5, before lysing with 0.6% SDS, 10 mM EDTA, 10 mM Tris-HCl, pH 7.5, for 24 h at 4°C. High molecular weight DNA was removed by centrifugation at 100,000 *g* for 50 min at 4°C. The supernatant containing low molecular weight DNA was digested with proteinase K for 1 h at 65°C, followed by extensive phenol extraction. The DNA was then recovered by ethanol precipitation and electroporated into *E. coli* strain MC1061/P3, and the transformants were selected on LB-amp/tet plates. Plasmid DNAs were purified from eight pools of these colonies (100 independent recombinant cDNAs/pool). Each pool of DNA was transfected into ldlF cells as DNA/DOGS complexes, and transfectants were selected by incubation at 39.5°C in medium G. Surviving colonies (~100–300 colonies from 1.2×10^6 transfected cells) were observed from ldlF cells transfected with seven of these eight pools. Two rounds of iterative subpooling and rescreening yielded a single positive clone (p*LDLF*-1) containing a 1.1-kb insert.

Stable p*LDLF*-1 transfectants of ldlF cells, designated ldlF[*LDLF*], were obtained by cotransfecting a DNA/DOGS complex of p*LDLF*-1 and PSV2neo into ldlF cells as described above. The transfectants were selected at the nonpermissive temperature and in medium G, and all of the surviving colonies (>100) were pooled and grown to mass culture.

Transfection of pLDLF-1 into Various Temperature-sensitive CHO Mutants

On day 0, ldlE cells were plated into six-well dishes at 180,000 cells/well in medium F at 34°C, ldlF and End 4 cells at 200,000 cells/well, ldlG cells at 250,000 cells/well, and ldlH cells at 300,000 cells/well. On day 2, the cells were cotransfected with mixtures of 1.98 μg p*LDLF*-1, 0.22 μg pSV2neo, and DOGS as described above, the amounts of reagents were scaled down proportionately from 100-mm dish to six-well dish transfections. After a 14-h incubation at 34°C, the cells were washed and incubated in medium F at 34°C for 24 h. The cells were then harvested and replated at low and high densities (40,000 cells/well and 400,000 cells/well) in six-well dishes in medium F and incubated at 34°C. 1 d later, the medium was replaced with medium G. To assess transfection efficiency (G418 resistance), we incubated the low density dishes at 34°C for 16 d. To assess complementation of temperature-sensitive lethality (G418 resistance and growth at the nonpermissive temperature), we incubated the high density dishes at 39.5°C for 16 d. The surviving cells were washed in PBS, fixed with methanol for 15 min, and stained with crystal violet.

Other Procedures

LDL Receptor Activity Assay. The receptor-mediated degradation of ^{125}I-LDL (10 μg protein/ml, 401 cpm/ng protein) was measured in medium D at 39.5°C as described previously (Krieger, 1983). The high affinity degradation values shown represent the differences between measurements made in the absence (duplicate determinations) and presence (single determinations) of excess unlabeled LDL (400 μg protein/ml) and are presented as nanograms of ^{125}I-LDL degraded in 5 h/mg of cell protein. Protein concentrations were determined by the method of Lowry et al. (1951).

DNA Sequencing and Sequence Analysis. Both strands of the insert in p*LDLF*-1 were sequenced using the dideoxy chain termination method with Sequenase (United States Biochemical Corp., Cleveland, OH). Surveys of the sequence databases and analyses of protein sequence motifs were performed using the BLAST network service at the National Center for Biotechnology Information (National Institutes of Health, Bethesda, MD) and the program MOTIFS (with PROSITE, version 10.2) in the Genetics Computer Group Sequence Analysis Software Package (version 7.3, Madison, WI; Altschul et al., 1990; Devereux et al., 1984).

Metabolic Labeling, Immunoprecipitations, and Protein Secretion. Metabolic labeling of cells with [^{35}S]methionine in medium I (medium D prepared using methionine-free Ham's F12), immunoprecipitation of LDL receptors from labeled cell lysates using an anti-LDL receptor polyclonal antibody (anti-C), electrophoresis, and autoradiography were performed as previously described (Laemmli, 1970; Kozarsky et al., 1986). To detect secretion of metabolically labeled proteins, we pulse labeled cells in six-well dishes for 30 min with 300 μCi/ml [^{35}S]methionine in medium I, washed the cells once with Ham's F12, and chased them for 2 h in 0.5 ml of medium D containing 1 mM unlabeled methionine, all at the indicated temperatures. The harvested chase media were clarified by centrifugation at 12,850 *g* for 60 min at 4°C, and to 24 μl from each sample we added 8 μl of 4× loading buffer. The specimens were then boiled for 5 min and loaded onto 5–15% polyacrylamide gradient gels for electrophoresis (Hobbie et al., manuscript submitted for publication).

Results

Temperature-dependent Ultrastructural Defects in ldlF Cells

Our previous analysis of the mutant phenotypes of ldlF cells suggested that major disruptions in Golgi function might, at least in part, account for the temperature-sensitive defects in protein transport through the secretory pathway (Hobbie et al., manuscript submitted for publication). To determine if this was the case, we compared the ultrastructure of the Golgi apparatus in wild-type CHO and ldlF cells at the permissive (34°C) and nonpermissive (39.5°C) temperatures. We used both immunofluorescence microscopy with antibodies to the Golgi-associated protein mannosidase II (Moremen and Touster, 1985; Lippincott-Schwartz et al., 1989) and electron microscopy. Fig. 1 *a* shows that mannosidase II immunoreactivity in ldlF cells at 34°C was distributed in a compact perinuclear pattern of short, rodlike structures, a distribution characteristic of the Golgi in CHO cells (Kao and Draper, 1992). Essentially identical results were observed for wild-type CHO cells at 34°C (not shown) and 39.5°C (Fig. 1 *b*). In contrast, the Golgi-associated immunostaining of mannosidase II in ldlF cells gradually dispersed into a diffuse punctate pattern after the cells were transferred to the nonpermissive temperature. After a 6-h incubation at 39.5°C, there was little classic perinuclear Golgi immunostaining (Fig. 1 *c*). The rate of loss of the perinuclear

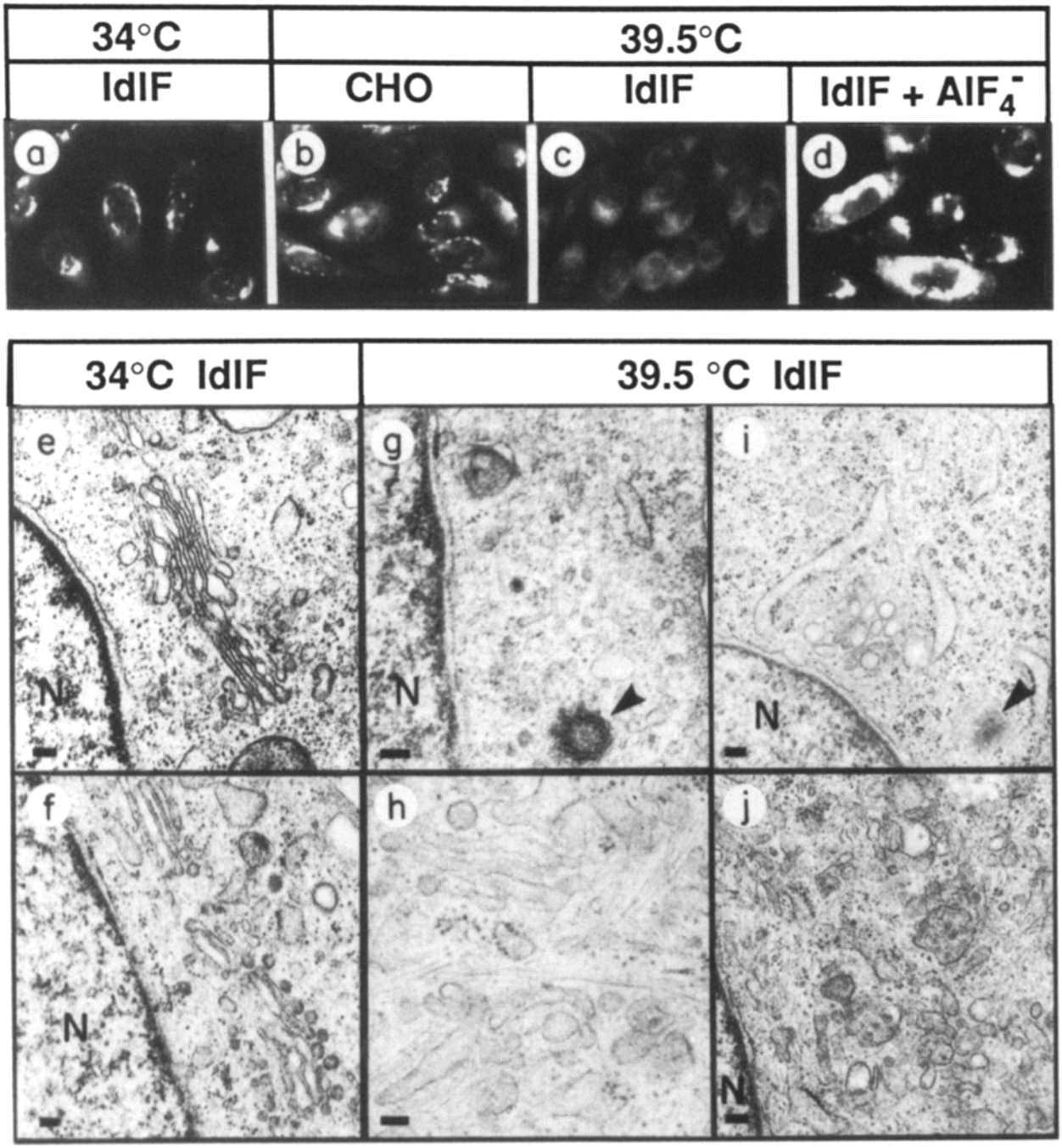

Figure 1. Structure of the Golgi apparatus in CHO cells and mutant ldlF cells at the permissive (34°C) and nonpermissive (39.5°C) temperatures. Nearly confluent monolayers of CHO and ldlF cells in medium B were incubated either at 34°C (*a*, *e*, and *f*) or 39.5°C for 6 h (*b*, *c*, *d*, *g*, *h*, *i*, and *j*) before fixation and analysis as described in Materials and Methods. Panels *a–d* show the cellular distribution of the Golgi marker mannosidase II detected by immunofluorescence microscopy using a polyclonal anti-mannosidase II antibody. In *d*, ldlF cells were incubated in medium B containing AlF_4^- (50 μM $AlCl_3$ and 30 mM NaF) for 10 min before the shift to 39.5°C and throughout the 6-h incubation at 39.5°C (+AlF_4^-). Panels *e–j* show the transmission electron micrographs of the perinuclear Golgi regions of the cells. The arrowheads in *g* and *i* indicate the locations of centrioles and *N* denotes the nucleus. Bars, 100 nm.

pattern of Golgi-associated immunostaining at 39.5°C appeared similar to that of the onset of defects in the posttranslational modifications of LDL receptors (Hobbie et al., manuscript submitted for publication). In other experiments with ldlF cells (not shown), we have also observed a temperature-dependent dispersion of both β-COP, a component of coatomers (Duden et al., 1991; Waters et al., 1991; Serafini et al., 1991; Stenbeck et al., 1993; Ostermann et al., 1993), and ldlCp, a peripheral Golgi-membrane protein required for several *medial* and *trans*-Golgi–associated processing reactions (Kingsley et al., 1986; Podos, S., P. Reddy, J. Ashkenas, and M. Krieger, manuscript in preparation).

Electron microscopy showed that, at the nonpermissive temperature, there were dramatic ultrastructural changes in

the Golgi apparatus (Fig. 1, *e–j*). At the permissive temperature (Fig. 1, *e* and *f*), the Golgi in ldlF cells comprised a series of characteristically stacked cisternae and budding vesicles located near the nucleus and centriole (e.g., see Farquhar and Palade, 1981; Zuber et al., 1991). In as little as 4 h after shifting (not shown) to the nonpermissive temperature, the Golgi began to dissociate into vesicles and tubules, which are seen in Fig. 1, *g–j* after a 6-h incubation at 39.5°C. In addition to the loss of the Golgi's structural integrity, at the nonpermissive temperature, we observed some dilation of the rough endoplasmic reticulum (Fig. 1 *i*) and the presence of cytoplasmic filaments (Fig. 1, *i* and *h*) that were not observed at 34°C.

Because heterotrimeric GTP-binding proteins have been implicated in Golgi function, we examined the effects of AlF_4^- on the Golgi disruption in ldlF cells. AlF_4^-, in combination with GDP, can act as a nonhydrolyzable GTP surrogate for the heterotrimeric, but not low molecular weight, GTP-binding proteins (Stow et al., 1991; Donaldson et al., 1991*b*; Bomsel and Mostov, 1992; Rothman and Orci, 1992; Pryer et al., 1992; Schwaninger et al., 1992; Pimplikar and Simons, 1993; Stenbeck et al., 1993; Wilson et al., 1993; Montmayeur and Borrelli, 1994). Inhibition of GTP hydrolysis or substitution of GTP with either nonhydrolyzable analogues (e.g., GTPγS) or structural surrogates (a GDP-AlF_4^- complex) appear to stabilize the Golgi's structure (Donaldson et al., 1991*a*). ldlF cells were treated with AlF_4^- (50 μM $AlCl_3$ and 30 mM NaF) at 34°C for 10 min, and then incubated for 6 h at 39.5°C in the presence of AlF_4^- before immunofluorescence analysis of mannosidase II localization. AlF_4^- prevented the temperature-dependent dispersion of Golgi-associated immunofluorescence (Fig. 1, compare *d* with *c*). Therefore, the *LDLF* gene product might influence Golgi structure by participating in a trimeric GTP-binding protein-dependent assembly or stabilization of Golgi-associated proteins, such as the coatomer proteins. Unfortunately, it was not possible to analyze the intracellular processing of newly synthesized proteins in cells treated with AlF_4^- using standard metabolic labeling, pulse-chase techniques (e.g., see below), because AlF_4^- inhibited incorporation of [^{35}S]methionine into these proteins (not shown).

Expression Cloning of ldlF

To identify the molecular basis of the defects in ldlF cells, we cloned a cDNA that corrects the characteristic defects in ldlF cells (see Materials and Methods). An unamplified cDNA expression library from wild-type CHO cells was prepared in the vector pcDNA I. ldlF cells were cotransfected (Behr et al., 1989; Loeffler et al., 1990) with mixture of this library and pSV2neo, which carries a resistance marker for G418. Transfected phenotypic revertants were selected by incubation at the nonpermissive temperature in medium containing G418. In one experiment, we obtained one independent surviving colony from $\sim 7 \times 10^7$ transfected cells. (We observed no survival of control ldlF cells transfected with the insert-free vector pcDNA I plus pSV2neo under these selection conditions.) Plasmids were recovered from the surviving transfectant using a COS cell fusion technique (Dawson et al., 1991; Naglich et al., 1992) and one, p*LDLF*-1, was used for further analysis. A pool of stable transfectants, designated ldlF[*LDLF*], were isolated after cotransfection of ldlF cells with p*LDLF*-1 and pSV2neo.

Correction of Pleiotropic Defects by Transfection with pLDLF-1

Six distinguishing characteristics of ldlF cells at the nonpermissive temperature are (*a*) dramatically reduced LDL receptor activity; (*b*) instability of cell surface LDL receptors; (*c*) abnormal posttranslational processing of proteins in the secretory pathway; (*d*) drastically reduced protein secretion; (*e*) death after prolonged incubation (>24 hr) at the nonpermissive temperature (Hobbie et al., manuscript submitted for publication); and (*f*) dissociation of the Golgi apparatus. Fig. 2 compares several of these properties of ldlF cells with those of transfected ldlF[*LDLF*] and wild-type CHO cells. Fig. 2 *a* shows the results of an LDL receptor activity assay in which ^{125}I-LDL degradation was used to measure, at the nonpermissive temperature, the end products of the LDL receptor pathway (surface binding, internalization, and lysosomal degradation [Goldstein et al., 1983; Krieger, 1983]). LDL receptor activity at 34°C was essentially normal (not shown, see Hobbie et al., manuscript submitted for publication). At 39.5°C, LDL receptor activity was extremely low in ldlF cells, but was restored to normal levels in the stable ldlF[*LDLF*] transfectant.

Because the loss of LDL receptor activity in ldlF cells at 39.5°C is closely correlated with receptor instability (Hobbie et al., manuscript submitted for publication), these data suggested that LDL receptor stability was restored to normal in the ldlF[*LDLF*] transfectants. To test this, we used a two-step procedure (see diagram in Fig. 2 *b*). First, cells were pulse-labeled with [^{35}S]methionine for 30 min and chased for 45 min (*chase #1*), both at 34°C. Under these conditions, newly synthesized receptor precursors were processed to their cell surface mature forms (Fig. 2 *b*: *m*, 155 kD) as previously described (Kozarsky et al., 1986; Hobbie et al., manuscript submitted for publication, and see below). The cells were then subjected to a second chase (#2) for ≤12 h at 39.5°C, and the receptors were immunoprecipitated and analyzed by gel electrophoresis and autoradiography (Kozarsky et al., 1986). There was little loss of receptor in wild-type CHO cells throughout the 12 h of chase #2 (Fig. 2 *b*, *top panel*). As previously described (Hobbie et al., manuscript submitted for publication), in ldlF cells (Fig. 2 *b*, *middle panel*), there was a significant loss of receptor by 9 h of chase #2, and very little receptor was detectable after 12 h. Fig. 2 *b* (*bottom panel*) shows that transfection of ldlF cells with p*LDLF*-1 restored normal LDL receptor stability.

Fig. 2 *c* shows the posttranslational processing of LDL receptors measured at the nonpermissive temperature using a metabolic labeling, pulse/chase, immunoprecipitation assay. As previously described, in wild type CHO cells the LDL receptor was synthesized in the ER as an endoglycosidase H–sensitive precursor (Fig. 2 *c*: *p*, ~125 kD) that was converted to a sialylated, cell surface–expressed, endo H–resistant mature form (Fig. 2 *c*: *m*, ~155 kD) after transport through and processing by the Golgi apparatus (Tolleshaug et al., 1982; Cummings et al., 1983; Kozarsky et al., 1986; Hobbie et al., manuscript submitted for publication). In untransfected ldlF cells at the nonpermissive temperature, the precursor was slowly converted to an intermediate form, *i*, which was previously demonstrated to be resistant to endoglycosidase H and essentially free of sialic acid (Hobbie et al., manuscript submitted for publication). In ldlF[*LDLF*] transfectants, LDL receptor processing was essentially re-

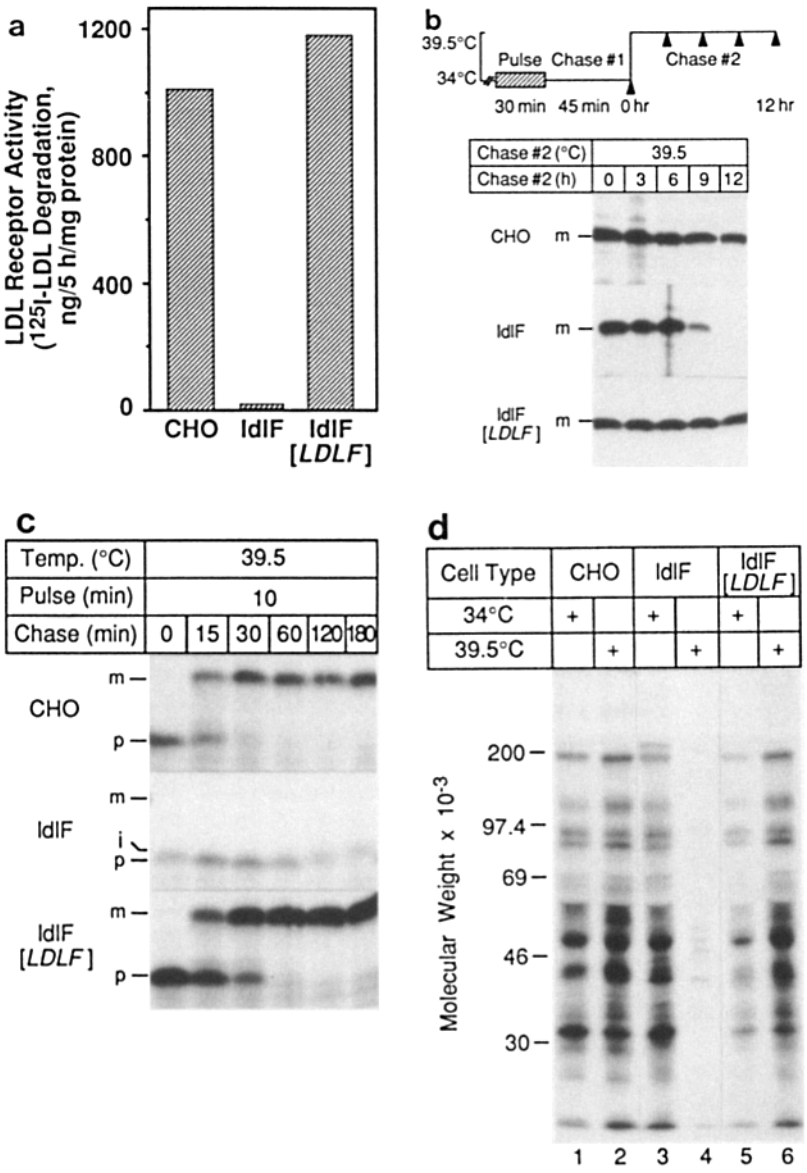

Figure 2. LDL receptor activity (*a*), stability (*b*), and posttranslational processing (*c*), and total protein secretion (*d*) by wild-type CHO, mutant ldlF, and transfectant ldlF[*LDLF*] cells at the nonpermissive temperature (39.5°C). (*a*) LDL receptor activity. On day 0, CHO and ldlF cells were plated at 60,000 cells/well in medium D at 34°C, and ldlF[*LDLF*] cells were plated at 80,000 cells/well in medium G at 39.5°C in 24-well dishes. On day 1, CHO and ldlF cells were shifted to 39.5°C. 12 h later, ^{125}I-LDL (10 μg protein/ml) was added in the absence (duplicate determinations) or presence (single determinations) of 400 μg protein/ml of unlabeled LDL and, after a 5-h incubation at 39.5°C, the amounts of high affinity ^{125}I-LDL degradation were determined as described in Materials and Methods. (*b*) LDL receptor stability. On day 0, cells were plated at 34°C in six-well dishes at 150,000 cells/well in either medium D (CHO, ldlF), or medium G (ldlF*[LDLF]*). On day 2, the cells were pulse labeled with 300 μCi/ml [^{35}S]methionine for 30 min at 34°C, washed once with Ham's F12 medium, and then chased for 45 min at 34°C in medium D containing 1 mM unlabeled methionine (*Chase #1*) to permit both maturation of the receptors to their 155-kD mature forms (*m*) and transport to the cell surface as previously described (Hobbie et al., manuscript submitted for publication, and see text). At time 0, the cells were refed with prewarmed (39.5°C) medium D containing 1 mM unlabeled methionine, and incubated at 39.5°C for the indicated times (*Chase #2*). The cells were then lysed, and the lysates subjected to immunoprecipitation with an anti-LDL receptor antibody as described in Materials and Methods. The immunoprecipitates were reduced with β-mercaptoethanol, and then were analyzed by 6% gel electrophoresis and autoradiography as described in Methods. (*c*) LDL receptor posttranslational processing. On day 0, CHO and ldlF cells were plated at 150,000 cells/well in medium D at 34°C, and ldlF[*LDLF*] cells were plated at 180,000 cells/well in medium G at 39.5°C in six-well dishes. On day 1, CHO and ldlF cells were shifted to 39.5°C for 12 h before pulse labeling all of the cells with 300 μCi/ml of [^{35}S]methionine at 39.5°C for 10 min. The cells were then washed and chased for the indicated times in medium D containing 1 mM unlabeled methionine. LDL receptors were immunoprecipitated from detergent-solubilized cell extracts with an antibody specific for the COOH-terminus of the LDL receptor, anti-C, reduced with β-mercaptoethanol, and analyzed by 6% polyacrylamide gel electrophoresis and autoradiography as described in Materials and Methods. The mobilities of the mature (*m*, 155 kD) and precursor (*p*, 125 kD) forms of the LDL receptors in wild-type cells, as well as the endoglycosidase H– and sialidase-resistant intermediate (*i*) form, are indicated. (*d*) Secretion of metabolically labeled proteins. On day 0, cells were plated at 34°C in six-well dishes at 150,000 cells/well in either medium D (CHO, ldlF), or medium G (ldlF*[LDLF]*). On day 1, cells were either shifted to 39.5°C or were maintained at 34°C for 13.5 h as indicated. On day 2, the cells were pulse labeled for 30 min with [^{35}S]methionine, and then washed and chased for 2 h in 0.5 ml of medium D containing 1 mM unlabeled methionine. The chase media were harvested, and total secretion of proteins into the medium was assessed by 5–15% gradient polyacrylamide gel electrophoresis and autoradiography as described in Materials and Methods.

stored to normal. (In ldlF cells, the processing of LDL receptors and other membrane proteins [e.g., vesicular stomatitis virus G protein, mannose-6-phosphage receptor], is defective at 39.5°C, but essentially normal at the permissive temperature [Hobbie et al., manuscript submitted for publication]).

Fig. 2 *d* compares total protein secretion by these cells at the permissive and nonpermissive temperatures. Cells were pulse-labeled with [^{35}S]methionine for 30 min, washed, and chased in unlabeled medium for 2 h before harvesting the media and analyzing their contents of newly synthesized protein by gel electrophoresis and autoradiography as previously described (Hobbie et al., manuscript submitted for publication). At 34°C, all three cell lines secreted many different metabolically labeled proteins. At 39.5°C, CHO cell secretion was somewhat greater than that at 34°C, secretion by ldlF mutant cells was dramatically reduced, and wild-type levels of secretion were observed in ldlF[*LDLF*] transfectants. Similar results have been observed after a 5-h chase (not shown). The dramatic decrease in secretion by

ldlF cells at 39.5°C was not caused by decreased total protein synthesis, because protein synthesis at this temperature was reduced by only about one quarter of that at 34°C (Hobbie et al., manuscript submitted for publication, and data not shown). These results, taken together with the restoration of normal mannosidase II and β-COP localization in the transfected cells (immunofluorescence data not shown) and the data described below, establish that transfection of ldlF cells with p*LDLF*-1 corrects all of the characteristic temperature-sensitive abnormal phenotypes in ldlF cells.

Plasmid p*LDLF*-1 could encode the *LDLF* gene itself or an extragenic suppressor of the mutant *LDLF* gene. Extragenic suppression caused by overexpression of transfected genes can occur (e.g., in yeast genetics studies; see Rine, 1991). Definitive resolution of this issue awaits the future sequencing of the *LDLF* gene in the mutant cells. Nevertheless, indirect complementation experiments suggest that the cDNA in p*LDLF*-1 may encode the *LDLF* gene. Plasmid p*LDLF*-1 was transfected into temperature-sensitive, conditional lethal CHO cell mutants representing five complementation groups, all of which exhibit temperature-sensitive defects in the secretory pathway: ldlE, ldlF, ldlG, ldlH, and End4 (Nakano et al., 1985; Wang et al., 1990; Malmstrom and Krieger, 1991; Presley et al., 1991; Zuber et al., 1991; Kao and Draper, 1992; Hobbie et al., manuscript submitted for publication; and see Materials and Methods). The mutant phenotypes of ldlE, ldlG, and ldlH cells have been directly compared with those of ldlF and shown to be similar, but not identical (Malmstrom and Krieger, 1991; Hobbie et al., manuscript submitted for publication; Guo, Q., A. Fisher, and M. Krieger, unpublished data). Cells in the End4 complementation group have been reported by others to exhibit temperature-sensitive defects in secretion and temperature-sensitive dissociation of the Golgi apparatus (Kao and Draper, 1992; Zuber et al., 1991). Thus, End4, at least in some respects, resembles ldlF, although they are genetically distinct. Fig. 3 shows the consequences of cotransfecting plasmids p*LDLF*-1 and pSV2neo into these mutants. The controls in the left panels show that all five classes of mutant could be transfected with comparable efficiency (selection in medium containing G418 at the permissive temperature). However, when the cells were incubated in the same selection medium at the nonpermissive temperature (*right panels*), only ldlF transfectants survived. There was complementation of the temperature-sensitive lethality of ldlF cells, but not of any of the other phenotypically related mutants. Thus, correction of the defect by plasmid p*LDLF*-1 was specific, and is most simply explained if the cDNA in p*LDLF*-1 corresponds to the defective gene, *LDLF*, in the ldlF cells.

cDNA Sequence

Fig. 4 *a* shows the nucleotide (*top*) and predicted amino acid (*bottom*) sequences of the cDNA insert in plasmid p*LDLF*-1. The sequence surrounding the ATG encoding the first methionine is consistent with that described by Kozak (1989) for initiator methionines; there are, however, no in-frame stop codons in the 39-base sequence 5′ of this methionine. The open reading frame predicts a 308-residue protein with a calculated mass of 34,523 D. Using the program MOTIFS and the PROSITE database (version 10.2 from Amos Bairoch, University of Geneva, Switzerland), as well as visual inspection, we identified several potential phosphorylation sites and one potential N-glycosylation site, all of unknown significance. We did not detect any other common sequence motifs or predicted secondary or tertiary structural elements, such as signal sequences, potential membrane spanning domains, nucleotide binding sites, heptad repeats, etc. An initial survey of available databases indicated that there were no known homologous genes (see below). However, there were four partial sequences determined from random cDNA cloning (expressed sequence tags) from humans, rice, *Arabidopsis*, and *C. elegans* whose sequence similarities to the p*LDLF*-1 sequence were sufficiently high to consider these to be homologous gene fragments from different species. Fig. 4 *b* shows an alignment of the predicted protein sequences and a consensus sequence. The human and rice sequences overlap with amino acid positions 1–168, and the

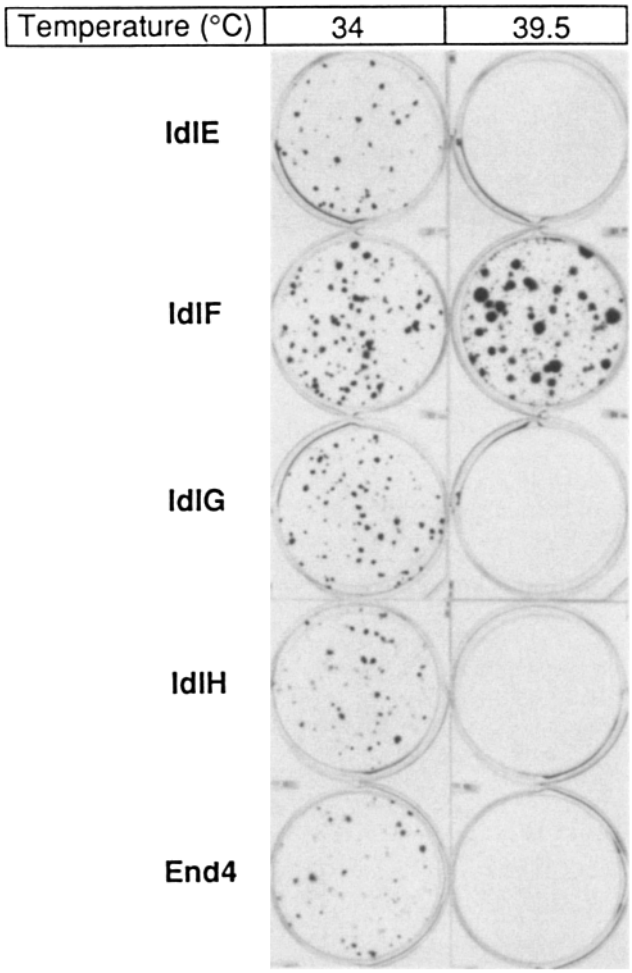

Figure 3. Effects of transfection with p*LDLF*-1 on the temperature-sensitive lethal phenotypes in five classes of CHO mutants: ldlE, ldlF, ldlG, ldlH, and End4. On day 0, the indicated temperature-sensitive, conditional lethal mutant CHO cells were plated at 34°C. On day 2, monolayers of each mutant were transfected with a mixture of p*LDLF*-1 (1.98 μg) and pSV2neo (0.22 μg) as described in Materials and Methods. The cells were subsequently incubated in medium G containing G418 either at 34°C for 16 d to monitor transfection efficiency (*left panels*) or at 39.5°C for 16 d to test for reversion of their temperature-sensitive lethal phenotypes (*right panels*). Surviving colonies were stained with crystal violet.

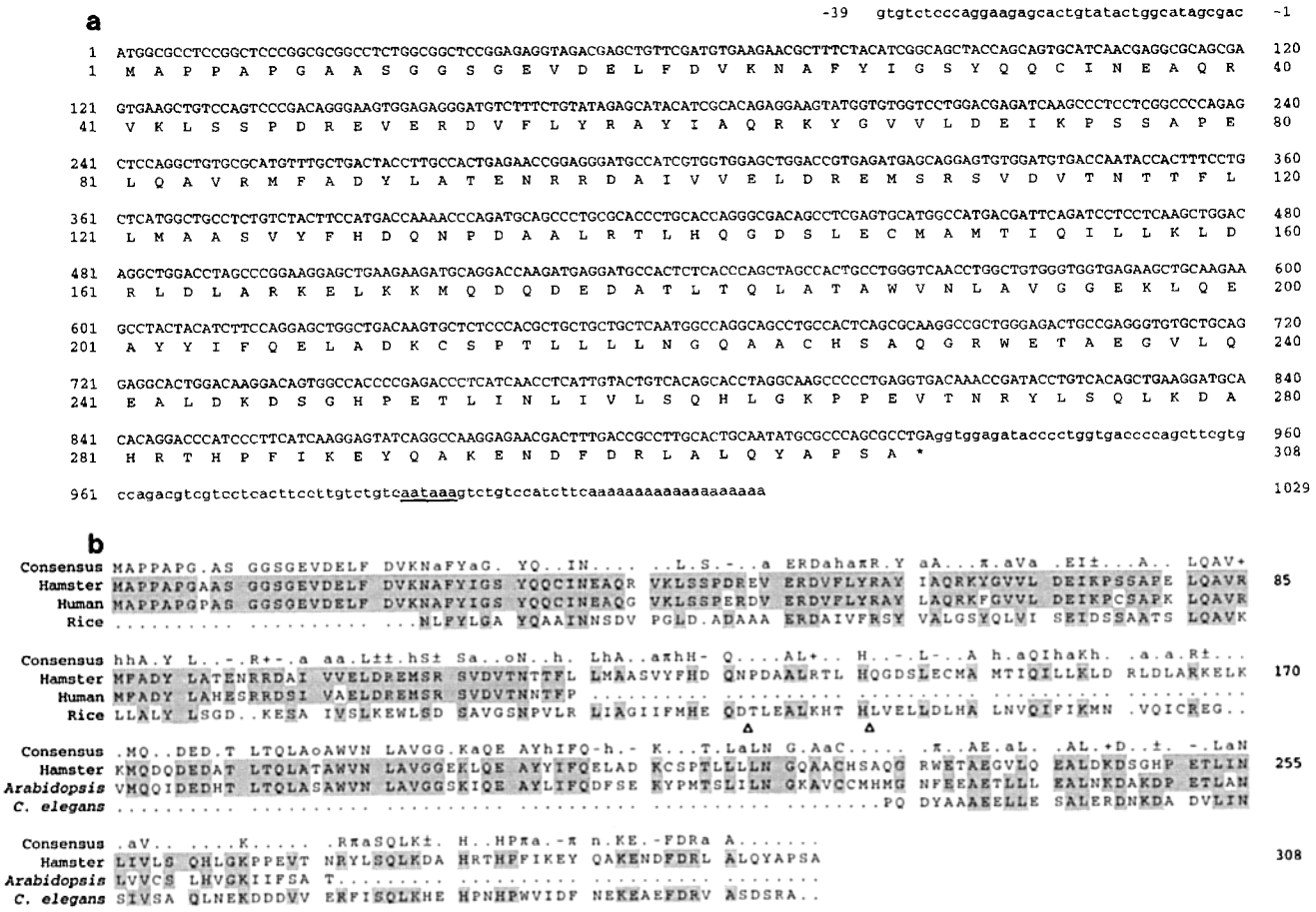

Figure 4. Sequence of p*LDLF*-1 and comparison with expressed sequence tags from four species. (*a*) Nucleotide (*upper line*) and predicted protein (*lower line*) sequence of *pLDLF*-1 cDNA. The cDNA insert in p*LDLF*-1 was cloned and sequenced as described in Materials and Methods. Nucleotides are numbered relative to the start of the putative initiation codon. A polyadenylation signal in the 3′ untranslated region is underlined. These data are available from EMBL under accession no. Z32554. (*b*) Comparison of predicted protein sequences of the hamster p*LDLF*-1 with those of expressed sequence tags from human (Adams et al., 1993, GenBank no. T08752), rice (GenBank no. D15415), *Arabidopsis* (GenBank no. T14110), and *C. elegans* (GenBank no. T00495) homologues: The sequences were aligned using the program PILEUP (Devereux et al., 1984) and visual inspection. EST sequences, which are incomplete cDNA sequences, are shown only for the predicted open reading frames corresponding to the p*LDLF*-1 sequence. Amino acid residues are numbered according to the hamster sequence. The human and rice sequences overlap with p*LDLF*-1 amino acid positions 1–168 and the *Arabidopsis* and *C. elegans* sequences overlap with positions 171–306. To account for presumed errors in the rice expressed sequence tag sequence, one base was deleted from or one inserted in the sequence at the positions indicated by triangles. Gaps to improve the alignment are indicated by dots. Amino acids matching in at least two of the five species are shaded. Consensus sites are indicated in the top row when all residues at each position are occupied by a single amino acid (*capital letter*) or a single class of residue (*a*, aliphatic [*A, I, L, V*]; *π*, aromatic (*F, W, Y*); *h*, hydrophobic, (*a, π, M*); +, positively charged (*H, K, R*); −, negatively charged (*D, E*); ±, charged (−, +); *o, S* or *T*; *n, Q* or *N*).

Table I. Amino Acid Sequence Identities and Similarities for the pLDLF-1 Gene in Five Species

Species 1	Species 2	Sequence overlap (no. of residues)	Identity (%)	Similarity* (%)
Hamster	Human	120	89	93
	Rice	142	34	64
	Arabidopsis	101	56	70
	C. elegans	78	36	59
Human	Rice	94	33	64
Arabidopsis	*C. elegans*	43	30	34

* Similarity was calculated by adding the percent of identical residues with the percent of residues falling into the same consensus groups, as defined in the legend to Fig. 4.

Arabidopsis and *C. elegans* sequences overlap with positions 171–306. Table I shows the strikingly high values of the pairwise sequence identities and similarities for the five sequences. This high conservation suggests that this gene probably plays a critical role in cell function. This is consistent with the temperature-sensitive, conditional lethal phenotype of ldlF cells and the apparently essential role of the gene for Golgi function.

pLDLF-1 Encodes ϵ-COP

Hara-Kuge et al. (1994) have very recently submitted to the Genbank database the sequence of bovine ϵ-COP (Genbank no. X76980). Its predicted protein sequence is also 308 residues and is 92% identical and 98% similar to that of the

hamster p*LDLF*-1 over its entire 308-residue length. Therefore, we and they have simultaneously and independently cloned the hamster and bovine ε-COP cDNAs. Thus, ε-COP corrects the defects in ldlF cells and may be the *LDLF* gene.

Discussion

ldlF cells are temperature-sensitive CHO cell mutants that have previously been shown to exhibit five distinctive characteristics at the nonpermissive temperature (39.5°C): (*a*) dramatically reduced LDL receptor activity; (*b*) instability of cell surface LDL receptors; (*c*) abnormal posttranslational processing of proteins in the secretory pathway, which suggest defects in intraGolgi and ER to Golgi membrane transport; (*d*) drastically reduced protein secretion, and (*e*) death after prolonged incubation (>24 h) at the nonpermissive temperature (Hobbie et al., manuscript submitted for publication).

In the current study, morphologic analysis using immunofluorescence and electron microscopy established that the classic structure of the Golgi apparatus (perinuclear stacked cisternae with budding vesicles) in ldlF cells at the permissive temperature (34°C) dissociated at 39.5°C into vesicles and tubules, and both integral (mannosidase II) and peripheral (β-COP and ldlCp) Golgi-associated proteins dispersed throughout the cytoplasm. (ldlCp is a peripheral Golgi membrane protein required for several *medial* and *trans*-Golgi-associated processing reactions [Kingsley et al., 1986; Podos, S., P. Reddy, J. Ashkenas and M. Krieger, manuscript in preparation]). The time of onset of the dissociation of the Golgi and the other temperature-sensitive defects in ldlF cells was relatively long (several hours at the nonpermissive temperature, also see Hobbie et al., manuscript submitted for publication). This presumably results from either a slow rate of inactivation of the *LDLF* gene product (ldlFp) or perhaps from the requirement that the mutant phenotypes can be fully expressed only after functional ldlFp molecules synthesized at the permissive temperature are replaced with inactive molecules made at the nonpermissive temperature.

AlF_4^- inhibited the temperature-dependent dispersion of Golgi-associated mannosidase II immunofluorescence. AlF_4^-, in combination with GDP, can act as a nonhydrolyzable surrogate for GTP in the heterotrimeric G proteins (e.g., $G\alpha_i$ and $G\alpha_s$) that appear to participate in multiple membrane transport reactions (Stow et al., 1991; Donaldson et al., 1991*b*; Bomsel and Mostov, 1992; Rothman and Orci, 1992; Pryer et al., 1992; Schwaninger et al., 1992; Pimplikar and Simons, 1993; Stenbeck et al., 1993; Wilson et al., 1993; Montmayeur and Borrelli, 1994). This suggests that the *LDLF* gene product might influence Golgi structure by participating in a trimeric GTP-binding protein-dependent assembly or stabilization of Golgi-associated proteins, such as the coatomer proteins (COPs). Stenbeck et al. (1993) have recently shown that the sequence of one of these, β′-COP, is homologous to the β-subunits of trimeric G proteins. Thus, AlF_4^-, and possibly the product of the *LDLF* gene, may act directly on coatomers or coatomer-associated proteins.

The effects on the Golgi apparatus of incubating ldlF cells at the nonpermissive temperature were reminiscent of those of incubating wild-type cells with brefeldin A (BFA), although the changes with BFA are more rapid (Takatsuki and Tamura, 1985; Fujiwara et al., 1988; Lippincott-Schwartz et al., 1989, 1990; Shite et al., 1990; Orci et al., 1991, and data not shown). BFA induces dissociation from the Golgi of β-COP (Donaldson et al., 1990), and presumably the other coat proteins that compose the coatomer, induces the vesiculation and tubulation of the Golgi complex, causes intermixing of contents of Golgi cisternae and the ER, and inhibits protein secretion. AlF_4^-, which interferes with Golgi dissociation in ldlF cells, also inhibits the BFA-induced dissociation of the Golgi and the release of COPs (Donaldson et al., 1991*a*).

The coatomer complex (α, β, β′, γ, δ, ε, and ζ COPs) can be found in the cytoplasm or assembled either on the surface of the Golgi membrane or on Golgi-derived, non-clathrin-coated vesicles (Duden et al., 1991; Waters et al., 1991; Serafini et al., 1991; Stenbeck et al., 1993; Ostermann et al., 1993). These vesicles are thought to play important roles in ER- and Golgi-associated intercompartmental membrane traffic (Orci et al., 1986, 1989; see Ostermann et al., 1993 and references cited therein). Mutation in the yeast homologue of γ-COP (Sec21p) can block ER to Golgi transport (Kaiser and Schekman, 1990; Stenbeck et al., 1992; Hosobuchi et al., 1992), as can the microinjection of antibodies to β-COP in animal cells (Pepperkok et al., 1993). The assembly of coatomers on membranes requires the action of ARFs, which are small GTP-binding proteins (Donaldson et al., 1992*b*; Palmer et al., 1993). BFA interferes with GDP-GTP exchange on ARFs (Donaldson et al., 1992*a*; Helms and Rothman, 1992), preventing GTP/ARF-dependent assembly of coatomers on membranes and disrupting Golgi structure and function.

Using a CHO cell cDNA expression library and complementation of the temperature-sensitive conditional lethality of ldlF cells, we isolated an expression vector, p*LDLF*-1, which when transfected in ldlF cells could correct all of their distinctive temperature-sensitive defects. The correction was specific in that p*LDLF*-1 could not correct the temperature-sensitive conditional lethality of four other CHO mutants with defects in intracellular membrane traffic: ldlE, ldlG, ldlH, and End4 (Nakano et al., 1985; Wang et al., 1990; Malmstrom and Krieger, 1991; Presley et al., 1991; Zuber et al., 1991; Kao and Draper, 1992; Hobbie et al., manuscript submitted for publication). It seems likely that the plasmid p*LDLF*-1 encodes the *LDLF* gene itself; however, additional studies will be required to address the alternative possibility that it encodes an extragenic suppressor of the mutant *LDLF* gene.

The predicted amino acid sequence of the hamster p*LDLF*-1 gene is virtually identical (92% identity; 98% similarity) to the very recently reported sequence of bovine ε-COP (Hara-Kuge et al., 1994). Therefore, this work provides the first direct genetic evidence that in animal cells ε-COP, and thus the coatomer complex (Kuge et al., 1993), can play a role both in establishing or maintaining Golgi structure and in mediating ER-through-Golgi transport, and that it can influence normal endocytic recycling of LDL receptors. The importance of ε-COP is highlighted not only by previous in vitro biochemical studies (e.g., Ostermann et al., 1993) and its ability to correct the temperature-sensitive conditional lethal defects in ldlF cells, but also by its very highly conserved sequence during evolution. The sequence similarities for hamster ε-COP compared to the partial sequences of its human, rice, *Arabidopsis*, and *C. elegans* homologues are 93, 63, 70, and 59%, respectively. ldlF cells will provide a

powerful tool for the detailed molecular analysis both of the structure and function of ε-COP (e.g., as a recipient of specifically mutated ε-COP genes) and of the role of coatomers in membrane transport (e.g., in in vitro transport assays).

The precise mechanism by which a defect in ε-COP could lead to the abnormalities seen in ldlF cells remains to be established. The effects of the mutation in ldlF cells on intracellular transport are complex and include a dramatically increased rate of degradation of cell surface LDL receptors and the progressive inhibition of Golgi-associated reactions at the nonpermissive temperature (Hobbie et al.). Initially after transfer to the nonpermissive temperature, there are disruptions in reactions associated with the most distal portions of the posttranslational processing pathway (*trans*-Golgi and *trans*-Golgi Network–associated glycosylation reactions); subsequently, there are disruptions in the more proximal steps associated with ER to *medial* Golgi transport. Thus, multiple reactions are disrupted at the nonpermissive temperature. It is reasonable to expect that defects in ε-COP in vivo could either directly or indirectly interfere with the assembly, association with membranes, and/or functions of coatomers. Some of the defects in ldlF cells (e.g., rapid degradation of LDL receptors) may arise as secondary consequences of primary disruptions in Golgi and/or ER structure and function. Alternatively, ε-COP and its associated proteins may participate in multiple, independent transport reactions in both the secretory and endocytic pathways. In this regard, it is interesting to note that the sequences of β-COP and ζ-COP are similar to those of the clathrin-associated proteins β-adaptin (Duden et al., 1991; Serafini et al., 1991) and AP17 and AP19 (Kuge et al., 1993), respectively. Previous studies have established that other proteins, e.g., NEM-sensitive fusion protein, serve as common components of the cell's intercompartmental transport machinery (Rothman and Orci, 1992; Wilson et al., 1989; Beckers et al., 1989; Diaz et al., 1989; Balch, 1990; Laurie and Robbins, 1991; Sollner et al., 1993). Furthermore, using in vitro assays, Peter et al., (1993) have recently shown that β-COP is essential for protein transport from the ER to the Golgi.

We and others have isolated a variety of mutant CHO cells with temperature-sensitive defects in the secretory and endocytic pathways (e.g., Robbins et al., 1983, 1984; Klausner et al., 1984; Marnell et al., 1984; Nakano et al., 1985; Roff et al., 1986; Colbaugh et al., 1988, 1989; Hughes-Ryser et al., 1988; Malmstrom and Krieger, 1991; Cain et al., 1991; Laurie and Robbins, 1991; Zuber et al., 1991; Hobbie et al., manuscript submitted for publication). The current studies represent the first identification of a gene, ε-COP, which can correct the complex abnormal phenotypes in such cells. Similar temperature-sensitive phenotypes in at least some of the other mutants might arise because of mutations in other COP genes or genes for COP-associated proteins. It also seems likely that molecular analysis of some of these mutants will define previously unrecognized genes required for intracellular vesicular transport. Genetic analysis of membrane transport in CHO cells should be particularly informative because the morphology of the Golgi apparatus in these cells is well defined and readily accessible for in vivo and in vitro ultrastructural and biochemical analyses. Therefore, mammalian somatic cell genetics provides a powerful method, which is complementary to biochemical and yeast genetics approaches, for the investigation of intracellular membrane traffic.

We thank Shangzhe Xu and Marsha Penman for excellent technical assistance; P. Reilly of the MIT Biomedical Microscopy Laboratory and D. Resnick for assistance; F. Solomon and R. Hynes for use of the fluorescence microscope in the Center for Cancer Research; J. R. Falck for the generous gift of the DOGS reagent; R. Draper for End4 cells; H. Lodish for COS-7 cells; R. Klausner and J. Donaldson for providing the anti-mannosidase II antibody and the anti-β-COP antibody (M3A5, originally from T. Kreis); S. Podos for examining the distribution of ldlCp in ldlF cells; and M. Brown, J. Goldstein, D. Russell, C. Kaiser, H. Ploegh, J. G. Naglich, J. E. Metherall, M. Evans, A. Pearson, S. Acton, and S. Podos for helpful discussions.

This work was supported by National Institutes of Health grants HL-41484, RR-05734 (BRS Shared Instrumentation Grant) and HL-08775 (a National Research Service Award postdoctoral fellowship to Q. Guo).

Received for publication 21 March 1994.

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