Insulin Increases the Cell Surface Concentration of \( \alpha_2 \)-Macroglobulin Receptors in 3T3-L1 Adipocytes

ALTERED TRANSIT OF THE RECEPTOR AMONG INTRACELLULAR ENDOCYTIC COMPARTMENTS*

(Received for publication, September 26, 1988)

Silvia Corvera, Douglas F. Graver, and Robert M. Smith
From the Department of Pathology and Laboratory Medicine, University of Pennsylvania Medical School, Philadelphia, Pennsylvania 19104-6089

The present study shows that insulin causes an increase in the binding of \( \alpha_2 \)-macroglobulin (\( \alpha_2 \)M) to 3T3-L1 adipocytes. Scatchard analysis of the binding at 4 °C indicated an approximate 2-fold increase in the number of \( \alpha_2 \)M binding sites, with no change in the apparent affinity of the receptor. In addition, a 2-3-fold increase in the binding of monoclonal antibody 2C6, which recognizes a component of the \( \alpha_2 \)M receptor, was found in cells treated at 37 °C with insulin and then KCN to inhibit receptor endocytosis. An increased cellular accumulation of \( \alpha_2 \)M was also observed in response to insulin. Interestingly, the increase in the rate of accumulation of \( \alpha_2 \)M was significantly smaller than the increase in the number of \( \alpha_2 \)M receptors on the cell surface, suggesting that the rate of ligand internalization or subsequent processing is altered in response to insulin. Ultrastructural analysis of the internalization pathway of the \( \alpha_2 \)M receptor was performed using colloidal gold-coupled 2C6 monoclonal antibody. Control cells incubated for 20 min at 37 °C with the gold-conjugated antibody displayed 40% of cellular gold particles on the cell surface and 60% within intracellular structures. In insulin-treated cells this proportion was reversed, with 64% of the particles being found on the cell surface, and only 36% within intracellular structures. Significant differences in the distribution of gold particles among intracellular structures were detected between control and insulin-treated cells. Whereas in control cells, 18% of the total cellular gold particles internalized into tubulovesicles and multivesicular bodies, in insulin-treated cells only 3% of the gold particles were found within these structures. These data indicate that the movement of this receptor between endocytic compartments is altered in response to insulin, and suggest that the effect of insulin to increase the cell surface concentration of \( \alpha_2 \)M receptors and the accumulation of \( \alpha_2 \)M is due, at least in part, to alterations in the endocytic portion of the receptor recycling pathway.

Within minutes after stimulation of cells by insulin, a 3-5-fold increase in the plasma membrane concentration of glucose transporters (1, 2), transferrin receptors (3-5), and IGF-

* This work was supported in part by Juvenile Diabetes Foundation Grant 5-24155 and National Institutes of Health Grant DK 19525.

1 The abbreviations used are: IGF-II, insulin-like growth factor-II; \( \alpha_2 \)M, \( \alpha_2 \)-macroglobulin; BSA, bovine serum albumin; Heps, 4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid.
the receptor, and in its movement among intracellular endo-
cytic compartments.

**Experimental Procedures**

α2-Macroglobulin and Monoclonal Antibody 2C6—α2-Macroglobu-
lin was isolated from outdated human plasma by the procedure of
Swenson and Howard (14). This procedure involves precipitation
with ammonium sulfate, shown to convert α2M to the electrophoret-
ically fast form which does not require further activation by primary
amino groups in order for binding to high affinity receptors to
occur (15). More than 85% of the preparation migrated in polyacryl-
amide gels as an 185-kDa polypeptide, indicating that relatively little
proteolysis occurred during the isolation procedure. Purified α2M was
iodinated using the Enzymobeads procedure (Bio-Rad) to a specific
activity of 0.8-1.2 Ci/mmol.

Monoclonal antibody 2C6 was obtained from Dr. John Hanover
(National Institutes of Health) and has been reported to interact with
the α2M receptor (13, 16). For conjugation to colloidal gold, the
antibody was extensively dialyzed against 2 mM boric acid/borax
buffer, pH 9.0, and added at a final concentration of 1.5 mg/100 ml
to a suspension of 15-nm gold particles prepared by the method of
Slot and Geuze (17). The preparation was stabilized with bovine
serum albumin (BSA), and diluted in 10 mM phosphate buffer, pH
7.4, containing 0.1% BSA. The unreacted antibody was removed by
column chromatography (Ultrogel AcA 44, LKB Products), and the
gold conjugate was concentrated by addition of 10 mL of buffer (YM100 mem-
branes). After sterilization by filtration through 0.22-μm filters, the
preparation was stored at 4°C and used within 10 days of preparation.

Experiments were performed using a final particle molarity of 10 nm.

**Cell Culture**—3T3-L1 cells were grown in 24- or 6-well multidishes
(Nunc, Denmark) for binding experiments or studies involving elec-
tron microscopy, respectively. Fibroblasts were seeded and fed every
2 days in Dulbecco's modified Eagle's medium supplemented with
0.75 mg/ml glutamine, nonessential amino acids (Gibco), 50 units/ml
penicillin, 50 μg/ml streptomycin, and 10% fetal calf serum (Hyclone),
and grown under 10% CO2. At confluence, differentiation was started by
the addition of medium containing 0.25 μM dexamethasone (Sigma)
and 0.5% isobutylmethylxanthine (Sigma). After 48 h, this
was replaced with fresh medium, which was not removed until exper-
iments were performed. Lipid droplets were observed in 80-85% of
the cells 4 days after initiating differentiation. Experiments were
performed on the sixth day of differentiation.

**Binding and Uptake of α2M and Monoclonal Antibody 2C6**—On the
sixth day of differentiation, monolayers were washed three times with
1 ml of Krebs-Ringer/Hepes buffer supplemented with 2 mM sodium
pyruvate and 2% BSA, and equilibrated for 30 min at 37°C. Insulin
(crystalline porcine, Eli Lilly) was then added at various concen-
trations for 100-1000 nM, and incubations were continued for
5 min at 12,000 x g. The pellets were then processed for electron
microscopy as described (18) and tissue blocks sectioned and exam-
ined for gold particles. Certain definitions were adhered to in the
analysis of gold particle distribution. Plasma membrane invaginations
which were coated in their cytoplasmic surface by a bristle structure
were defined as coated pits. Gold particles were also detected in small
membrane invaginations which were smooth in their cytoplasmic
surface, and were called smooth invaginations. Membrane enclosed
structures within the cytoplasm were defined as vesicles. Those with
a bristle coating were defined as coated vesicles. Smaller vesicles
were round, had a clear lumen, and lacked a bristle coat. Tubulovesicles
were slightly larger, had a clear lumen, and an elongated or irregular
contour. Multivesicular bodies were large vesicles that contained
small vesicular structures within their lumen, but no discernible
electron-dense material. Lysosomes were defined by the presence of
electron dense material. Approximately 50 photogra-
phs were taken from each tissue block, and two to three separate
tissue blocks were analyzed for each condition in each of three
experiments. The gold particles found in different structures were
tallied, and the results expressed as a percentage of total gold particles
counted. These ranged from 400-700/condition in three different
experiments, in which independent preparations of gold-2C6 were
employed. Similar results were obtained in each experiment.

**Results**

The binding of 125I-α2M to the cell surface of 3T3-L1 cells
on day 6 of differentiation was analyzed. Cell monolayers
were incubated with increasing concentrations of the ligand
at 4°C, to prevent receptor endocytosis. After 4 h, the amount
of α2M specifically bound to the cell monolayers was mea-
ured. The binding of α2M to control cell monolayers saturated
at a concentration of approximately 0.5 nM (Fig. 1, left panel).
Scatchard analysis of the saturation isotherm indicated a Kd
of 0.1 nM, and a maximal binding of 16 fmol/106 cells. These
values are similar to those reported for cultured human fibro-
blasts. Alternatively, after insulin treatment KCN was added at a
concentration of approximately 50 μM insulin for 10 min at 37°C as described under
"Experimental Procedures." Cells were chilled to 4°C and incubated
for 4 h with α2M at the concentrations indicated. Nonspecific binding
was measured in the presence of their lumen. Approximate approximately 20% of the total radioactivity bound. Plotted in the
left panel are the means ± S.E. of 4 independent experiments
performed in triplicate. Right panel represents the Scatchard analysis of the data shown in the left panel.

![Fig. 1. Saturation analysis of the binding of 125I-α2M to control 3T3-L1 cells.](image-url)
binding, from 16 to 24 fmol/10^6 cells. No consistent difference.

Specific uptake were treated for 10 min with 20% L-cell 
2100 nM insulin, α2M was then added at a final concentration of 1 nM (time 0) in the presence or absence of 5 μM unlabeled α2M and specific cell-associated radioactivity was assessed at the times indicated. Non-specific uptake of α2M by control (○) or insulin-treated (●) cells after 40 min incubation is depicted by the isolated symbols. Plotted are the means ± S.E. of 5 independent experiments performed in triplicate.

FIG. 2. Uptake of α2M by control or insulin-treated 3T3-L1 adipocytes. 3T3-L1 adipocyte monolayers at day 6 of differentiation were treated for 10 min with (●—●) or without (○—○) 100 nM insulin. α2M was then added at a final concentration of 1 nM (time 0) in the presence or absence of 5 μM unlabeled α2M and specific cell-associated radioactivity was assessed at the times indicated. Non-specific uptake of α2M by control (○) or insulin-treated (●) cells after 40 min incubation is depicted by the isolated symbols. Plotted are the means ± S.E. of 5 independent experiments performed in triplicate.

FIG. 3. Binding of monoclonal antibody 2C6 to control or insulin-treated 3T3-L1 adipocytes. Left panel, 3T3-L1 adipocyte monolayers were incubated at 4 °C in the presence of the indicated concentrations of monoclonal antibody 2C6, followed by goat anti-rat second antibody and 125I-protein A as described under “Experimental Procedures.” The other half were incubated with 1 nM 125I-α2M for 4 h. Fig. 3, right panel, shows that insulin increased the cell surface binding of antibody 2C6 and α2M by approximately 2- and 1.8-fold, respectively. Half-maximal stimulation of both antibody binding and α2M binding occurred at 1–5 nM insulin. These data support the notion that antibody 2C6 binds to the α2M receptor, and that the insulin-mediated increase in α2M binding is due to an approximately 2-fold increase in the number of receptors on the cell surface.

The discrepancy between the increase in cell surface receptors and the initial rate of accumulation of α2M suggests that insulin may cause a relative decrease in the rate of internalization or subsequent processing of α2M. However, the possibility remains that the comparison between the receptor numbers, measured at 4 °C, and the uptake of α2M, measured at 37 °C, may not be valid if some redistribution of the receptors occurs at 4 °C. To address this possibility, we measured the number of cell surface receptors in cells that had been treated with KCN at 37 °C. This treatment inhibits receptor internalization, and allows the measurement of cell surface receptor numbers at higher temperatures (3, 6). Binding of antibody 2C6 to control or insulin-treated KCN-poisoned cells, assessed as described under “Experimental Procedures,” was of 119 ± 5 and 421 ± 21 cpm, respectively (data are means ± S.E. of 2 independent experiments performed in triplicate). The values obtained in parallel experiments in which antibody binding was measured at 4 °C were 478 ± 28 and 1013 ± 78 cpm for control or insulin-treated cells, respectively (means ± S.E.). These results suggest that redistribution of receptors

protein recognized by antibody 2C6 represents the α2M receptor (13). Fig. 3, left panel, shows that antibody 2C6 bound to the surface of 3T3-L1 cells, reaching saturation at approximately 10 μg/ml. The effect of insulin on the binding of this monoclonal antibody to the cell surface was compared to the effect of insulin on α2M binding. Cell monolayers were preincubated for 10 min at 37 °C with increasing concentrations of insulin, and chilled to 5 °C. Half of the wells were incubated with 10 μg/ml of antibody 2C6, followed by a second antibody and 125I-protein A as described under “Experimental Procedures.” The other half were incubated with 1 nM 125I-α2M for 4 h. Fig. 3, right panel, shows that insulin increased the cell surface binding of antibody 2C6 and α2M by approximately 2- and 1.8-fold, respectively. Half-maximal stimulation of both antibody binding and α2M binding occurred at 1–5 nM insulin. These data support the notion that antibody 2C6 binds to the α2M receptor, and that the insulin mediated increase in α2M binding is due to an approximately 2-fold increase in the number of receptors on the cell surface.

The discrepancy between the increase in cell surface receptors and the initial rate of accumulation of α2M suggests that insulin may cause a relative decrease in the rate of internalization or subsequent processing of α2M. However, the possibility remains that the comparison between the receptor numbers, measured at 4 °C, and the uptake of α2M, measured at 37 °C, may not be valid if some redistribution of the receptors occurs at 4 °C. To address this possibility, we measured the number of cell surface receptors in cells that had been treated with KCN at 37 °C. This treatment inhibits receptor internalization, and allows the measurement of cell surface receptor numbers at higher temperatures (3, 6). Binding of antibody 2C6 to control or insulin-treated KCN-poisoned cells, assessed as described under “Experimental Procedures,” was of 119 ± 5 and 421 ± 21 cpm, respectively (data are means ± S.E. of 2 independent experiments performed in triplicate). The values obtained in parallel experiments in which antibody binding was measured at 4 °C were 478 ± 28 and 1013 ± 78 cpm for control or insulin-treated cells, respectively (means ± S.E.). These results suggest that redistribution of receptors

FIG. 3. Binding of monoclonal antibody 2C6 to control or insulin-treated 3T3-L1 adipocytes. Left panel, 3T3-L1 adipocyte monolayers were incubated at 4 °C in the presence of the indicated concentrations of monoclonal antibody 2C6, followed by goat anti-rat second antibody and 125I-protein A as described under “Experimental Procedures.” Specific radioactivity bound to the monolayers was assessed. Plotted are the means ± S.E. of 2 independent experiments performed in duplicate. Right panel, monolayers were incubated for 10 min at 37 °C with the indicated concentrations of insulin and chilled to 4 °C. The specific binding of 1 nM 125I-α2M (●) or of 10 μg/ml antibody 2C6 (●) were then assessed as described under “Experimental Procedures.” Plotted are the means ± S.E. of 3 independent experiments performed in duplicate.

S. Corvera, D. F. Graver, and R. M. Smith, unpublished observations.
to the cell surface indeed occurs at 4 °C, both in control and in insulin-treated cells. Nevertheless, the results indicate that the previously observed 2-fold increase in cell surface receptor numbers in response to insulin is not an artifact of this redistribution. In fact, an approximately 3-fold increase in cell surface receptor numbers in response to insulin was observed in KCN-treated cells.

We sought to identify the structures along the endocytic/recycling pathway that might be regulated by insulin to bring about an increase in the cell surface concentration of α2M receptors. Control or insulin-treated cell monolayers were incubated for 20 min at 37 °C with saturating concentrations of antibody 2C6 conjugated to 15-nm colloidal gold particles. In both control or insulin-treated cells, gold particles were detected at the cell surface in undifferentiated plasma membrane, coated pits, or smooth invaginations. Intracellular gold particles were found in coated vesicles, smooth uncoated vesicles proximal to the plasma membrane, tubulovesicles, multivesicular bodies, and large vesicles containing electron dense material which resembled lysosomes (Fig. 4). Quantitative analysis of the distribution of gold particles was performed as described under "Experimental Procedures." This analysis revealed that after 20 min of incubation of control cell monolayers with gold-2C6 antibody, 60% of the gold particles were intracellular and approximately 40% were localized on the cell surface. In contrast, in insulin-treated cells this proportion was reversed, with only 36% of the gold particles found inside the cell and 64% on the cell surface. The distribution of gold particles among cell surface and intracellular structures of control or insulin-treated cells is shown in Fig. 5. The percentage of total cellular particles on the cell surface was significantly higher in insulin-treated cells. These particles did not selectively accumulate in any specific plasma membrane structure, but were distributed among membrane, coated pits, and smooth membrane invaginations in a proportion similar to that observed in control cells. In contrast, significant differences in the distribution of particles among intracellular structures could be detected between control and insulin-treated cells. Whereas in control cells 18% of the total cellular particles were found within tubulovesicles and multivesicular bodies (equivalent to 30% of intracellular particles), insulin-treated cells contained only 3% of total cellular gold particles within these structures (equivalent to 8% of intracellular particles). In addition, the number of particles associated to lysosome-like structures, which amounted to 9% of total particles in control cells decreased to 3% upon insulin treatment. These results suggest that the movement of receptors among endocytic compartments may be altered in response to insulin, and this effect may serve to shunt a large population of receptors into the recycling pathway. The effect of insulin on the distribution of α2M receptors is consistent with its effects on the binding and accumulation of α2M, and with the possibility that the insulin-mediated increase in the cell surface concentration of α2M receptors results from a decrease in the intracellular transit time of the receptor, and/or a decrease in its rate of internalization.

**DISCUSSION**

The main finding in this paper is that insulin increases the cell surface concentration of receptors for α2M, and stimulates the accumulation of this ligand. Together with previous data indicating that insulin increases the cell surface concentration of the receptors for transferrin (3–5) and IGFI-II/Man-6-P (6, 7), these results indicate that insulin affects the distribution of numerous receptors that internalize and recycle through the clathrin-coated pit/coated vesicle pathway. This effect may be important in mediating the overall anabolic growth-potentiating effects of this hormone, by promoting an increased rate of nutrient and growth factor uptake.

The physiological role of α2M is not completely understood. This protein is considered an important scavenger of circu-
Insulin Action on a2M Receptors

Regulating proteases, inhibiting their activity against high molecular weight polypeptides and retrieving them into the cell for degradation (reviewed in Ref. 21). Moreover, α2M has been shown to suppress protease secretion by some cell types (22). In addition, α2M binds polypeptide growth factors with high affinity (23, 24), and thereby produces effects on cell growth. Whether or not these properties of α2M relate to the metabolic or growth promoting effects of insulin is presently unknown.

Several mechanisms could contribute to the insulin-mediated increase in the cell surface concentration of the receptors for transferrin, IGF-II/Man-6-P, and α2M. For example, the exocytosis of a latent pool of intracellular receptors could increase their concentration on the cell surface (4, 5). Alternatively, the endocytic portion of the pathway could be altered by insulin, in a way that would increase the half-life of the receptor on the cell surface, and decrease its intracellular half-life. This mechanism is consistent with studies which indicate that the effects of insulin and insulin-like growth factor I to increase the cell surface concentration of transferrin receptors are at least in part due to a decrease in the rate of receptor endocytosis (4). In addition, the rate of receptor internalization, but not the rate of exocytosis, appears to determine the number of cell surface transferrin receptors expressed in murine erythroleukemic cells (25). The biochemical analysis presented in this paper shows that the initial rate of accumulation of α2M is increased only 30%, under conditions where the number of cell surface receptors is increased almost 2-fold (Figs. 1 and 2 and "Results"), suggesting that insulin causes a relative decrease in the rate of receptor endocytosis.

The internalization pathway followed by the receptor in control or insulin-treated cells was studied using a monoclonal antibody to a component of the α2M receptor. We have employed a protocol in which control or insulin-treated cells are incubated at 37 °C in the presence of high concentrations of gold-labeled antibody. This protocol differs from most ultrastructural studies of receptor endocytosis, in which cells are equilibrated at 4 °C with the labeled ligand prior to increasing the temperature to allow endocytosis to occur. This latter type of protocol was precluded by our observation that cooling cells to 4 °C elicits a redistribution of α2M receptors ("Results"). Our data are consistent with previous studies which have delineated the intracellular pathway followed by α2M in 3T3-L1 cells (26), in that gold particles are found associated with coated pits, coated and uncoated vesicles, tubulovesicles, and multivesicular bodies. Few gold particles were found in lysosome-like structures, consistent with the notion that the receptor does not enter these structures under physiological conditions. Thus, although antibody 2C6 has been reported to cause down-regulation of the α2M receptor in Swiss 3T3 cells (19), no qualitative changes in the intracellular pathway of this receptor appear to occur in response to antibody binding. The ultrastructural analysis presented in this paper revealed two interesting effects of insulin. First, that the proportion of particles remaining on the cell surface is greater in insulin-treated cells compared to controls. These data are consistent with those obtained in the study of α2M accumulation (Fig. 2), in that they suggest a relative decrease in the rate of receptor internalization in response to insulin. Second, whereas in control cells gold particles accumulate in tubulovesicles and multivesicular bodies, in insulin-treated cells significantly fewer particles were found within these structures. The total number of multivesicular bodies did not appear to be decreased, indicating that it is the transit of the gold-antibody to these structures which is altered by insulin treatment. Although we cannot rule out the possibility that the antibody increases the concentration of receptors that enter late endocytic compartments under basal conditions, it is apparent that under these conditions insulin inhibits the movement of the receptor into these structures. Under physiological conditions, an analogous effect could decrease the intracellular transit time of the receptors and thereby cause a steady-state increase in their cell surface concentration. Because these studies do not provide information on the intracellular pathway of α2M itself, the consequences of the changes in the transit of the receptor on α2M accumulation in multivesicular bodies or lysosomes are not known. It will be interesting to investigate whether this effect of insulin on the subcellular distribution of α2M receptors is accompanied by structural modifications of the receptor structure similar to those that have been shown to occur on the IGF-II/Man-6-P receptor (27).

In summary, it has been shown that insulin treatment of differentiated 3T3-L1 cells causes an increase in the number of cell surface receptors for α2M, and a concomitant stimulation of the uptake of this ligand. Insulin appears to cause a relative decrease in the rate of α2M receptor internalization. Ultrastructural analysis of the uptake and transit of the α2M receptor suggests that insulin may inhibit the movement of this receptor among intracellular endocytic structures. Both of these mechanisms may operate to increase the number of α2M receptors on the cell surface.

Acknowledgments—We thank Dr. John Hanover (National Institutes of Health) for providing the 2C6 monoclonal antibody, Drs. Michael Prystowsky and Steven Spitalnik for the use of equipment in their laboratories, and Dr. Leonard Jarett for his support and discussion of this work. The excellent technical assistance of Neelima Shah is greatly appreciated.

REFERENCES

1. Cushman, S. W., and Wardazala, L. J. (1980) J. Biol. Chem. 255, 4758-4762
2. Suzuki, K., and Kono, T. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 2642-2645
3. Davis, R. J., Corvera, S., and Czech, M. P. (1986) J. Biol. Chem. 261, 8708-8711
4. Davis, R. J., Faucher, M., Racaniello, L. K., Carruthers, A., and Czech, M. P. (1987) J. Biol. Chem. 262, 13126-13134
5. Tannen, L. L., and Lienhard, G. E. (1987) J. Biol. Chem. 262, 8975-8980
6. Wardzala, L. S., Simpson, I. A., Rechler, M. M., and Cushman, S. W. (1984) J. Biol. Chem. 259, 8373-8383
7. Oka, Y., Rozek, L. M., and Czech, M. P. (1985) J. Biol. Chem. 260, 9435-9442
8. Watts, C. (1985) J. Cell Biol. 100, 633-637
9. Willingham, M. C., Pastan, I. H., Sahagian, G. J., Jourdian, G. W., and Neufeld, E. F. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 6967-6971
10. Oka, Y., and Czech, M. P. (1986) J. Biol. Chem. 261, 9090-9093
11. Willingham, M. C., Maxfield, F. R., and Pastan, I. H. (1979) J. Cell Biol. 82, 614-620
12. Goldberg, R. I., Smith, R. M., and Jarett, L. (1987) J. Cell. Physiol. 133, 203-209
13. Hanover, J. A., D’Souza, P., August, T., Pastan, I., and Willingham, M. C. (1986) J. Biol. Chem. 261, 16732-16737
14. Swenson, R. P., and Howard, J. B. (1979) J. Biol. Chem. 254, 4459-4466
15. Hanover, J. A., Rudick, J. E., Willingham, M. C., and Pastan, I. (1983) Arch. Biochem. Biophys. 227, 570-579
16. Hughes, E. N., and August, J. T. (1981) J. Biol. Chem. 256, 664-671
17. Slot, J. W., and Geuze, H. J. (1986) Eur. J. Biochem. 158, 87-93
18. Smith, R. M., Cobb, M. H., Rosen, O. M., and Jarett, L. (1985) J. Cell. Physiol. 123, 167-179
19. Van Deenen, L. L. L., and Van Den Berghe, H. (1979) J. Biol. Chem. 254, 5155-5160
20. Hanover, J. A., Cheng, S., Willingham, M. C., and Pastan, I. H. (1983) J. Biol. Chem. 258, 370-377
21. Harper, P. C., and Brower, M. S. (1983) *Ann. N. Y. Acad. Sci.* 421, 1-9
22. Johnson, W. J., Pizzo, S. V., Imber, M. J., and Adams, D. O. (1982) *Science* 218, 574-576
23. O'Connor-McCourt, M. D., and Wakefield, L. M. (1987) *J. Biol. Chem.* 262, 14090-14099
24. Raines, E. W., Bowen-Pope, D. F., and Ross, R. (1984) *Proc. Natl. Acad. Sci. U. S. A.* 81, 3424-3428
25. Mulford, C. A., and Lodish, H. F. (1988) *J. Biol. Chem.* 263, 5455-5461
26. Tran, D., Carpentier, J. L., Sawano, F., Gorden, P., and Orci, L. (1987) *Proc. Natl. Acad. Sci. U. S. A.* 84, 7957-7961
27. Corvera, S., and Czech, M. P. (1988) *Proc. Natl. Acad. Sci. U. S. A.* 85, 7314-7318