Recent results have shown that autophagic sequestration in the human colon cancer cell line HT-29 is controlled by the pertussis toxin-sensitive heterotrimeric $G_{13}$ protein. Here we show that transfection of an antisense oligodeoxynucleotide to the $a_{13}$-subunit markedly inhibits autophagic sequestration, whereas transfection of an antisense oligodeoxynucleotide to the $a_{2}$-subunit does not change the rate of autophagy in HT-29 cells. Autophagic sequestration was arrested in cells transfected with a mutant of the $a_{2}$-subunit ($Q204L$) that is restricted to the GTP-bound form. In $Q204L$-expressing cells, 3-methyladenine-sensitive degradation of long lived [$^{14}$C]valine-labeled proteins was severely impaired and could not be stimulated by nutrient deprivation. Autophagy was also reduced when dissociation of the $\beta\gamma$ dimer from the GTP-bound $a_{13}$-subunit was impaired in cells transfected with the G203A mutant. In contrast, a high rate of pertussis toxin-sensitive autophagy was observed in cells transfected with an $a_{13}$-subunit mutant ($S47N$) which has an increased guanine nucleotide exchange rate and increased preference for GDP over GTP. Cells that express pertussis toxin-insensitive mutants of either wild-type $a_{13}$-subunit ($C351S$) or $S47N$ $a_{13}$-subunit ($S47N/C351S$) exhibit a high rate of autophagy.

The role of heterotrimeric G proteins ($a\beta\gamma$) as signal transducers is well known (1–3). Their interaction with activated sense oligodeoxynucleotide to the $a_{13}$-subunit markedly inhibits autophagic sequestration, whereas transfection of an antisense oligodeoxynucleotide to the $a_{2}$-subunit does not change the rate of autophagy in HT-29 cells. Autophagic sequestration was arrested in cells transfected with a mutant of the $a_{2}$-subunit ($Q204L$) that is restricted to the GTP-bound form. In $Q204L$-expressing cells, 3-methyladenine-sensitive degradation of long lived [$^{14}$C]valine-labeled proteins was severely impaired and could not be stimulated by nutrient deprivation. Autophagy was also reduced when dissociation of the $\beta\gamma$ dimer from the GTP-bound $a_{13}$-subunit was impaired in cells transfected with the G203A mutant. In contrast, a high rate of pertussis toxin-sensitive autophagy was observed in cells transfected with an $a_{13}$-subunit mutant ($S47N$) which has an increased guanine nucleotide exchange rate and increased preference for GDP over GTP. Cells that express pertussis toxin-insensitive mutants of either wild-type $a_{13}$-subunit ($C351S$) or $S47N$ $a_{13}$-subunit ($S47N/C351S$) exhibit a high rate of autophagy.

Membrane fusion is also required during macroautophagy, a major cellular catabolic pathway whose terminus is the lysosomal compartment (15–17). Autophagy is a nonselective process which begins with the sequestration of various organelles and fractions of the cytosol into a membrane probably derived from the rough endoplasmic reticulum (18) or from another organelle called a phagophore (19, 20) to form closed vacuoles (early autophagosomes). After maturation (see Ref. 20 and references therein for a recent discussion), these vacuoles acquire lysosomal enzymes by either direct fusion with lysosomes (21–23) or late endosomes/prelysosomes (24). We have previously shown that the impairment of N-linked glycoprotein processing (25–27) in undifferentiated HT-29 cells is the consequence of a constitutive autophagic pathway (28). Recently, we have shown that autophagic sequestration is controlled by a $G_{13}$ protein in this cell line (29). The regulation of autophagic sequestration by GTPases does not seem to be restricted to HT-29 cells since it has been reported that GTPyS inhibits the de novo formation of autophagic vacuoles in permeabilized rat hepatocytes (30). An important step toward the understanding of the control of autophagy is to identify the state of activation of the $G_{13}$ protein (i.e. nucleotide binding) that regulates autophagic sequestration. In the present study using site-directed mutagenesis and stable cell transfections, we demonstrate that autophagic sequestration is switched on when the $G_{13}$ protein is bound to GDP. In contrast, autophagic sequestration is inhibited when the $G_{13}$ protein is bound to GTP. Autophagy is also reduced when dissociation of the $\beta\gamma$ dimer from the GTP-bound $a_{13}$-subunit is inhibited. Using $G_{13}$ pertussis toxin (PTX)$^\dagger$-resistant mutants we show that the GDP-bound form of $G_{13}$ must attach to an intracellular membrane in order to initiate autophagic sequestration.

**EXPERIMENTAL PROCEDURES**

*Reagents—PTX, 3-MA, and all other chemicals were purchased from Sigma. Cell culture reagents and Genetin (G418) were from Life Technologies, Inc. (Ergany, France). Nitrocellulose membranes were from Schleicher & Schuell (Dassel, Germany). The bicinchoninic acid (BCA) kit was from Pierce. Rat cDNAs encoding the $a_{13}$- and $a_{2}$-subunits were kindly provided by Dr. R. Reed (John Hopkins University, Baltimore, MD). Enzymes for cloning and sequencing were from Life Technologies, Inc. (Cergy Pontoise, France), and the eurycaryote expression vectors pcDNA3 and pBK/CMV were from Invitrogen (San Diego, CA) and Stratagene, respectively. Oligonucleotide-directed mutagenesis kits were obtained from Promega (Madison, WI). Synthetic oligonucleotides were from Eurogentec (Seraing, Belgium). Polyclonal antibodies to $a_{13,2}$- and $a_{13,3}$-subunits were from NEN Dupont (Les Ulis, France). Each antibody was raised against a decapetptide from the C-terminal of each subunit (31). The radioisotopes $\alpha^{25}$S-dATP (1000 Ci/mmol), $[^3H]$raffinose (5–15 Ci/mmol), and GTPyS (1000 Ci/mmol), $[\gamma^{32}$P]GTPyS (1000 Ci/mmol), and the ECL$^\dagger$ Western blotting

$^\dagger$ The abbreviations used are: PTX, pertussis toxin; 3-MA, 3-methyladenine; PAGE, polyacrylamide gel electrophoresis; GTPyS, guanosine 5'-O-(thiotriphosphate); GDPβS, guanosine 5'-O-β,γ-impotent (thiodiphosphate).
Regulation of Autophagy by Trimeric \(G_i3\) Protein

**Immunoblotting and Quantitation of G Protein \(\alpha\)-Subunits—**Cell homogenates were prepared as previously reported (29). Proteins (100 μg) were resolved by SDS-PAGE and transferred to nitrocellulose. The membrane was incubated for 1 h in blocking buffer (ECL\({\textsuperscript{\textregistered}}\) Western blotting detection kit), then with either anti-\(\alpha_i\) (1/1,000), anti-\(\alpha_g\) (1/500), or anti-\(\alpha_S\) (1/1,000) diluted in Tris-buffered saline, pH 7.6, containing 0.1% Tween-20 (TBS-T) for 1 h. After washing with TBS-T, bound IgG was labeled with horseradish peroxidase anti-rabbit IgG (1/10,000). Blots were then washed twice with TBS-T and coaxed for 60 s with the chemiluminescence solution (ECL\({\textsuperscript{\textregistered}}\) detection kit). The membranes were exposed to Kodak X-Omat AR film for 10–40 s.

The quantity of \(\alpha_i\)-subunits in each cell population was evaluated by densitometric analysis of autoradiograms of the nitrocellulose membranes. Stably transfected cell populations used in this study were selected in the following way: cells transfected with either for the wild-type \(\alpha_g\)-subunit or its different mutants have a 2.8–3.0-fold overexpression of the \(\alpha_i\)-subunit when compared to untransfected cells. Cells transfected with the plasmid containing anti-sense oligodeoxynucleotides to \(\alpha_i\) and \(\alpha_g\)-subunits have a 0.15-fold expression of \(\alpha_i\)- and \(\alpha_g\)-subunits when compared to untransfected cells.

**Autophagic Sequestration of \([H]Raffinose—**\([H]\)Raffinose sequestration was monitored as previously reported (28) using a modification of the method of Seglen et al. (39). Briefly, cells (5 × 10\(^5\))/50 μl) were incubated for 15 min at 37°C with 2 μCi of \([H]\)raffinose, subjected to electronionization (at ambient temperature), and incubated at 4°C for 30 min, followed by 15 min at 37°C. Cells were then washed twice with phosphate-buffered saline in cold. Single-stranded DNA (1 μl) was obtained from untransfected, \(G_i2\)- and \(G_i3\)-subunits with 0.1% Lubrol WX. Sequencing of lactate dehydrogenase—Lactate dehydrogenase (LDH) was measured as previously reported (28) following the method described by Kopitz et al. (40). Briefly, cells were gently washed three times with phosphate-buffered saline (pH 7.4) and then twice with homogenization buffer (50 mM potassium phosphate, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 500 μg/ml bovine serum albumin, 0.1% Tween 20, pH 7.5) and centrifuged at 70,000 \(\times\) g for 60 min. The radioactivity associated with the pellet and total homogenate was measured by liquid scintillation counting. Sequestration of lactate dehydrogenase—Lactate dehydrogenase was assayed by measuring the oxidation of NADH with pyruvate as a substrate at 340 nm.

**Measurement of the Degradation of Long Lived Proteins—**HT-29 cells were incubated for 18 h at 37°C with 0.2 μCi/ml \([\text{3}^{\text{H}}]\)leucine. Unincorporated radioactivity was removed by three rinses with phosphate-buffered saline (pH 7.4). Cells were then incubated either in complete medium or in nutrient-free medium (without amino acids and in absence of fetal calf serum) plus 0.1% of bovine serum albumin. Both media were supplemented with 10 μl cold valine. When required, 10 mM 3-MA was added throughout the chase period. After the 1st h of incubation, at which time short lived proteins were being degraded, the medium was replaced with the appropriate fresh medium, and incubation was continued for an additional 4 h. Cells were then incubated with 0.5 μl of phosphate-buffered saline and the radiolabeled proteins in the 4-h medium, and cells were incubated in 10% trichloroacetic acid, 1% phosphotungstic acid (pH 4) at 4°C. The precipitated proteins were separated from the soluble radioactivity by centrifugation at 600 \(\times\) g for 10 min then dissolved in 1 ml of Soluene 350. The rate of protein degra-
Fig. 1. Autophagic sequestration is inhibited in HT-29 cells transfected with an antisense oligodeoxynucleotide to the \( \alpha_{i2} \)-subunit. Panel A, Western blotting of \( \alpha_{i2} \), \( \alpha_{i3} \), and \( \alpha_{i}\)-subunits in untransfected cells (control), cells transfected with antisense oligodeoxynucleotide to \( \alpha_{i2} \)-subunit (AS \( \alpha_{i2} \)) and antisense oligodeoxynucleotide to \( \alpha_{i}\)-subunit (AS \( \alpha_{i} \)). Panel B, autophagic sequestration was determined in untransfected cells (control), \( \alpha_{i2} \)-subunit overexpressing cells, cells transfected with antisense oligodeoxynucleotide to \( \alpha_{i3} \)- and \( \alpha_{i}\)-subunits, respectively. In panel B values are the mean \( \pm \) S.D. \((n = 5)\).

Table 1

| Cell population | Autophagic sequestration of LDH | Autophagic sequestration of LDH |
|-----------------|-------------------------------|-------------------------------|
|                 | Leupeptin | Leupeptin + 3-MA | Leupeptin + PTX |
| Untransfected cells | 6.12 ± 0.3 \(^a\) | 0.48 ± 0.05 | 0.64 ± 0.07 |
| \( \alpha_{i2} \)-overexpressing cells \(^b\) | 6.04 ± 0.4 | 0.52 ± 0.05 | 0.58 ± 0.04 |
| \( \alpha_{i3} \)-overexpressing cells \(^b\) | 11.85 ± 1.1 | 0.42 ± 0.05 | 0.49 ± 0.04 |
| \( \alpha_{i2} \) antisense \(^c\) | 5.95 ± 0.3 | 0.51 ± 0.05 | 0.57 ± 0.06 |
| \( \alpha_{i3} \) antisense \(^c\) | 0.70 ± 0.4 | 0.44 ± 0.05 | 0.45 ± 0.04 |
| S47N-expressing cells \(^b\) | 12.27 ± 0.8 | 0.86 ± 0.07 | 0.78 ± 0.08 |
| G203A-expressing cells \(^b\) | 1.66 ± 0.3 | 0.45 ± 0.05 | 0.46 ± 0.05 |
| Q204L-expressing cells \(^b\) | 0.55 ± 0.05 | 0.42 ± 0.04 | 0.46 ± 0.05 |
| C351S-expressing cells \(^b\) | 11.54 ± 0.5 | 0.75 ± 0.07 | 11.21 ± 0.3 |
| S47N/C351S-expressing cells \(^b\) | 12.04 ± 0.4 | 0.82 ± 0.07 | 11.50 ± 0.3 |

\(^a\) Values are the mean \( \pm \) S.D. \((n = 5)\).
\(^b\) Selected clones of HT-29 cells have a 2.8-3-fold increase of either \( \alpha_{i2} \)- or \( \alpha_{i3} \)-subunits when compared to the relevant \( \alpha_{i} \)-subunit in untransfected cells.
\(^c\) Selected clones of HT-29 cells expressed only 10–15% of the relevant \( \alpha_{i} \)-subunit in untransfected cells.

**RESULTS**

**Effect of \( \alpha_{i} \) Antisense Oligodeoxynucleotides on Autophagic Sequestration**—Autophagic sequestration has been shown to be stimulated when the \( \alpha_{i3} \)-subunit was overexpressed in HT-29 cells (29). We speculated that a decreased expression of this subunit would suppress the autophagic sequestration. To answer this question, we have transfected HT-29 cells with a plasmid containing an antisense oligodeoxynucleotide of 39 bases of the 5’-noncoding region immediately upstream of and including the ATG codon. Fig. 1A shows that after transfection of the \( \alpha_{i2} \)-subunit antisense oligodeoxynucleotide the expression of the \( \alpha_{i3} \)-subunit was reduced by 85% in HT-29 cells (see also Table 1). The effect of the \( \alpha_{i2} \)-subunit antisense oligodeoxynucleotide was specific since the expression of the \( \alpha_{i} \)-subunit was not modified in transfected cells (Fig. 1A). The expression of the \( \alpha_{i3} \)-subunit was not investigated since it has been shown that this subunit is not expressed in intestinal cells (41). Similarly when cells were transfected with an \( \alpha_{i2} \)-subunit antisense oligodeoxynucleotide, only the expression of the \( \alpha_{i2} \)-subunit was reduced (Fig. 1A). In both cases the expression of the \( \alpha_{i} \)-subunit was not modified in transfected cells (Fig. 1A).

The autophagic sequestration capacity of cells transfected with antisense oligodeoxynucleotides was measured in two ways (28, 40): (i) the rate of sequestration of the cytosolic enzyme lactate dehydrogenase (Table I) and (ii) the sequestration of radiolabeled [\( ^3H \)]raffinose (Fig. 1B). The autophagic sequestration capacity of cells transfected with \( \alpha_{i2} \)-subunit antisense oligodeoxynucleotide was similar to that observed in parental cells (Table I and Fig. 1B). In contrast both lactate dehydrogenase and raffinose sequestrations were severely impaired in cells transfected with \( \alpha_{i3} \)-subunit antisense oligodeoxynucleotide (Table I and Fig. 1B). These results confirmed that the \( \alpha_{i3} \) protein is essential for autophagic sequestration. However, these experiments provide no information on the nucleotide requirement for this G protein to either stimulate or abrogate autophagic sequestration.

**The GTP-bound Form of the \( \alpha_{i3} \)-Subunit Inhibits the Autophagic Sequestration**—Several mutations in the conserved nucleotide binding regions of GTPases including \( \alpha \)-subunits of trimeric G proteins have been characterized (for a review, see Ref. 42). We have chosen a site-directed mutagenesis strategy to investigate the influence of nucleotide-binding regions to the \( \alpha_{i3} \) protein on autophagic sequestration (see also next paragraph). The first mutant we constructed was the Q204L mutant. This mutation has been shown to stabilize the \( \alpha_{i3} \)-subunit in its GTP-bound form (43).

Autophagic sequestration was measured in Q204L-expressing cells as described in Fig. 1 and Table I. Both lactate dehy-
digenase and \[^{3}H\text{]raffinose}\] sequestrations were almost totally inhibited in Q204L expressing cells (Table I; Fig. 2A). Accordingly the degradation of \[^{14}C\text{]valine}\]-labeled long-lived proteins was dramatically reduced in Q204L-expressing cells either when measured in complete medium or in nutrient-free medium (see Fig. 2B). In contrast nutrient deprivation stimulated the 3-MA-sensitive degradation of radiolabeled proteins (3-MA, a potent inhibitor of the macroautophagic sequestration) (44) in untransfected (2.58 ± 0.13 versus 3.40 ± 0.18%/h) and wild-type \(\alpha_{i3}\)-subunit-overexpressing cells (6.10 ± 0.25 versus 7.5 ± 0.44%/h).

The GTP binding to \(\alpha\)-subunits leads to its dissociation from the \(\beta\gamma\) dimer. Thus to investigate whether or not the dissociation of the \(\beta\gamma\) dimer from the GTP-bound form of the \(\alpha_{i3}\)-subunit is important in inhibiting autophagic sequestration, we have transfected HT-29 cells with the G203A \(\alpha_{i3}\)-subunit mutant. This mutant is equivalent to the G203A mutant of the \(\alpha_{i2}\)-subunit (45), the G204A mutant of the \(\alpha_{i3}\)-subunit (46) and to the G226A mutant of the \(\alpha_{i3}\)-subunit (37). This last mutant cannot dissociate from the \(\beta\gamma\) complex despite the presence of bound GTP (37, 47). We have demonstrated the absence of \(\beta\gamma\) dimer dissociation from the G203A \(\alpha_{i3}\)-subunit mutant by examination of the sedimentation rate of the G203A \(\alpha_{i3}\)-subunit in sucrose gradients in the presence or absence of GTPγS (Fig. 3). Wild-type \(\alpha_{i3}\)-subunit sedimented more rapidly in the absence of the activating ligand than in its presence. This change in sedimentation rate is characteristic of the dissociation of \(\alpha\)-subunits from \(\beta\gamma\) subunits (37, 38). In contrast, GTPγS did not change the sedimentation rate of the G203A \(\alpha_{i3}\)-subunit mutant. This negative result was not due to the inability of the GTP analog to bind to the G203A \(\alpha_{i3}\)-subunit mutant. When sucrose gradient sedimentation experiments were carried out in the presence of \[^{35}S\text{]GTP}\gamma\text{S}\) we detected radioactivity in a position corresponding to trimeric (G203A) \(G_{i3}\), whereas no radioactivity was present at this position when the wild-type \(\alpha_{i3}\)-subunit was used (Fig. 3). Radioactivity under the peak with the lower sedimentation coefficient could correspond to any GTP-binding proteins, in-

![Image](image1.png)

**Fig. 1.** *Autophagy in cells that express Q204L and G203A \(\alpha_{i3}\)-subunit mutants.* A, autophagic sequestration of \[^{14}C\text{]raffinose}\] was determined in Q204L and G203A \(\alpha_{i3}\)-subunit-expressing cells as described in the legend of Fig. 1. Parallel experiments were performed in wild-type \(\alpha_{i3}\)-subunit overexpressing and untransfected cells (control). B, the rate of \[^{14}C\text{]valine}\] labeled long-lived proteins was measured in cells incubated either in complete medium (C) or nutrient-free medium (NF) as described under “Experimental Procedures.” Experiments were performed in presence or absence of 10 mM 3-MA. Data are expressed as the percentage of cellular protein degraded in 4 h. The values reported in panels A and B are the mean ± S.D. of five determinations.
2.1%, and 0.6–0.8% in untransfected, wild-type raffinose sequestration per hour was 4.0–4.5%, 13–14%, 1.9–2.1%, and 0.6–0.8% in untransfected, wild-type α3-subunit-expressing cells (Table I and Fig. 2A). The rate of sequestration was around (closed symbols in panels A and B) or presence of 100 μM GDPβS (open symbols in panels A and B). Each sample was then applied to linear 5–20% sucrose gradients with marker proteins. After trichloroacetic acid precipitation, fractions were analyzed by electrophoresis in SDS gels and immunoblotted with anti-α3 antibody as detailed under “Experimental Procedures.” Results were analyzed as described in the legend to Fig. 3. Arrows indicate the position of the marker proteins used: 1, cytochrome c; 2, carbonic anhydrase; 3, bovine serum albumin.

These results indicate that the G203A mutant is unable to undergo the conformational change to dissociate the α3-subunit from the β2 dimer as previously demonstrated for the G226A α3-mutant (37, 47).

Autophagic sequestration was reduced in G203A-expressing cells when compared to untransfected cells or wild-type α3-subunit-expressing cells (Table I and Fig. 2A). The rate of raffinose sequestration per hour was 4.0–4.5%, 13–14%, 1.9–2.1%, and 0.6–0.8% in untransfected, wild-type α3-subunit, G203A-, and Q204L-expressing cells, respectively. In each cell population studied the rate of sequestration of lactate dehydrogenase (Table I) and ornithine decarboxylase (data not shown), two cytosolic enzymes, was similar to those reported for [3H]raffinose (see also next paragraph). This result demonstrates that autophagic sequestration in HT-29 cells is nonselective toward cytosolic markers as has been previously reported for rat hepatocytes (40). Accordingly the rate of 3-MA-sensitive protein degradation was greatly reduced in G203A-expressing cells and not stimulated by nutrient deprivation (Fig. 2B).

The GDP-bound Form of the α3-Subunit Stimulates Autophagic Sequestration—The results reported above suggested that the GDP-bound form of G203A switches on autophagic sequestration. To confirm that the trimeric form of GDP-bound G203A stimulates the autophagic sequestration, we constructed another α3-subunit mutant. This mutant, S47N is equivalent to the S54N mutant of the α3-subunit (48). This last mutant was shown to have an increased guanine nucleotide exchange rate and increased preference for GDP over GTP (48). When membrane preparations were primed with 1 μM GTPγS and then incubated with an excess of GDPβS, the S47N mutant and wild-type α3-subunits behaved differently in sucrose gradients. The wild-type α3-subunit sedimented as a monomer (Fig. 4, panels A and B) or presence of 100 μM GTPγS (open symbols in panels A and B). Each sample was then applied to linear 5–20% sucrose gradients with marker proteins. After trichloroacetic acid precipitation, fractions were analyzed by electrophoresis in SDS gels and immunoblotted with anti-α3 antibody as detailed under “Experimental Procedures.” Results were analyzed as described in the legend to Fig. 3. Arrows indicate the position of the marker proteins used: 1, cytochrome c; 2, carbonic anhydrase; 3, bovine serum albumin.

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type αi3-subunit overexpressing cells when measured either in complete medium or in nutrient-free medium. Here again the 3-MA-sensitive degradation of radiolabeled proteins was stimulated by nutrient deprivation (Fig. 5B).

Membrane Coupling Is Required in Order for the GDP Occupied αi3-Subunit to Switch on Autophagic Sequestration—The results reported above suggest that when the αi3-subunit is occupied by GDP it stimulates autophagic sequestration. However, we have reported that ADP-ribosylation of Gαi3 is a strong inhibitor of autophagy in HT-29 cells (29). ADP-ribosylation of Gαi, by PTX, which is most efficient when the αi3-subunit is coupled to βγ (50), blocks the interaction of the α-subunits of Gαi-proteins with their receptors and consequently prevents the receptor-mediated exchange of GDP for GTP (51).

In order to further study the roles of ADP-ribosylation and GDP binding to the αi3-subunit, we have constructed two mutants of the αi3-subunit insensitive to PTX modification (52). After transfection, clones having a 3-fold overexpression of the αi3-subunits were selected (Table I), and the expression of the subunits was monitored before and after PTX treatment by high resolution urea SDS-PAGE (35). As expected, the migration of proteins from both C351S- and S47N/C351S-expressing mutants was unaffected by PTX treatment (Fig. 6, upper panel). C351S- and S47N/C351S-expressing mutants have a rate of autophagic sequestration similar to that of cells overexpressing the wild-type αi3-subunit and S47N αi3-subunit mutant (Fig. 6, lower panels; see also Figs. 2 and 5 and Table I). PTX treatment strongly inhibits autophagic sequestration in untransfected cells, overexpressing wild-type αi3-subunit and in S47N-expressing cells (Fig. 6, lower panels, and see Ref. 29), whereas autophagic sequestration was unaffected by PTX treatment in C351S- and C351S/S47N-expressing cells (Fig. 6, lower panels, and Table I) but inhibited by 3-MA (Table I). These results suggest that 3-MA and PTX act on different targets to inhibit autophagic sequestration and confirm that Gαi3 is the only PTX-sensitive G protein involved in the control of autophagic sequestration in HT-29 cells.

DISCUSSION

Our recent results have shown that the autophagic pathway is controlled at the sequestration step by a Gαi3 protein (29). Data presented here extend our understanding of this new function for trimeric G proteins. Although it cannot be completely excluded that the Gαi3 protein controls other catabolic pathways (e.g. microautophagy, direct uptake of cytosolic fractions by lysosomes) in HT-29 cells, several experiments indicate that Gαi3 governs the macroautophagic pathway and more probably the sequestration step: (i) in the different cell populations studied the sequestration of cytosolic markers was greatly reduced by 3-MA which is commonly recognized as an inhibitor of the macroautophagic sequestration step (44). (ii) 3-MA-sensitive degradation of long lived proteins is stimulated by nutrient-deprivation, a condition known to enhance the macroautophagic pathway (53, 54), exclusively in cells that express either the wild-type αi3-subunit (Fig. 2B), the S47N mutant αi3-subunit (Fig. 5B), or PTX-insensitive mutant αi3-subunits (data not shown). In contrast, the rate of 3-MA-sensitive protein degradation is not modified by nutrient deprivation in cells that express the GTP-bound form of the αi3-subunit (Fig. 2B). (iii) 3-MA-sensitive lysosomal degradation of N-linked glycoproteins substituted with endoplasmic reticulum-type oligosaccharides is dependent upon the level of expression of the αi3-subunit (29) and of the guanine nucleotide exchange on the αi3-subunit (data not shown). Nevertheless, in order to unequivocally correlate Gαi3 protein expression and activation to the macroautophagic pathway, morphometric studies are in progress to evaluate the fractional volume of autophagic vacuoles in each HT-29 cell population.

Transfection of cells with antisense oligodeoxyribonucleotides gives strong additional support to the key role of Gαi3 in the control of the autophagic pathway. We have found that an 85% reduction of the expression of the αi3-subunit was sufficient to cause 90% inhibition of autophagic sequestration in HT-29 cells (see Table I). A similar reduction of the expression of the αi3-subunit has been shown to inhibit the adenyl cyclase response by more than 70% in different cell systems (32, 55). The stimulatory effect of Gαi3 in autophagy is due to intracellular events because, as previously reported for the wild-type αi3-subunit (29), all generated mutants of the αi3-subunit are predominantly associated with intracellular membranes. These observations must now be added to the mounting evidence for the intracellular distribution of trimeric G protein subunits in

E. Ogier-Denis, C. Bauvy, and P. Codogno, manuscript in preparation.
demonstrated that GTP is required for the dissociation of Gb, where it should be noted that GαQ or GαR, and two splice variants of GαQ were found associated with Golgi membranes isolated from rat liver (60).

From the results reported in the present study we can hypothesize a mechanism by which Gαi controls the autophagic sequestration. When Gαi is occupied by GDP it can interact with a putative membrane receptor. This interaction would lead to autophagic sequestration. The fact that PTX, which inhibits the interaction of Gi proteins with their receptors (51), will require the characterization of membrane components interacting with Gαi. However, the function of trimeric G proteins, including Gαi, in membrane remodeling is complex and probably does not exclusively depend upon the interaction with membrane proteins but also requires the recruitment of cytosolic proteins (61, 62).

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