Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 5049–5052, June 1992
Biochemistry

Cloning of a Rab3 isotype predominately expressed in adipocytes

GIULIA BALDINI*, TOBIAS HOHL*, HERBERT Y. LIN*†, AND HARVEY F. LODISH*†

*Whitehead Institute for Biomedical Research, Nine Cambridge Center, Cambridge, MA 02142; and †Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139

Contributed by Harvey F. Lodish, February 19, 1992

ABSTRACT **We have isolated the cDNA for Rab3D, an additional member of the small molecular weight GTP-binding protein family. Rab3D message is abundant in mouse adipocytes. It is increased during differentiation of 3T3-L1 cells into adipocytes, temporally coincident with the appearance of the insulin-sensitive glucose transporter GLUT4. Rab3D is a close homolog of Rab3A, which is found on the cytoplasmic surface of neurosecretory vesicles and which may be involved in their regulated secretion. Since our previous work showed that in permeabilized adipocytes nonhydrolizable GTP analogs mimic insulin in triggering exocytosis of GLUT4-containing vesicles, Rab3D may be involved in the insulin-induced exocytosis of GLUT4-containing vesicles in adipocytes.**

Insulin stimulates glucose uptake in fat, muscle, and heart; this process is defective in non-insulin-dependent diabetes mellitus (1). In adipocytes, this is due mainly to translocation of two glucose transporter isotypes, GLUT4 (expressed only in fat and muscle) and the ubiquitously expressed GLUT1 (2–8) from trans-Golgi or endosome tubulovesicular organelles to the plasma membrane (9–12). A GTP-binding protein seems to be involved in GLUT4 translocation because guanosine 5′-[γ-thio]triphosphate and other nonhydrolyzable GTP analogs mimic insulin in inducing a shift of GLUT4 to the plasma membrane in permeabilized fat cells (30). Small molecular weight GTP-binding proteins have been proposed to regulate other types of vesicular traffic in eukaryotic cells (14, 15).

3T3-L1 mouse fibroblasts differentiate to adipose cells when induced by appropriate culture conditions (16). At the fourth day of the differentiation program, the rate of 2-deoxyglucose transport is stimulated 10-fold by insulin, in contrast to the 2-fold stimulation observed in undifferentiated 3T3-L1 fibroblasts. This induction correlates with the appearance of GLUT4 message and protein (17). We reasoned that if a small GTP-binding protein is involved in the insulin-stimulated recruitment of GLUT4 at the cell surface, it will appear or be increased during differentiation of the 3T3-L1 cells. Utilizing a PCR (polymerase chain reaction) strategy, we have cloned from an adipocyte-specific subtractive library an additional member of the *Rab* gene family, termed *Rab3D*,‡ that exhibits this property.

METHODS

Cell Culture. 3T3-L1 mouse fibroblasts (a gift of Howard Green, Boston) were differentiated to 3T3-L1 adipocytes as described (16).

RNA Preparation. Ten to 20 100-mm-diameter cell culture dishes of 3T3-L1 cells at different stages of differentiation were harvested, washed with ice-cold phosphate-buffered saline, and resuspended in 25 ml of proteinase K buffer [0.5% SDS/0.1 M NaCl/1 mM EDTA/20 mM Tris·HCl, pH 7.4/400 μg of proteinase K (Boehringer)] per ml. The cells were homogenized for 60 sec with a Polytron homogenizer and incubated at 37°C for 1 hr. The samples were then adjusted to 0.4 M NaCl and incubated at room temperature for 1 hr with 0.5 ml of oligo(dT)-cellulose (Collaborative Research) equilibrated in high-salt buffer (0.1% SDS/0.4 M NaCl/1 mM EDTA/10 mM Tris·HCl, pH 7.4). The resin was washed three times with high-salt buffer and loaded on a Poly Prep column (Pharmacia), and the RNA was eluted in low-salt buffer (0.1% SDS/1 mM EDTA/10 mM Tris·HCl, pH 7.4).

3T3-L1 Adipocyte-Specific Subtractive cDNA Library. This entire procedure is essentially as described (18). In brief, cDNA libraries from 3T3-L1 adipocytes and fibroblasts were constructed in the vector pcDNA-1 (Invitrogen, San Diego) and maintained in *Escherichia coli* cells. Single-stranded copies of the two libraries were derived by superinfection with the M13 helper phage RV1 (a gift of Brian Seed, Massachusetts General Hospital, Boston). The 3T3-L1 fibroblast (preadipocyte) single-stranded DNA library was sonicated to a mean length of ≈500 base pairs (bp), conjugated to biotin, and hybridized in excess to the nonsonicated 3T3-L1 adipocyte single-stranded DNA library. The hybridization mixture contained in a 50-μl volume: 25 μg of biotinylated 3T3-L1 preadipocyte DNA library, 2.5 μg of 3T3-L1 adipocyte DNA library, 2 μg of poly(A), 2 μg of poly(C), 50% formamide, 5× SSC (1× = 0.15 m NaCl/0.015 M sodium citrate, pH 7), 100 mM sodium phosphate, 1 mM EDTA, and 0.1% SDS. The mixture was heat-sealed in a 50-μl capillary and incubated at 100°C for 1 min and then at 42°C for 20 hr. The hybridized inserts, common to the two libraries, were removed by affinity chromatography on an avidin-containing resin as described (18). The resulting single-stranded adipocyte-specific DNA library was then converted to double-stranded DNA by T7 DNA polymerase as follows. The DNA was annealed to 1.6 pmol of T7 primer in a 10-μl reaction mixture containing 20 mM Tris·HCl (pH 7.4) and 10 mM $MgCl_2$, heated at 65°C for 2 min, and cooled to 30°C for 30 min. This mixture was then brought to a final volume of 20 μl containing each dNTP at 0.150 mM, 0.5 mM dithiothreitol, 0.025 μg of bovine serum albumin per ml, and 20 units of T7 DNA polymerase (Biolabs, Northbrook, IL) and was incubated at 37°C for 30 min. The T7 DNA polymerase reaction mixture was used directly to transform competent FG2 bacteria by electroporation with a Gene Pulser (Bio-Rad).

Screening of the Adipocyte-Specific Subtractive Library. Bacterial clones expressing the subtracted cDNA library were screened with a ^{32}P-labeled PCR product. The primers for the PCR reaction were: 5′-TGGGA$\frac{C}{T}$ACNGCNGGNCA$\frac{A}{G}$GA-3′(sense; N = A, C, G, or T) corresponding to the G-3 region Trp-Asp-Thr-Ala-Gly-Gln-Glu conserved in Rab and Ras proteins (19) and 5′-AGGCTGCAG$\frac{A}{G}$TCNNN$\frac{C}{T}$TT$\frac{A}{G}$T-TNCC-3′(antisense), corresponding to the G-4 region Gly-Asn-Lys-Xaa-Asp (19). Both regions are common to all Rab

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Abbreviation: GLUT, glucose transporter.

‡The sequence reported in this paper has been deposited in the GenBank data base (accession no. M89777).

proteins. DNA from the subtracted library (40 ng) and 1 μM antisense primer in 100 μl of reaction mix (GeneAmp PCR core reagents, Perkin–Elmer/Cetus) containing 1.5 mM $MgCl_2$ were subjected to the following temperature cycles: 1 cycle of 94°C for 2 min, 25°C for 3 min, and 50°C for 2 min, followed after addition of 1 μM sense primer by 29 cycles of 94°C for 2 min, 55°C for 1 min, and 72°C for 20 sec. The subtracted library was transferred to Biotrans nylon membranes (ICN) which were pretreated and then hybridized at high stringency (50% formamide in the hybridization solution) with the ^{32}P-labeled PCR probe (10^6 dpm·ml^{-1}, random-primed labeling system) as described (20) and washed twice for 20-min periods in 2× SSC containing 0.1% SDS (50°C) and in 0.1× SSC containing 0.1% SDS (50°C). The *Bam*HI-*Sph* I cDNA inserts from the positive clones were labeled by random priming as above and used as probes for RNA blot-hybridization (Northern) analysis. The selected insert, corresponding to Rab3D, was subcloned into the replicative form of M13mp18 and M13mp19 (Pharmacia) and sequenced on both strands by the dideoxy chain-termination method in combination with Sequenase version 2.0 (United States Biochemical). The computation for sequence comparison was performed at the National Center for Biotechnology Information by using the BLAST network service.

Northern Blot Analysis. One microgram of poly(A)$^+$ RNA prepared from 3T3-L1 cells on the indicated days of the differentiation program (16, 21) or from different tissues was fractionated on a formaldehyde/agarose gel (1.0%) and then blotted and fixed onto a Biotrans nylon membrane. The blots were hybridized with the indicated probes that were ^{32}P-labeled as above. The hybridization and washing conditions, at 42°C and 50°C, respectively, were as described (6). When indicated, blots were hybridized with a 117-base oligonucleotide encoding the unique Rab3D 5′-terminal region (the underlined sequence from nucleotide 3 to 120 shown in Fig. 1). For this region the nucleotide identity with other Rab3 proteins is only 45–55%. The 117-base oligonucleotide was generated by a PCR with Rab3D cDNA as a template and 1 μM of the primers 5′-CGAGCGGCCGCCAGTGTGC-3′ (sense), corresponding to nucleotides 3–21 in the 5′ untranslated Rab3D nucleotide sequence, and 5′-AGCAGTTTGAACATGTAGTC-3′ (antisense), corresponding to the deduced amino acids at positions 20–26, Asp-Tyr-Met-Phe-Lys-Leu-Leu. The conditions for the PCR were as above, with the following temperature cycle for 29 cycles: 94°C for 2 min, 50°C for 1 min, and 72°C for 20 sec.

RESULTS

cDNA libraries were constructed from 3T3-L1 preadipocyte fibroblasts and differentiated adipocytes in the vector pcDNA-1 (18). After subtraction of the adipocyte library with the preadipocyte one, the enrichment for GLUT4 cDNA was >100-fold (not shown).

Small molecular weight eukaryotic GTP-binding proteins share multiple regions of homology. In region G-3 and G-4, the sequences Trp-Asp-Thr-Ala-Gly-Gln-Glu and Gly-Asn-Lys-Xaa-Asp are strictly conserved in all members of the Rab GTPases involved in vesicular transport (19). Two sets of degenerate oligonucleotides corresponding to these amino acid sequences were used as primers in the PCR reaction to amplify specific cDNAs from the 3T3-L1 subtracted library. As expected, the product of the PCR reaction migrated in a 2% agarose gel as a single band of approximately 200 bases. This radiolabeled probe was utilized to screen the subtracted 3T3-L1 library. Forty of the 10,000 inserts tested positive and 13 unique clones were selected after cross-hybridization analysis of the inserts. Northern blot analysis showed that two identical clones of 2.3 kilobases (kb) hybridized with two RNA species of 2.3 and 4.0 kb, both induced after 3T3-L1 adipose conversion. The new clone was designated Rab3D.

Fig. 1 shows the nucleotide and the deduced amino-acid sequence of Rab3D. Rab3D has, as expected from the cloning strategy, the conserved amino acid motifs (underlined in Fig. 1) of the Rab proteins in regions G-3 and G-4 (19).

In Fig. 2 the deduced amino acid sequence of Rab3D is compared to that of all the other known members of the Rab3 family (22, 23). From residue Asp-16 to residue Lys-191, the amino acid identity among murine Rab3D and bovine Rab3A, Rab3B, and Rab3C is, respectively, 88%, 86%, and 85%. Rab3D has <54% identity to other Rab proteins. However, in the boxed amino- and carboxyl-terminal regions of Rab3D, respectively, <25% and <28% of the residues are identical to the other Rab3 proteins; these differences are not due to species variability because in these regions the three Rab3As are >90% identical, as are the two cloned Rab3Bs.

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                                                5' CCCGAGCGGCCGCCAGTGTGCTCTAAAGCGAGATCCCACTGAG 43

    ATGGCATCCGCTAGTGAGCCCCCTGCCAGCCCAAGAGACGCTGCTGACCAGAACTTTGACTACATGTTCAAACTGCTCTTGATCGGGAAC 133
  1 MetAlaSerAlaSerGluProProAlaSerProArgAspAlaAlaAspGlnAsnPheAspTyrMetPheLysLeuLeuleuIleGlyAsn

    AGCAGCGTGGGCAAGACATCCTTCCTGTTCCGCTATGCCGATGACTCCTTCACCCCTGCCTTCGTGAGCACAGTGGGCATCGACTTCAAG 223
 31 SerSerValGlyLysThrSerPheLeuPheArgTyrAlaAspAspSerPheThrProAlaPheValSerThrValGlyIleAspPheLys

    GTCAAGACGGTCTACCGACATGACAAGAGGATCAAGCTGCAGATTTGGGACACAGCAGGCCAGGAGCGCTACCGGACAATCACCACGGCC 313
 61 ValLysThrValTyrArgHisAspLysArgIleLysLeuGlnIleTrpAspThrAlaGlyGlnGluArgTyrArgThrIleThrThrAla

    TACTATCGCGGAGCTATGGGTTTCCTGCTCATGTATGACATCGCCAACCAGGAGTCCTTCACCGCGGTGCAGGACTGGGCTACGCAGATC 403
 91 TyrTyrArgGlyAlaMetGlyPheLeuLeuMetTyrAspIleAlaAsnGlnGluSerPheThrAlaValGlnAspTrpAlaThrGlnIle

    AAAACCTATTCCTGGGACAATGCCCAGGTAATCCTCGTGGGGAACAAGTGTGACCTGGAAGACGAACGGGTCGTACCTGCTGAGGATGGC 493
121 LysThrTyrSerTrpAspAsnAlaGlnValIleLeuValGlyAsnLysCysAspLeuGluAspGluArgValValProAlaGluAspGly

    CGGAGGCTCGCCGATGATCTTGGTTTTGAGTTCTTTGAGGCCAGCGCCAAGGAGAATATCAATGTGAAGCAGGTGTTCGAGCGCCTGGTG 583
151 ArgArgLeuAlaAspAspleuGlyPheGluPhePheGluAlaSerAlaLysGluAspIleAsnValLysGlnValPheGluArgLeuVal

    GACATCATCTGTGACAAGATGAATGAATCCCTGGAACCCAGCTCCAGCCCAGGCAGCAATGGAAAAGGCCCAGCCCTTGGGGATACCCCA 673
181 AspIleIleCysAspLysMetAspGluSerLeuGluProSerSerSerProGlySerAsnGlyLysGlyProAlaLeuGlyAspThrPro

    CCCCCACAGCCGAGCAGCTGCAGCTGTTAG 703
211 ProProGlnProSerSerCysSerCysEnd
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FIG. 1. Nucleotide and predicted encoded amino acid sequences of the mouse Rab3D cDNA. The amino acid sequences of regions G-3 and G-4, conserved in all known members of the Rab family (19) are underlined. The nucleotide sequence in the Rab3D 5′-terminal region (positions 3–120, underlined) was amplified by PCR and used as a specific probe to detect Rab3D mRNA.

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Rat     3A     1 MAS ATDSRYGQKESS DQNFDYMFKILIIGNSSVGKTSFLFRYADDSFTPAFVSTV
Bovine  3A       MAS ATDARYGQKESS DQNFDYMFKILIIGNSSVGKTSFLFRYADDSFTPAFVSTV
Human   3A       MAS ATDSRYGQKESS DQNFDYMFKILIIGNSSVGKTSFLFRYADDSFTPAFVSTV
Bovine  3B       MAS VTDGKAGVKDAS DQNFDYMFKLLIIGNSSVGKTSFLFRYADDTFTPAFVSTV
Human   3B       MAS VTDGKHGVKDAS DQNFDYMFKLLIIGNSSVGKTSFLLRYADDTFTPAFVSTV
Bovine  3C       MAS AQDARYGQKDSS DQNFDYMFKLLIIGNSSVGKTSFLFRYADDSFTSAFVSTV
Murine  3D       MAS ASEPPASPRDAA DQNFDYMFKLLLIGNSSVGKTSFLFRYADDSFTPAFVSTV

Rat     3A    56 GIDFKVKTIYRNDKRIKLQIWDTAGQERYRTITTAYYRGAMGFILMYDITNEESF
Bovine  3A       GIDFKVKTIYRNDKRIKLQIWDTAGQERYRTITTAYYRGAMGFILMYDITNEESF
Human   3A       GIDFKVKTIYRNDKRIKLQIWDTAGQERYRTITTAYYRGAMGFILMYDITNEESF
Bovine  3B       GIDFKVKTVYRHEKRVKLQIWDTAGQERYRTITTAYYRGAMGFILMYDITNEESF
Human   3B       GIDFKVKTVYRHEKRVKLQIWDTAGQERYRTITTAYYRGAMGFILMYDITNEESF
Bovine  3C       GIDFKVKTVFKNEKRIKLQIWDTAGQERYRTITTAYYRGAMGFILMYDITNEESF
Murine  3D       GIDFKVKTVYRHDKRIKLQIWDTAGQERYRTITTAYYRGAMGFLLMYDIANQESF

Rat     3A   111 NAVQDWSTQIKTYSWDNAQVLLVGNKCDMEDERVVSSERGRQLADHLGFEFFEAS
Bovine  3A       NAVQDWSTQIKTYSWDNAQVLLVGNKCDMEDERVVSSERGRQLADHLGFEFFEAS
Human   3A       NAVQDWSTQIKTYSWDNAQVLLVGNKCDMEDERVVSSERGRQLADHLGFEFFEAS
Bovine  3B       NAVQDWATQIKTYSWDNAQVILVGNKCDMEEERVVPTEKGRLLAEQLGFDFFEAS
Human   3B       NAVQDWATQIKTYSWDNAQVILVGNKCDMEEERVVPTEKGQLLAEQLGFDFFEAS
Bovine  3C       NAVQDWSTQIKTYSWDNAQVILVGNKCDMEDERVISTERGQHLGEQLGFEFFETS
Murine  3D       TAVQDWATQIKTYSWDNAQVILVGNKCDLEDERVVPAEDGRRLADDLGFEFFEAS

Rat     3A   166 AKDNINVKQTFERLVDVICEKMSESL DTADLAVTGAKQGPQ L TDQQAPPHQD CAC
Bovine  3A       AKDNINVKQTFERLVDVICEKMSESL DTADPAVTGAKQGPQ L TDQQAPPHQD CAC
Human   3A       AKDNINVKQTFERLVDVICEKMSESL DTADPAVTGAKQGPQ L SDQQVPPHQD CAC
Bovine  3B       AKENISVRQAFERLVDAICDKMSDTL DT DPSLLGTSKNTR L SDTPPLLQQN CSC
Human   3B       AKENISVRQAFERLVDAICDKMSDSL DT DPSMLGSSKNTR L SDTPPLLQQN CSC
Bovine  3C       AKDNINVKQTFERLVDIICDKMSESL ET DPAITAAKQNTR L KETPPPPQPN CGC
Murine  3D       AKENINVKQVFERLVDIICDKMNESL EP SSSPGSNGKGPA L GDTPPPQPSS CSC
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FIG. 2. The deduced amino acid sequence of Rab3D is compared with the sequences of the other Rab3 isotypes. Boldface type indicates residues identical in all Rab3 isoforms from all species. Boxed are regions unique to Rab3D; in these regions the three Rab3As and two Rab3Bs are >90% identical.

Fig. 3 shows Northern blot analyses of Rab3D and GLUT4 induction during differentiation of 3T3-L1 cells. Both the 2.3- and the 4.0-kb transcripts were labeled when Northern blots were probed either with the entire Rab3D cDNA insert or (not shown) with a PCR product encoding the unique 5′ nucleotide region of Rab3D mRNA (nucleotides 3–120 of the Rab3D sequence in Fig. 1). Both Rab3D messages are present at every stage of differentiation of 3T3-L1 cells. However, between days 2 and 4 of adipose conversion, there is a 4- to 5-fold increase in the abundance of both Rab3D messages; this coincides with the appearance of GLUT4 mRNA.

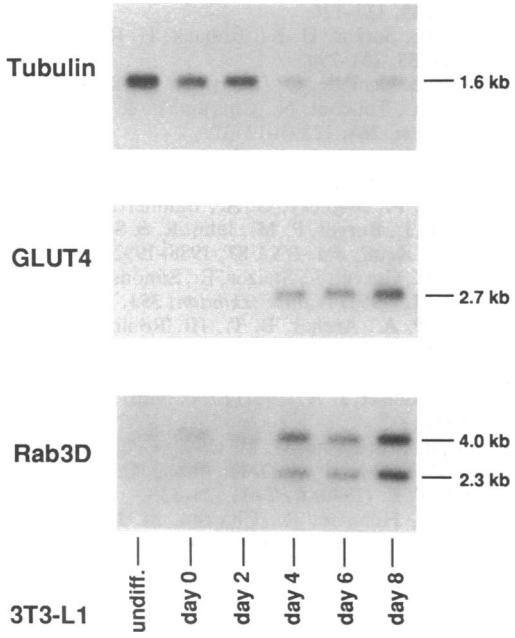

FIG. 3. Northern blot analysis of Rab3D, GLUT4, and tubulin mRNAs expressed during differentiation of 3T3-L1 fibroblasts. Each lane was loaded with 1 μg of poly(A)$^+$ RNA prepared from 3T3-L1 cells on the indicated day of the differentiation program (16). The same signal obtained in all samples probed with the tubulin cDNA indicates that approximately equal amounts of RNA were loaded on the gels. undiff., Undifferentiated.

Fig. 4 shows the tissue distribution of Rab3D by Northern blot analysis. Rab3D message is most abundant in fat and is present at lower levels in muscle and heart. In contrast to GLUT4, Rab3D message is also expressed, albeit at low levels, in non-insulin-sensitive tissues such as lung and spleen. In contrast to Rab3A, which is expressed only in nervous and neuroendocrine tissues, Rab3D is poorly expressed in brain.

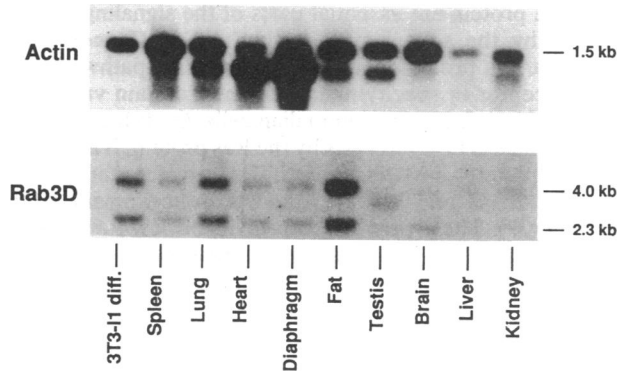

FIG. 4. Northern blot analysis of Rab3D mRNA tissue distribution. The blot was hybridized with a PCR 117-base product encoding the unique Rab3D 5′-terminal region (underlined nucleotides from position 3 to 120 in Fig. 1). A parallel blot is hybridized with an actin cDNA as the control for equal loading of RNA. Each lane was loaded with 1 μg of poly(A)$^+$ RNA prepared from the indicated tissues.

DISCUSSION

Here we describe the cloning and properties of an additional member of the Rab protein family, Rab3D. The majority of the deduced Rab3D amino acid sequence is identical to that of Rab3A, with the important exceptions of the amino- and carboxyl-terminal regions.

Rab3A is specifically targeted to neuronal and neuroendocrine synaptic vesicles and seems to be involved in regulated exocytosis triggered by membrane depolarization induced by the arrival of an action potential or by hormonal stimulation (24). The similarity of Rab3D to Rab3A suggests that it too is involved in regulated exocytosis. It has been proposed that the variable carboxyl-terminal region of GTP-binding proteins determines their correct targeting to specific subcellular organelles (25). The striking differences between Rab3D and the other members of the Rab3 family at their carboxyl-terminal regions suggest that Rab3D could be targeted in adipocytes to a population of vesicles undergoing induced exocytosis. Most likely these vesicles contain GLUT4, since this transporter is abundant in endosome and trans-Golgi membranes adjacent to the plasma membrane (11, 12).

Rab3D has at its carboxyl terminus the motif Cys-Xaa-Cys common to all members of the Rab3 family and to other GTP-binding Rab proteins involved in vesicular traffic. The sequence Cys-Xaa-Cys is necessary for the hydrophobic attachment of Rab3A to membranes (26), which occurs after geranylgeranylation of both cysteine residues and esterification of the carboxyl-terminal residue (27). These residues could be similarly modified in Rab3D.

During differentiation of 3T3-L1 cells, there is an increase in the level of the insulin receptor as well as the acquisition of an exocytotic pathway for insulin-sensitive translocation of glucose transporters to the plasma membrane. The increase in expression of Rab3D during differentiation, the relative abundance of its message in adult fat tissue, and its structural similarities to another exocytic GTP-binding protein, Rab3A, indicate that Rab3D might be involved in GLUT exocytosis. The presence of Rab3D in non-insulin-sensitive tissues indicates that Rab3D could also be involved in other regulated exocytotic systems.

Since guanosine 5′-[γ-thio]triphosphate and other nonhydrolyzable GTP analogs mimic insulin in inducing a shift of GLUT4 to the plasma membrane in permeabilized fat cells (30), activation of Rab3D presumably involves exchange of GTP for GDP and presumably is triggered by insulin activation of the insulin receptor protein-tyrosine kinase. In two well-studied invertebrate cells, a Ras protein is part of the intracellular signal transduction pathway induced by a receptor protein-tyrosine kinase: In specification of the *Drosophila* R7 photoreceptor cell, both Ras and a presumptive GTP–GDP exchange protein are essential parts of the signaling pathway induced by the sevenless receptor, and in *Caenorhabditis elegans* a Ras protein is part of the signaling pathway of the let-23 receptor in specifying induction of certain vulval cells (reviewed in ref. 28). In mammalian cells, GAP-Ras, a protein that activates GTP hydrolysis by the Ras protein, is a substrate for the platelet-derived growth factor receptor protein-tyrosine kinase and binds to the cytosolic domain of that receptor (29). These provide precedents of how Rab3D might be activated by the insulin receptor protein-tyrosine kinase.

We thank Brian Seed for advice and help in making the subtractive library and Bernard Thorens for helpful discussion. This research was supported by National Institutes of Health Grants GM-49516 and HL-41484. G.B. was a postdoctoral fellow supported by Juvenile Diabetes International Foundation.

1. Simpson, I. A. & Cushman, S. V. (1986) *Annu. Rev. Biochem.* **55**, 1059–1089.
2. Muekler, M., Caruso, C., Baldwin, S. A., Panico, M., Blench, I., Morris, H. R., Allard, W. J., Lienhardt, G. E. & Lodish, H. F. (1985) *Science* **229**, 941–945.
3. Birnbaum, M. J., Haspel, H. C. & Rosen, O. M. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 5784–5788.
4. Birnbaum, M. J. (1989) *Cell* **57**, 305–315.
5. James, D. E., Strube, M. & Muecker, M. (1989) *Nature (London)* **338**, 83–87.
6. Charron, M. J., Brosius, F. C., Alper, S. L. & Lodish, H. F. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 2535–2539.
7. Fukumoto, H., Kayano, T., Buse, J. B., Edwards, Y., Pilch, P. F., Bell, G. I. & Seino, S. (1989) *J. Biol. Chem.* **264**, 7776–7779.
8. Kaestner, K. H., Christy, R. J., McLenithan, J. C., Braiterman, L. T., Cornelius, P., Pekala, P. K. & Lane, M. D. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 3150–3154.
9. Cushman, S. V. & Wardzala, L. J. (1980) *J. Biol. Chem.* **255**, 4758–4762.
10. Suzuki, K. & Kono, T. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 2542–2545.
11. Smith, R. M., Charron, M. J., Shah, N., Lodish, H. F. & Jarett, L. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 6893–6897.
12. Slot, J. W., Geuze, H. J., Gigengack, S., Lienhard, G. E. & James, D. E. (1991) *J. Cell Biol.* **113**, 123–135.
13. Goud, B., Salminen, A., Walworth, N. C. & Novick, P. J. (1988) *Cell* **53**, 735–768.
14. Chavrier, P., Parton, R. G., Hauri, H. P., Simons, K. & Zerial, M. (1990) *Cell* **62**, 317–329.
15. Sudhof, T. C. & Jahn, R. (1991) *Neuron* **6**, 665–677.
16. Frost, S. C. & Lane, D. J. (1984) *J. Biol. Chem.* **260**, 2646–2652.
17. de Herreros, A. G. & Birnbaum, M. J. (1989) *J. Biol. Chem.* **264**, 19994–19999.
18. Duguid, J. R., Rohwer, R. G. & Seed, B. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 5738–5742.
19. Bourne, H. R., Sanders, D. A. & McCormick, F. (1991) *Nature (London)* **349**, 117–126.
20. Thorens, B., Sarkar, H. K., Kaback, H. R. & Lodish, H. F. (1988) *Cell* **55**, 281–290.
21. Green, H. & Kehinde, O. (1973) *Cell* **1**, 113–116.
22. Zahraoui, A., Touchot, N., Chardin, P. & Tavitian, A. (1989) *J. Biol. Chem.* **264**, 12394–12401.
23. Matsui, Y., Kikuchi, A., Kondo, J., Hishida, T., Teranishi, Y. & Takai, Y. (1988) *J. Biol. Chem.* **263**, 11071–11074.
24. Mollard, G. F., Mignery, G. A., Baumert, M., Perin, M. S., Hanson, T. J., Burger, P. M., Jahn, R. & Sudhof, T. C. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 1988–1992.
25. Chavrier, P., Gorvel, J., Steizer, E., Simons, K., Gruenberg, J. & Zerial, M. (1991) *Nature (London)* **353**, 769–772.
26. Johnston, P. A., Archer, B. T., III, Robinson, K., Mignery, G. A., Jahn, R. & Sudhof, T. C. (1991) *Neuron* **7**, 101–109.
27. Farnsworth, C. C., Kawata, M., Yoshida, Y., Takai, Y., Gelb, M. H. & Glomset, J. A. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 6196–6200.
28. Greenwald, I. & Rubin, G. M. (1992) *Cell* **68**, 271–281.
29. Kaplan, D. R., Morrison, D. K., Wong, G., McCormick, F. & Williams, T. L. (1990) *Cell* **61**, 125–133.
30. Baldini, G., Hohman, R., Charron, M. J. & Lodish, H. F. (1991) *J. Biol. Chem.* **266**, 4037–4040.