Estrogen receptor-negative Hs578T human breast cancer cells secrete insulin-like growth factor binding protein (IGFBP)-3 and IGFBP-4 as major binding protein (BP) species. Our previous immunohistochemical studies (Oh, Y., Müller, H. L., Pham, H., Lamson, G., and Rosenfeld, R. G. (1992) Endocrinology 131, 3123–3125) have demonstrated the existence of cell surface-associated IGFBP-3 and release of cell surface-associated IGFBP-3 into conditioned media by addition of IGF peptide in Hs578T cells. In this study, we have demonstrated that IGFBP-3 binding on the cell surface is specific and receptor-mediated, by showing: 1) a dose-dependent increase of IGFBP-3 binding by addition of divalent cations (CaCl₂ and MnCl₂); 2) dose-dependent competition of ¹²⁵I-IGFBP-3.ECo₃ (≥80% competition at 100 nM), but not by IGFBP-1 or fibronectin.

In addition, exogenous IGFBP-3 treatment resulted in a significant inhibitory effect on monolayer growth of Hs578T cells. This inhibitory effect of IGFBP-3 was shown to be specific and IGF-independent by demonstrating: 1) dose-dependent inhibition on cell growth (60% inhibition at 20 nM) and inhibition on DNA synthesis (10 nM; p < 0.05, 20 nM; p < 0.005) by exogenous IGFBP-3.ECo₃, but not by IGFBP-1; 2) absence of stimulatory effects on monolayer cell growth by either native IGFs or IGF analogs which have significantly decreased affinity for IGFBPs, but retain full affinity for type 1 and 2 IGF receptors; 3) significant diminution of the IGFBP-3 inhibitory effects on monolayer growth by coinucubation with native IGFs, but not by coinucubation with IGF analogs with decreased affinity for IGFBP-3.

In conclusion, exogenous IGFBP-3 shows specific binding on the cell surface and can inhibit Hs578T cell monolayer growth by itself, suggesting the existence of specific membrane-associated proteins or receptors for IGFBP-3. Furthermore, IGF-I and -II can attenuate inhibitory effect of IGFBP-3 by forming IGF-IGFBP-3 complexes, thereby preventing cell surface binding of IGFBP-3.

Insulin-like growth factors, IGF-I and IGF-II, are peptide mitogens for multiple cell lines, including human breast cancer cells (1–3). They share structural similarity with insulin and have their own high affinity receptors on the cell membrane (4). The mitogenic actions of both IGF-I and -II appear to be mediated through the type 1 IGF receptor, which has high affinity for both IGF-I and -II and low affinity for insulin (5–6). In contrast, the type 2 IGF receptor, with high affinity for IGF-II and significantly lower affinity for IGF-I, is identical to the mannose 6-phosphate receptor, which is involved in the transport of lysosomal enzymes; its role in mediating IGF action is controversial (7–9).

The IGFs, but not insulin, also have high affinity for a family of IGF-binding proteins (IGFBPs), six of which have been cloned and sequenced (10–15). IGFBP-1 through IGFBP-6 are found in many body fluids and in the conditioned media (CM) of a wide variety of cell types, where they modulate IGF peptide activity in a complex manner (16–20). Although the IGFBPs are presumed to regulate access of IGFs to their receptors, their precise biological roles remain unclear, since both stimulatory and inhibitory actions have been reported under varying conditions (21–25). IGFBP-3 is the major binding protein in human serum and serves as a storage depot for IGFs (26), resulting in an increase of the half-life of IGFs in the circulation, and control IGF access to extravascular spaces (26, 27). A variety of extrahepatic cell types synthesize and secrete IGFBP-3 (28–38). Thus, the presence of IGFBP-3 in the cellular microenvironment can directly modulate IGF actions. Interestingly, it has been reported that the patterns of IGFBP production in human breast cancer cells correlate with estrogen receptor (ER) status. ER-positive cell lines produce IGFBP-2 as major BP species, whereas ER-negative cells produce IGFBP-3 as the major IGFBP (39). Furthermore, recent reports have demonstrated that purified mouse IGFBP-3 can bind to the chick embryo fibroblast cell surface and inhibit cell growth (40). This IGFBP-3 effect was speculated to be an IGF receptor-independent action (41). In addition, several reports have demonstrated that cell growth inhibitors, such as TGF-β and trans-retinoic acid can increase IGFBP-3 production in human fetal fibroblasts (42) and human breast cancer cells (43).

It was speculated that the inhibitory effects on cell growth of these factors were partly mediated through IGFBP-3 action, but whether the IGFBP-3 inhibitory effect is IGF-dependent...
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remains unclear. We have previously reported the cell surface association and dissociation of IGFBP-3 in Hs578T human breast cancer cells. To further characterize the mechanism of IGFBP-3 action in vitro, we have investigated the role of IGFBP-3 in breast cancer cells. To further characterize the mechanism of IGFBP-3 action in vitro, we have investigated the role of IGFBP-3 in breast cancer cells. To further characterize the mechanism of IGFBP-3 action in vitro, we have investigated the role of IGFBP-3 in breast cancer cells. To further characterize the mechanism of IGFBP-3 action in vitro, we have investigated the role of IGFBP-3 in breast cancer cells.

**MATERIALS AND METHODS**

**Peptides and Proteins**—Recombinant human IGF-I was purchased from Bachem (Torrance, CA), and recombinant human IGF-II and insulin were provided by Lilly. IGF-I analog ([Gln5,Ala6,Tyr7,Leu8])IGF-I) was a generous gift from Dr. M. A. Cascieri (Merck) (44). An IGF-I-insulin hybrid was generously provided by Drs. J. F. Caro and S. H. Tager (University of Chicago) (45). [Leu6]IGF-II is a synthetic IGF-II analog with a leucine for tyrosine substitution at amino acid position 6; it has normal affinity for IGFBPs and the type 2 IGF receptor. The product was purified by gel filtration to remove polymeric byproducts followed by additional HPLC steps. Active [Gln5,Ala6,Tyr7,Leu8,Leu9]IGF-II was monitored during purification by a type 2 IGF receptor binding assay using rat placental membranes. HPLC purified hIGFBP-1 from human amniotic fluid was kindly provided by Dr. D. R. Powell (Baylor College of Medicine, Houston, TX) (47). Recombinant hIGFBP-3 was the generous gift of S. H. Tager (University of Chicago) (45). [Leu6]IGF-II was monitored during purification by a type 2 IGF receptor binding assay using rat placental membranes. HPLC purified hIGFBP-1 from human amniotic fluid was kindly provided by Dr. D. R. Powell (Baylor College of Medicine, Houston, TX) (47). Recombinant hIGFBP-3 was kindly provided by Dr. D. R. Powell (Baylor College of Medicine, Houston, TX) (47). Recombinant hIGFBP-3 was kindly provided by Dr. D. R. Powell (Baylor College of Medicine, Houston, TX) (47). Recombinant hIGFBP-3 was kindly provided by Dr. D. R. Powell (Baylor College of Medicine, Houston, TX) (47).

**Characterization**—Immunoprecipitations were performed essentially as described previously, but with antibodies generated against glycosylated hIGFBP-3 (aIGFBP-3,3g1) and nonglycosylated hIGFBP-3 (aIGFBP-3,3ng1). The product was purified by gel filtration to remove polymeric byproducts followed by additional HPLC steps. Active [Gln5,Ala6,Tyr7,Leu8,Leu9]IGF-II was monitored during purification by a type 2 IGF receptor binding assay using rat placental membranes. HPLC purified hIGFBP-1 from human amniotic fluid was kindly provided by Dr. D. R. Powell (Baylor College of Medicine, Houston, TX) (47).

**RESULTS**

Characterization of Secreted IGFBP-3 and Membrane-associated IGFBP-3—Fig. 1 presents a Western ligand blot of the IGFBP-3 present in the CM and solubilized membranes of the human breast cancer cell line Hs578T. Hs578T cells secrete a 41-kilodalton (kDa), 39-kDa, and 24-kDa IGFBP-3 species as major IGFBP-3 species and a 28-kDa IGF-binding species as a minor BP form. The 41- and 39-kDa IGFBP-3 species, which were found in both the CM (lane 4) and solubilized membranes (lane 7) are similar to human serum IGFBP-3 (lane 1) by migration pattern in ligand blots. Indeed, when CM or solubilized membranes were immunoprecipitated with polyclonal antibodies aIGFBP-3,3g1 and aIGFBP-3,3ng1, raised against glycosylated recombinant human IGFBP-3 (IGFBP-3,3g1) and nonglycosylated IGFBP-3 (IGFBP-3,3ng1), respectively (lanes 5-6 and 8-9), only the 41- and 39-kDa species were immunoprecipitated, as was true for human serum IGFBP-3 (lanes 2 and 3). Thus, both antibodies recognized identical IGFBP-3 species in cell membranes and in CM.

Deglycosylation studies with Endo-F demonstrated that the...
41- and 39-kDa species were two differentially glycosylated forms of IGFBP-3, which were both reduced to a 29-kDa core protein size, consistent with that of IGFBP-3.  

Visual inspection of the gel following Endo-F treatment is also shown (solubilized membranes, 14966 protein size, consistent with that of IGFBP-3).  

peared upon treatment with Endo-F, suggesting that it is the forms of IGFBP-3, which were both reduced to a 29-kDa core

Indeed, all of the deglycosylated IGFBP-3 forms from human serum, Hs578T CM, and solubilized membranes were immunoprecipitated with the IGFBP-3 antibody, αIGFBP-3ngl (lanes 15-17). Furthermore, the 28-kDa minor species disappeared upon treatment with Endo-F, suggesting that it is the glycosylated form of the 24-kDa IGFBP presumed to be IGFBP-4 (lanes 4 and 11) (54).  

To further investigate membrane-associated IGFBP-3, crude microsomal membranes were prepared. Cells were grown in DMEM with 10% fetal bovine serum until 90% confluent. Cells were then washed, changed into new serum, serum, Hs578T CM, and solubilized membranes were immunoprecipitated with antibodies (Ab1, Ab2). Two μl of human serum (lanes 2 and 3), 100 μl of Hs578T CM collected at 72 h (lanes 5, 6), or solubilized membranes (lanes 8 and 9) were immunoprecipitated with 5 μl of antibodies. Additional samples were treated with Endo-F (human serum, lane 10; Hs578T CM, lane 11; IGFBP-3cmp, lane 12; IGFBP-3ε col, lane 13; solubilized membranes, lane 14). Immunoprecipitation with αIGFBP-3ngl after Endo-F treatment is also shown (solubilized membranes, lane 15; pooled CM, lane 16; human serum, lane 17).

ever, when samples were incubated in the presence of [Leu27]IGF-II, which competes for occupancy of the IGFBPs, but not for the type 1 IGF receptor, a typical 135-kDa α-subunit and 270-kDa α-α dimer were readily identified (lanes 11 and 12). Thus, because of the higher binding affinity of IGFBP-3 for IGFs, the type 1 IGF receptor is normally "masked," but can be unmasked by low concentrations of [Leu27]IGF-II. This competition by membrane-associated IGFBP-3 for receptor binding has undoubtedly caused difficulties in previous attempts to interpret IGF receptor data from Hs578T cells (19) and other cells. Thus, IGF analogs which have high binding affinity only for IGFBPs may be useful in identifying masked IGF receptors. In Fig. 2B, [125I]IGF-II also bound primarily to membrane associated IGFBP-3 (lanes 1 and 6); addition of excess unlabeled IGF-I revealed a 250-kDa type 2 IGF receptor, presumably by displacing [125I]IGF-II from IGFBP-3 (lanes 2 and 3). As expected, incubation with unlabeled IGF-II and [Leu27]IGF-II displaced radioligand from both type 2 IGF receptors and membrane-associated IGFBP-3, at concentrations of 20 and 200 ng/ml (lanes 4, 5, 7, and 8).

These data indicate that IGFBP-3 expressed in Hs578T cells exists as a secreted form in CM and as a membrane-associated form on the cell surface. IGFBP-3 can be identified by its deglycosylation pattern and by immunoprecipitation with IGFBP-3-specific antibodies, αIGFBP-3gl and αIGFBP-3ngl.

**Mechanism for Cell Surface Association of IGFBP-3**—Our previous immunohistochemical studies have demonstrated that Hs578T cell surface binding of IGFBP-3 was increased by CaCl2 and decreased by EDTA and that cell surface-associated IGFBP-3 was released into CM following binding of IGF peptide (55, 56). To further investigate the factors that regulate IGFBP-3 binding on the cell surface, we performed binding assays to Hs578T cell monolayers using [125I]IGFBP-3ε col. Fig. 3A presents the effects of Ca2+ and Mn2+ on [125I]IGFBP-3ε col binding to Hs578T cell monolayers. Monolayers were incubated with or without indicated reagents and [125I]IGFBP-3ε col for 2.5 h at 15 °C. Binding of labeled IGFBP-3...
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determine nonspecific binding of IGFBP-3 (51). As can be seen in the inset, the competition by serum IGFBP fractions was dose-dependent; the maximum decrease in labeled IGFBP-3E. coli binding to the cell surface was observed with 10% serum IGFBP fractions. Therefore, nonspecific binding was determined in the presence of 10% human serum IGFBP fractions from G-50 acid chromatography. Fig. 3B shows that 125I-IGFBP-3E. coli binding was displaced by unlabeled IGFBP-3, with an IC50 of 12 nM and with >80% competition at 100 nM. When purified human IGFBP-1 and human fibronectin were used to compete with labeled IGFBP-3E. coli, no competition was observed at concentrations up to 50 nM for IGFBP-1 and 20 nM for fibronectin.

Cell Growth Inhibition by IGFBP-3—Further studies were performed to investigate the biological significance of IGFBP-3 binding to the cell surface. We have demonstrated previously that insulin, IGF-I, and IGF-II did not stimulate proliferation of Hs578T cells, despite their potent mitogenic effects on other breast cancer cell lines (1-3) and speculated that the absence of IGF effects could reflect interference by endogenous IGFBPs. Therefore, we tested the effect of endogenously secreted IGFBP-3 and exogenously added IGFBP-3E. coli on Hs578T cell proliferation by using the IGF-I analog, [Gln3,Ala4,Tyr15,Leu16]IGF-I. As shown in Fig. 4A, neither IGF-I nor [Gln3,Ala4,Tyr15,Leu16]IGF-I, which has significantly decreased affinity for IGFBPs, but retains full affinity for IGF receptors, stimulated cell growth at concentrations up to 20 nM. This implies that the absence of IGF effects on cell growth is not due to interference by endogenously secreted IGFBPs. Nevertheless, when exogenous IGFBP-3E. coli was added, it showed a significant inhibitory effect on monolayer growth of Hs578T cells (10 and 20 nM; p < 0.005). This inhibitory effect of exogenous IGFBP-3E. coli was dose-dependent, with 60% inhibition at a concentration of 20 nM. Cell growth inhibition was specific for IGFBP-3; exogenous IGFBP-1 showed no significant inhibitory effect at concentrations up to 20 nM. In addition, the inhibitory effect of IGFBP-3 could be demonstrated on DNA synthesis, using a [3H]thymidine incorporation assay, as shown in Fig. 4B. As expected, IGFBP-3E. coli, but not IGFBP-1, showed an inhibitory effect on DNA synthesis (10 nM; p < 0.05, 20 nM; p < 0.005).

FIG. 2. Autoradiogram of 125I-IGF-I or -II cross-linked to crude microsomal membranes of Hs578T cells. Hs578T crude microsomal membranes were cross-linked with either 125I-IGF-I (A) or 125I-IGF-II (B) in the presence or absence of cold IGF peptides and IGF analogs. 100 µg of membranes were either untreated (C, control) or preincubated with 20 or 200 ng/ml IGF-I, IGF-II, IGF/insulin hybrid, [Gln3,Ala4,Tyr15,Leu16]IGF-I, and [Leu16]IGF-II for 30 min at 23 °C, before 125I-IGF (150,000 cpm) binding and cross-linking. Solubilized complexes (reducing conditions) were separated by SDS-PAGE on a 6% gel. Large arrows indicate IGFBP-3, whereas small arrows indicate α-subunit and α- dimer of the type 1 IGF receptor (A), or the type 2 IGF receptor (B).

to the cell surface was increased by CaCl2 and MnCl2 in a dose-dependent manner, with an approximately 1.5-fold increase following coinoculation with CaCl2 at a concentration of 1 mM (p < 0.001) and a 3.5-fold increase following coinoculation with 10 mM MnCl2 (p < 0.001). These findings indicate that divalent cations facilitate IGFBP-3 binding to the cell surface and suggest that IGFBP-3 binding is a specific and receptor-mediated event, such as the interaction of fibronectin with its receptor (57). Further characterization of specific IGFBP-3 binding to the cell surface was performed in the presence of 1 mM CaCl2, as demonstrated in Fig. 3B. Because of the limited supply of recombinant human IGFBP-3, we used partially purified human serum IGF binding protein fractions derived from G-50 acid column chromatography to
**DISCUSSION**

Our previous studies demonstrated cell surface-associated IGFBP-3 in Hs578T cells, by use of cell monolayer affinity cross-linking and by immunoperoxidase staining of the cell surface with anti-IGFBP-3 antibodies. Nonreceptor-mediated post-translational regulation of IGFBP-3 by IGF peptides resulted from the release of cell surface-associated IGFBP-3 into CM by the binding of IGFs to IGFBP-3 (55). These results additionally indicated that the cell surface binding of IGFBP-3 is specific and is facilitated by divalent cations in Hs578T human breast cancer cells. Our current studies further demonstrate that IGFBP-3, itself, is a cell growth inhibitor, whose mechanism is IGF-independent.

Several investigators have previously postulated IGFBP-3 binding on the cell surface of different cell lines, such as bovine dermal fibroblasts (28), human neonatal fibroblasts (58), chick embryo fibroblasts (59), rat sertoli cells (60), and Hs578T human breast cancer cells (55). This cell surface binding of IGFBPs was also observed with IGFBP-1 in porcine aortic smooth muscle cells (61) and IGFBP-5 in human fetal dermal fibroblast cells (62). The mechanism of the cell surface binding for IGFBP-5 is unclear, but the association of IGFBP-1 was speculated to result from membrane receptors of the integrin protein family, which would recognize the arginine-glycine-aspartic acid (RGD) tripeptide sequence in...
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** FIG. 4. Effects of IGFBP-3E colI, IGFBP-1, IGF-I, and \([\text{Gln}^3, \text{Ala}^4, \text{Tyr}^{15}, \text{Leu}^6]\)IGF-I on monolayer growth (A) and DNA synthesis (B) in Hs578T cells. A, cells were grown in 12-millwell plates until 69% confluent and maintained in serum-free media for 72 h without or with various concentrations of IGFBP-3E colI (A), IGFBP-1 (○), IGF-I (●), or \([\text{Gln}^3, \text{Ala}^4, \text{Tyr}^9, \text{Leu}^5]\)IGF-I (○). B, cells were grown in serum-free media in 12-millwell plates without or with various concentrations of IGFBP-3E colI (○) or IGFBP-1 (●) (see "Materials and Methods"). DNA synthesis was estimated at 24.5 h as the actual disintegrations/min of tritiated thymidine incorporation after a 1.5-h pulse of \(^3\text{H}\) thymidine. Statistical significance in comparison with control values is indicated by * (p < 0.05) or ** (p < 0.005).

IGFBP-1 (63). However, IGFBP-3 lacks this RGD sequence, and fibronectin and IGFBP-1 show no competition for IGFBP-3 cell surface binding, indicating that the mechanism of IGFBP-3 cell surface binding is not mediated through fibronectin receptors and is different from that of IGFBP-1. Recently, Baxter and co-workers (58) reported that heparin, which releases proteins attached to cell surface proteoglycans, could displace IGFBP-3 binding on human neonatal fibroblast cell surfaces. They suggested that the association of IGFBP-3 with proteoglycans occurs as a result of interaction with a proteoglycan in the membrane or matrix of the cell, rather than interaction with a specific receptor for IGFBP-3 (58). It is likely that negatively charged heparin can bind to IGFBP-3, consequently preventing IGFBP-3 binding to the cell surface, mimicking the effect of IGFs on IGFBP-3 binding to the cell surface. It should be noted that even though fibronectin (64) and fibroblast growth factors (65) bind to cell surface proteoglycans through their heparin binding domain, they also interact with their own specific receptors on the cell surface, indicating the existence of multiple binding sites, which may also be true for IGFBP-3.

Recently, Harel and co-workers (40) reported that mouse IGFBP-3 (mIGFBP-3) inhibited DNA synthesis and growth of mouse 3T3 fibroblasts and chick embryo fibroblasts. Stimulation of DNA and RNA synthesis, not only by IGF-I, but also by serum and phorbol-12-myristate-13-acetate, was inhibited by IGFBP-3 (40). Further studies showed that mIGFBP-3 can bind to chick embryo fibroblasts membranes with low affinity. Binding of IGFBP-3 to the membrane was specific, and binding sites per cell were estimated at 60,000 (59). We have recently reported that the growth of human IGFBP-3 transfected mouse Balb/c fibroblast cells is significantly slower (2.5-fold) than in control cells transfected with vector alone (66). When transfected cells were grown in insulin-containing media (5 μg/ml), growth rates of the IGFBP-3 transfected cells were not restored to control levels.
even though the expressed IGFBP-3 does not bind insulin. These results suggest that exogenous or endogenous IGFBP-3 has inhibitory effects on cell growth, which, as in our studies, may be IGF-independent. However, such experiments were performed in non-human models, using IGFBP-3 from a different species; additionally, fibroblasts have true IGF effects, mediated through IGF receptors. Therefore, it is difficult to determine whether the IGFBP-3 inhibitory effects derive entirely from the inhibition of IGF-dependent actions (e.g. by sequestration of the IGF peptides) or are independent events.

Accordingly, the Hs578T human breast cancer cell system is an excellent model for IGFBP action. These cells are not stimulated by IGF-I, IGF-II, or IGF analogs. It is of note that Hs578T cells contain the Harvey (H)-ras oncogene, possessing the genetic alteration of a substitution of aspartic acid for glycine at position 12 (67). Ras proteins are part of a large family of guanosine triphosphatases (GTPases) whose function is to act as a switch along essential cellular pathways (68). Recent reports have demonstrated that GTPase-activating protein (GAP) becomes physically associated with, and phosphorylated by, the activated tyrosine kinase-containing receptors, such as the insulin receptor (69), platelet-derived growth factor receptor (70), and EGF receptor (71). After stimulation with ligands, there is an inhibition of ras protein, resulting from its escape from down-regulation by GAP.

As schematized in Fig. 6, it is tempting to speculate that the ras protein is a dominant downstream element in tyrosine kinase-containing receptor pathways. The activated oncogenic ras protein overides the cell proliferative effects of insulin (3), IGFs (3), and EGF (73) in Hs578T cells. The lack of responsiveness of these cells to IGF-I thus enables one to investigate the IGF-independent actions of IGFBP-3. The inhibition of Hs578T cell replication by IGFBP-3 is IGFBP-3-specific and appears to result from cell surface association of IGFBP-3. Indeed, we are faced with the interesting situation where rather than IGFBPs modulating IGF actions, IGF peptides attenuate the inhibitory effects of IGFBP-3, by forming an IGF-IGFBP-3 complex and thereby preventing cell association of the binding protein (55).

It is of note that there is a strong correlation between IGFBP-3 production and estrogen receptor status in human breast cancer cells (39). In addition, TGF-β (74) and trans-retinoic acid (43, 75) inhibit breast cancer cell growth. TGF-β production is inhibited by estrogen and insulin treatment, but growth-inhibitory anti-estrogens and glucocorticoids strongly stimulate its secretion (76). Interestingly, these Hs578T inhibitors stimulate IGFBP-3 production in human fetal fibroblasts (58) and MCF-7 ER-positive breast cancer cells (43). It is reasonable to speculate that these inhibitory effects are, in part, mediated through IGFBP-3-specific actions, in addition to the blocking of IGF actions. In this respect, the production and proteolysis of IGFBP-3 as an inhibitor is tightly regulated to balance net growth rate in vivo.

REFERENCES

1. Furlanetto, R. W., and DiCarlo, J. N. (1984) Cancer Res. 44, 2122–2128
2. Huff, K. K., Knabbe, C., Lindsay, R., Lippman, M. E., and Dickson, R. B. (1988) Mol. Endocrinol. 2, 200–208
3. De Leon, D. D., Wilson, D. M., Powers, M., and Rosenfeld, R. G. (1992) (Growth Factors 6, 327–336
4. Czech, M. P. (1990) Cell 59, 235–238
5. Conover, C. A., Miera, P., Hintz, R. L., and Rosenfeld, R. G. (1987) Biochem. Biophys. Res. Commun. 139, 501–506
6. Oh, Y., Benuza, M. W., Pham, H. M., Smanik, P. A., Smith, M. C., and Rosenfeld, R. G. (1991) Biochem. Biophys. Res. Commun. 179, 249–254
7. Roth, R. A. (1988) Science 239, 1269–1271
8. Itoh, I., Ohkuni, Y., Ogata, E., and Kojima, I. (1987) Biochem. Biophys. Res. Commun. 142, 275–286
9. Mucsi, C. P., Kohn, E. C., Grubh, J. H., Sly, W. S., Oh, Y., Muller, H. L., Rosenfeld, R. G., and Helman, L. J. (1992) J. Biol. Chem. 267, 9000–9004
10. Bruckman, A., Groffen, C., Kortleve, D. J., Geurts van Kessel, A., and Drop, S. L. S. (1988) EMBO J. 7, 2417–2423
