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Substitution of the *erbB-2* oncoprotein transmembrane domain activates the insulin receptor and modulates the action of insulin and insulin-receptor substrate 1

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ABSTRACT **The mechanism through which insulin binding to the extracellular domain of the insulin receptor activates the intrinsic tyrosine kinase in the intracellular domain of the protein is unknown. For the c-*neu*/*erbB-2* (c-*erbB-2*) protooncogene, a single point mutation within the transmembrane (TM) domain converting Val-664 to Glu (*erbB-2*$^{V\rightarrow E}$) results in elevated levels of tyrosine kinase activity and cellular transformation. We report the construction of a chimeric insulin receptor in which the TM domain of the receptor has been substituted with that encoded by *erbB-2*$^{V\rightarrow E}$. When expressed in Chinese hamster ovary cells this chimeric receptor displays maximal levels of autophosphorylation and kinase activity in the absence of insulin. This activity results in an increase in the level of insulin-receptor substrate 1 phosphorylation but a down-regulation in insulin-receptor substrate 1 protein and desensitization to insulin stimulation of glycogen synthesis. By contrast, basal levels of DNA synthesis are elevated to levels ≈60% of those observed in serum-stimulated cells. Overexpression of chimeric insulin receptors containing the c-*erbB-2* TM domain or a single point mutation in the insulin receptor TM domain of Val-938 → Asp, on the other hand, shows none of these alterations. Thus, the TM domain encoded by *erbB-2*$^{V\rightarrow E}$ contains structural features that can confer ligand-independent activation in a heterologous protein. Constitutive activation of the insulin receptor results in a relative increase in basal levels of DNA synthesis, but an apparent resistance to the metabolic effects of insulin.**

The insulin receptor is composed of two α and two β subunits disulfide-linked to form an $\alpha_2\beta_2$ native heterotetrameric structure. The α subunit is entirely extracellular and contains the ligand-binding domain. The β subunit has an extracellular domain, a 23-amino acid transmembrane (TM) domain, and an intracellular domain that contains a tyrosine kinase. Binding of insulin to the α subunit results in a rapid autophosphorylation on tyrosine residues in the β subunit, thus activating the receptor kinase to phosphorylate other substrates (1, 2). This process of ligand binding with subsequent transmission of the signal through the plasma membrane and activation of an intrinsic kinase is a common event among many cell-surface growth-factor receptors (3–7).

We (8) and others (9, 10) have been investigating the role of the TM domain in the signal-transduction process in the insulin receptor. Results from these studies demonstrate that, in general, there is a broad tolerance for structural changes or substitutions within the TM domain while ligand-activated functions are preserved and suggest that the TM domain may not play a major role in communication of the signal through the membrane but may act primarily to anchor the protein in the lipid bilayer. However, recently we showed that substitution of the wild-type insulin receptor (IR^{WT}) TM domain with that from the c-*neu*/*erbB-2* (c-*erbB-2*) protooncogene results in a partial, ligand-independent activation of the receptor kinase *in vitro*, although in intact cells this chimeric receptor appears to function normally (8). The c-*erbB-2* protooncogene encodes for an epidermal growth factor-receptor-related protein. The oncogenic form of the protein is encoded by a sequence containing a point mutation within the TM-encoding domain converting Val-664 → Glu (*erbB-2*$^{V\rightarrow E}$) causing constitutive activation of the associated tyrosine kinase, which leads to cellular transformation (11–14). Recent work by Longo *et al.* (10) suggests that making a similar point mutation at an analogous position in the insulin receptor may produce partial activation of the insulin receptor kinase. In this report we analyzed the effects of substituting the insulin receptor TM domain with that encoded by c-*erbB-2* (IR/TM$^{c\text{-}erb}$), containing a single-base mutation of the insulin receptor (IR/TM$^{V\rightarrow D}$) similar to that encoded by *erbB-2*$^{V\rightarrow E}$ and a chimeric insulin receptor containing the complete *erbB-2*$^{V\rightarrow E}$-encoded TM domain (IR/TM$^{erbV\rightarrow E}$). We find that only the latter receptor displays complete ligand-independent activation of the receptor tyrosine kinase both *in vitro* and in intact cells. We also find that this constitutive activation of the insulin receptor modulates insulin-receptor substrate 1 (IRS-1) protein level and phosphorylation state, DNA synthesis, and insulin-stimulated glycogen synthesis.

MATERIALS AND METHODS

***In Vitro* Mutagenesis of the Insulin Receptor cDNA and Transfection of Chinese Hamster Ovary (CHO) Cells.** Construction of the expression vectors containing the cDNAs for IR^{WT}, the insulin receptor containing a Val-938 → Asp substitution (IR/TM$^{V\rightarrow D}$), and the insulin receptor containing a substitution of the IR^{WT} TM domain with that encoded by the c-*erbB-2* protooncogene (IR/TM$^{c\text{-}erb}$) is described elsewhere (8). For construction of IR/TM$^{erbV\rightarrow E}$ a 1.9-kb *Pst* 1 fragment from the IR/TM$^{c\text{-}erb}$ cDNA construct was used as a template for the *in vitro* mutagenesis reactions with a mutagenic oligonucleotide coding for a Val → Glu conversion as depicted in Fig. 1. An expression vector containing the mutated insulin receptor cDNA was cotransfected, along with a plasmid coding for neomycin resistance (Neo), into CHO cells. Clonal cell lines were isolated from neomycin-

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Abbreviations: CHO, Chinese hamster ovary cells; TM, transmembrane; IR^{WT}, wild-type insulin receptor; IR/TM$^{V\rightarrow D}$, insulin receptor containing a Val-938 → Asp substitution; IR/TM$^{c\text{-}erb}$, insulin receptor containing the TM domain from the ErbB-2 protooncoprotein; IR/TM$^{erbV\rightarrow E}$, insulin receptor containing the TM domain from the ErbB-2 oncoprotein; IRS-1, insulin-receptor substrate 1; BSA, bovine serum albumin.

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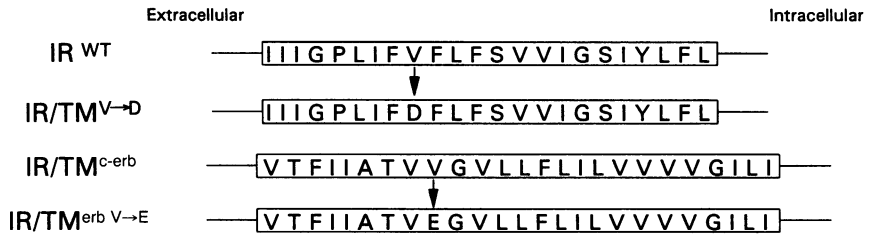

FIG. 1. Amino acid sequence of the mutated/chimeric insulin receptor TM domains. IR/TM$^{c\text{-}erb}$, a chimeric insulin receptor with a substitution of the wild-type TM domain with that from c-*erbB-2*; IR/TM$^{erbV\rightarrow E}$, a chimeric insulin receptor with a substitution of the wild-type TM domain with that from the ErbB-2 oncoprotein.

resistant colonies by using fluorescence-activated cell sorting followed by cloning by limiting dilution as described (8, 15). The CHO cells were maintained in Ham's F-12 medium/10% fetal bovine serum and in a humidified atmosphere of 5% CO_2.

Insulin-Stimulated Phosphorylation in Intact Cells. Monolayers of CHO cells either mock-transfected (Neo) or expressing the indicated receptor cDNA were serum starved overnight in F-12 medium/0.1% insulin-free bovine serum albumin (BSA). Insulin-stimulated autophosphorylation was assessed after incubation with or without 100 nM insulin for 1 min. The cells were then harvested in 50 mM Hepes, pH 7.6/1% Triton X-100/150 mM NaCl/protease/phosphatase inhibitors as described (15). Insoluble material was removed by centrifugation, and the supernatants were immunoprecipitated with a human specific monoclonal antireceptor antibody 83-14 (from Kenneth Siddle, Cambridge, U.K.) and protein A (Pansorbin). Alternatively, whole-cell lysates were prepared by solubilization in SDS/PAGE sample buffer/100 mM dithiothreitol, boiled for 3 min, sonicated, and boiled for an additional 3 min. Proteins from either preparation were separated by SDS/PAGE, transferred to nitrocellulose membranes, and treated sequentially with anti-phosphotyrosine antibodies and ^{125}I-labeled protein A (16). After extensive washing the membranes were subjected to autoradiography. The autoradiographs were analyzed by using a Molecular Dynamics laser scanning densitometer and accompanying software.

***In Vitro* Kinase Assays.** *In vitro* kinase assays were done by using immunocomplexed insulin receptors prepared with the 83-14 antibody from cells incubated with or without 100 nM insulin for 4 min. The kinase reactions were initiated by the addition of a peptide substrate (Y628) derived from the IRS-1 sequence and 50 μM [γ-^{32}P]ATP to the immunocomplexed receptors (17). The reactions were allowed to proceed for 4 min at room temperature and terminated by adding 5% trichloroacetic acid/BSA at 1 mg/ml. Insoluble material was pelleted by centrifugation, and aliquots of the supernatant were spotted onto phosphocellulose paper followed by extensive washing in 150 mM H_3PO_4. Radioactivity incorporated was determined by counting Cerenkov radiation.

Quantitation of IRS-1. IRS-1 protein levels were determined in cells expressing Neo, IR^{WT}, and two independently isolated clonal cell lines (C1 and C2) expressing IR/TM$^{erbV\rightarrow E}$. The various cell lines were harvested, as described above, for immunoprecipitations, and aliquots of the lysates containing equal amounts of protein were incubated with a polyclonal antibody raised against an N-terminal peptide of IRS-1. The precipitated proteins were treated as described above for immunoblotting and probed with an antibody raised against a C-terminal IRS-1 peptide. Bands corresponding to IRS-1 were quantitated by scanning laser densitometry.

Insulin-Stimulated Growth and Metabolic Assays. For insulin-stimulated DNA synthesis, cells were grown in 24-well cluster trays and serum-starved for 3 days in Ham's F-12 medium/0.1% BSA. The cells were stimulated with the indicated concentration of insulin (in triplicate) for 15 hr followed by a 1-hr incubation with [^{3}H]thymidine (1 μCi per well; 1 Ci = 37 GBq). Trichloroacetic acid-precipitable material was collected on glass-fiber filters, the filters were washed several times with ice-cold trichloroacetic acid, and the radioactivity was determined by liquid scintillation counting. For insulin-stimulated glycogen synthesis, confluent monolayers of cells were incubated for 3 hr in low-glucose serum-free Ham's F-12 medium/0.1% BSA, stimulated with the indicated concentrations of insulin (in duplicate) for 45 min, and then incubated with [^{14}C]glucose (0.5 μCi per well) for an additional 45 min. Ethanol-precipitable material was collected on glass-fiber filters, the filters were washed with excess ethanol, and radioactivity, representing [^{14}C]glucose incorporation into glycogen, was determined by liquid scintillation counting.

RESULTS

To study the potential role of the TM domain in insulin receptor activation, three mutant insulin receptors, in addition to the IR^{WT}, were used: IR/TM$^{c\text{-}erb}$ contains the entire TM domain from the normal c-*erbB-2* protooncogene substituted for the IR^{WT} TM domain (Fig. 1). IR/TM$^{V\rightarrow D}$ contains a point mutation in the insulin receptor TM domain converting Val-938 to Asp, thus placing a charged amino acid in an analogous location to the activating mutation in the *erbB-2*$^{V\rightarrow E}$ oncogene. The third mutant, IR/TM$^{erbV\rightarrow E}$, is a chimeric receptor in which the TM domain from the *erbB-2* oncogene replaces the IR^{WT} TM domain and thus contains the c-*erbB-2* TM domain plus the activating point mutation found in the ErbB-2 oncoprotein. All three mutant receptors were expressed to similar levels (1–4 $\times$ 10^6 receptors per cell) in CHO cells and proteolytically processed normally into α and β subunits. In addition, all three mutant receptors bound insulin with an affinity ($K_d \approx 1$ nM) similar to that of IR^{WT} (data not shown).

Receptor Phosphorylation. Both IR/TM$^{c\text{-}erb}$ and IR/TM$^{V\rightarrow D}$ displayed normal insulin-stimulated receptor autophosphorylation similar to that seen for cells expressing IR^{WT}, as determined by immunoblots of anti-insulin-receptor immunoprecipitates from extracts of intact cells probed with anti-phosphotyrosine antibodies (Fig. 2*A* and ref. 8). By comparison, IR/TM$^{erbV\rightarrow E}$ receptors displayed near-maximal phosphorylation on tyrosine residues in the absence of insulin with little or no further stimulation after addition of 100 nM insulin (Fig. 2 *A* and *B*). *In vitro* kinase assays using immunoaffinity-purified wild-type or mutant insulin receptors and a peptide substrate derived from native IRS-1 sequence showed that the receptor kinase was constitutively activated as well (Fig. 2*C*). The V_{max} value for kinase reactions done without insulin stimulation for IR/TM$^{erbV\rightarrow E}$ was similar to that seen for IR^{WT} after activation with insulin, whereas the K_m value was not altered from IR^{WT}. Thus, the TM domain from erb$^{V\rightarrow E}$ conferred constitutive activation to

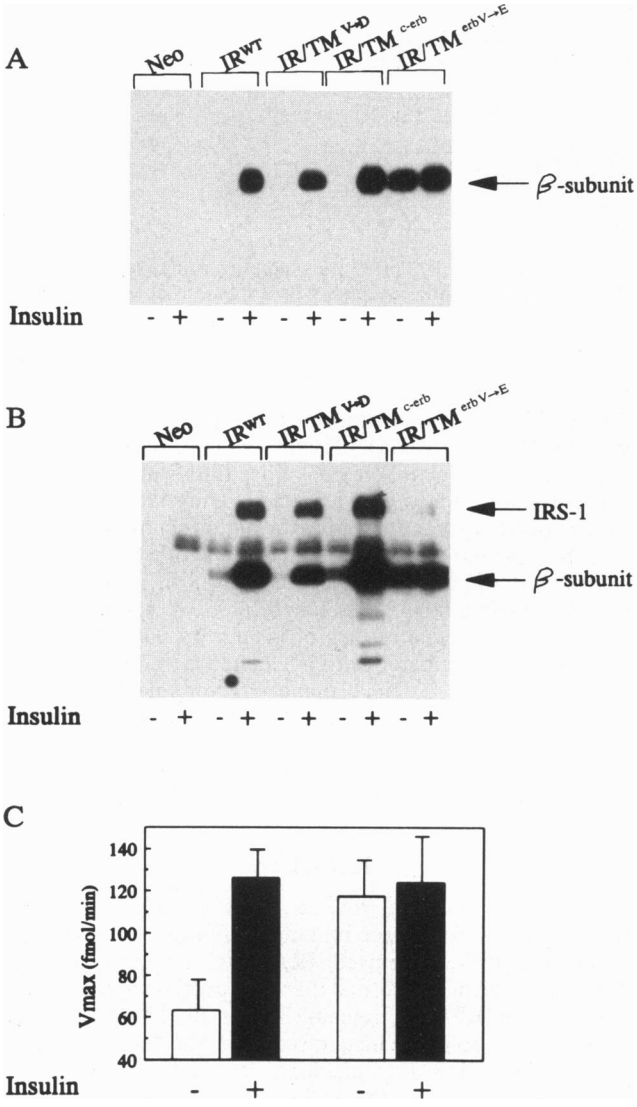

FIG. 2. Autophosphorylation and kinase activity in CHO cells expressing normal and mutant insulin receptors. Cultures of CHO cells either mock-transfected (Neo) or expressing the wild-type (IR^{WT}) or mutant ($IR/TM^{V\rightarrow D}$, $IR/TM^{c\text{-}erb}$, and $IR/TM^{erbV\rightarrow E}$) human insulin receptors were incubated with or without 100 nM insulin for 1 min. Immunoblots prepared from equal numbers of immunoprecipitated insulin receptors (*A*) or equal amounts of protein from whole-cell lysates (*B*) were probed with antiphosphotyrosine antibodies followed by incubation with ^{125}I-labeled protein A and after extensive washing were subjected to autoradiography. Arrows indicate the insulin receptor β subunit and IRS-1. (*C*) *In vitro* phosphorylation of an IRS-1-derived peptide substrate using immunocomplexed insulin receptors isolated from control (−) or insulin-stimulated (+) cells expressing either IR^{WT} (left set of columns) or $IR/TM^{erbV\rightarrow E}$ (right set of columns). Values indicate the average V_{max} ± SD from independent experiments.

the insulin receptor kinase, as measured by both autophosphorylation and phosphorylation of an exogenous substrate.

Phosphorylation of IRS-1. In intact cells, the insulin receptor phosphorylates its endogenous substrate IRS-1 on multiple tyrosine residues, and this results in the activation of downstream signals, ultimately producing both metabolic and growth effects (1, 18–20). To determine the effects of constitutive insulin receptor kinase activation on IRS-1 phosphorylation, immunoblots of whole-cell lysates prepared from cells expressing IR^{WT}, $IR/TM^{c\text{-}erb}$, and $IR/TM^{V\rightarrow D}$ were probed with anti-phosphotyrosine antibodies. These blots demonstrated an ≈20-fold increase in insulin-stimulated tyrosine phosphorylation of IRS-1 (Fig. 2*B*). In comparison with the $IR/TM^{erbV\rightarrow E}$ cells, phosphorylation of IRS-1 was slightly increased (≈2-fold) in the basal state but showed only a minor further increase (<2-fold) after insulin addition. Thus, the maximum level of insulin-stimulated IRS-1 phosphorylation was reduced ≈80% when compared with cells expressing IR^{WT}.

In light of the apparent discrepancy between constitutive kinase activity of the $IR/TM^{erbV\rightarrow E}$ receptor and reduced IRS-1 phosphorylation, we quantitated the levels of IRS-1 protein in the transfected CHO cells. With the use of an anti-IRS-1 antibody directed against the N-terminal 15 amino acids to immunoprecipitate IRS-1 from cell extracts, followed by probing immunoblots of the immunoprecipitates with a C-terminal antibody, there was an ≈80% decrease in IRS-1 protein levels in two independently isolated clonal cell lines expressing $IR/TM^{erbV\rightarrow E}$ (Fig. 3*A*). This observed decrease in IRS-1 protein is similar to that seen in cells expressing the IR^{WT} that have been chronically treated with insulin (Fig. 3*B*). Thus, the decrease in IRS-1 phosphorylation in $IR/TM^{erbV\rightarrow E}$ cells was due to a decrease in the level of substrate protein rather than to an altered substrate recognition because the K_m values for the peptide substrate were similar for both IR^{WT} and $IR/TM^{erbV\rightarrow E}$.

Insulin-Regulated Bioeffects. To determine the effects of a constitutively activated receptor on insulin-stimulated metabolic and growth-related events, we measured the incorporation of [^{14}C]glucose into glycogen and [^{3}H]thymidine incorporation into DNA, respectively. As we have reported (21), CHO cells expressing the normal IR^{WT} display a leftward shift in the insulin dose–response curve and a 2-fold increase in the maximum insulin-stimulated glycogen synthesis when compared with the control neomycin-resistant cells (Fig. 4). Cells expressing $IR/TM^{erbV\rightarrow E}$ displayed similar basal levels of glycogen synthesis as cells expressing IR^{WT}, but no stimulation was produced by insulin addition, indicating that these cells were desensitized to insulin for this biological response.

In cells expressing IR^{WT}, low concentrations (1 nM) of insulin stimulated the incorporation of thymidine into DNA to ≈60% of the levels seen when these cells are stimulated with fetal bovine serum (Fig. 5). Two independent clones of cells expressing $IR/TM^{erbV\rightarrow E}$ revealed a decrease in maximal serum stimulation, as compared with cells expressing IR^{WT}. In addition, these cells were insensitive to insulin stimulation, and the basal levels of thymidine incorporation were elevated as compared with their maximal serum stimulation.

DISCUSSION

The mechanism by which the point mutation in the *erbB-2*$^{V\rightarrow E}$-encoded TM domain confers kinase activation and transformation is uncertain. Using chemical cross-linking, Wiener *et al.* (22) found that compared with the native ErbB-2 protein, the oncogenic form (ErbB-$2^{V\rightarrow E}$) exists in an aggregated state at the cell surface, potentially mimicking ligand-induced receptor oligomerization. A model for aggregation based on dimer formation between two TM α-helices of the ErbB-2 oncoprotein has been proposed by Sternberg and Gullick (23), in which Glu-664 provides a critical hydrogen bond that stabilizes dimer formation. Detailed mutagenesis studies of the *erbB-2*$^{V\rightarrow E}$-encoded TM domain have shown that primary sequence surrounding Glu-664 and its position within the TM domain are both critical for kinase activation and transformation (12, 14). In this report we show that substitution of the entire TM domain from *erbB-2*$^{V\rightarrow E}$ into a heterologous protein (the insulin receptor) results in full ligand-independent activation of the kinase. In view of the evidence that dimer formation and receptor transphosphor-

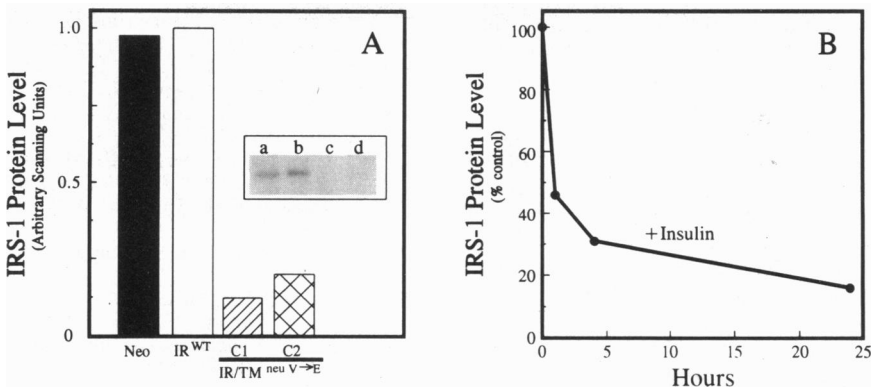

FIG. 3. Quantitation of IRS-1. (*A*) Relative amounts of IRS-1 protein from Neo, IR^{WT}, or two independently isolated clonal cell lines expressing $IR/TM^{erbV\rightarrow E}$ (C1 and C2) were determined by scanning laser densitometry of an autoradiograph of an immunoblot probed with an anti-IRS-1 antibody. (*Inset*) Representative autoradiograph. Lanes: a, Neo; b, IR^{WT}; and c and d, $IR/TM^{erbV\rightarrow E}$ C1 and C2, respectively. (*B*) CHO cells expressing IR^{WT} were treated with 200 nM insulin for the indicated times, and IRS-1 protein was quantitated as described above.

ylation are important for tyrosine kinase activation generally (3) and for the insulin receptor specifically (24), it seems likely that introduction of the TM from *erbB-2*$^{V\rightarrow E}$ promotes dimerization by direct TM–TM interaction between $\alpha\beta$ heterodimers within the holoreceptor complex and may account for the observed receptor activation. Our findings predict that similar mutations would activate other receptor tyrosine kinases.

As discussed above, the position of the Val → Glu mutation and the surrounding amino acids in the *erbB-2* TM domain are imperative for ligand-independent kinase activation (12, 14). Our results with the insulin receptor are in agreement because we and others (8, 9) have shown that neither the single mutations in $IR/TM^{V\rightarrow D}$ or $IR/TM^{V\rightarrow E}$ nor substitution with the TM domain from ErbB-2 confers activation of the insulin receptor in intact cells. Similar point mutations corresponding to the activating *erbB-2*$^{V\rightarrow E}$ in the TM domain of the epidermal growth factor receptor-encoding gene also had no effect on epidermal growth factor receptor kinase activity or signaling properties (25, 26). In contrast, Longo *et al.* (10) have shown that in some circumstances a Val → Asp substitution within the insulin receptor TM domain may partially activate the kinase. However, this increase in basal autophosphorylation was only minor, and these receptors still required insulin stimulation for full activation.

The other major findings of this study are that introduction of the constitutively active insulin receptor into cells results in down-regulation of IRS-1 protein, desensitization of glycogen synthesis, and elevated basal levels of DNA synthesis. Previous studies using cultured rat adipocytes and rat hepatoma cells indicate that long-term exposure of cells to insulin (6–24 hr) can desensitize some metabolic responses. These effects include a loss in insulin-stimulated glucose uptake and glycogen synthase activation and a decrease in the number of cell-surface insulin receptors (27, 28). Recently, Rice *et al.* (29) found that chronic insulin treatment of 3T3-L1 adipocytes also resulted in down-regulation of IRS-1. We have found similar results in studies using Fao rat hepatoma cells (data not shown) and show here that this effect is also seen

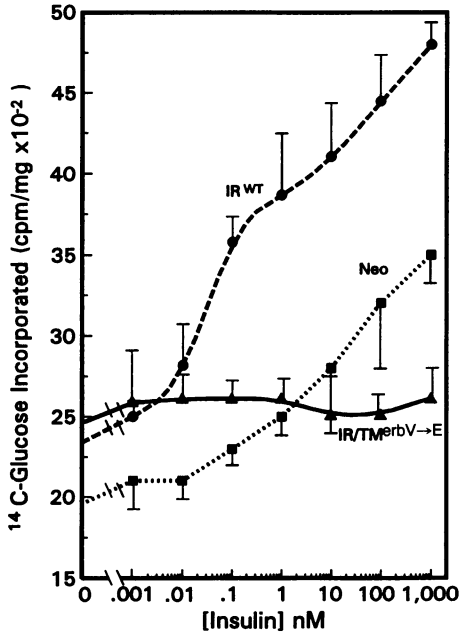

FIG. 4. Insulin-stimulated glycogen synthesis in CHO cells expressing normal and mutant insulin receptors. Confluent monolayers of cells were stimulated (in duplicate) with the indicated concentrations of insulin and then incubated with [^{14}C]glucose (0.5 μCi per well; 1 Ci = 37 GBq). Ethanol-precipitable radioactivity representing [^{14}C]glucose incorporation into glycogen was collected, as described, and quantitated by liquid scintillation counting. Each point represents the average cpm incorporated per mg of cell protein ± SEM from three independent experiments.

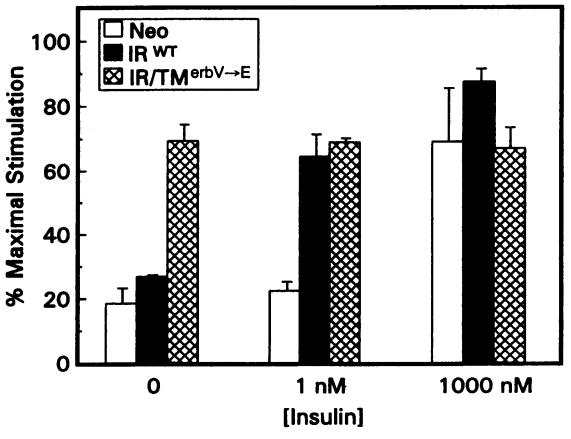

FIG. 5. Insulin-stimulated DNA synthesis. Cells were grown in 24-well cluster trays and serum-starved for 3 days in Ham's F-12 medium/0.1% BSA. The cells were stimulated with the indicated concentration of insulin (in triplicate) for 15 hr followed by a 1-hr incubation with [^{3}H]thymidine (1 μCi per well). Data are presented as the percent maximal stimulation that was determined by stimulation with 10% fetal bovine serum (the maximal serum-stimulated values were 2580 cpm ± 504 and 991 cpm ± 262 for IR^{WT} and $IR/TM^{erbV\rightarrow E}$, respectively). Each value represents the average ± SEM from three independent experiments.

in CHO cells (Fig. 3*B*). In the present study we show that expression of IR/TM$^{erbV\rightarrow E}$ causes a loss of insulin-stimulated glycogen synthesis and DNA synthesis both occurring at a time when IRS-1 is down-regulated. Presently, the role of IRS-1 in insulin-stimulated glycogen synthesis is unclear. However, recent studies in which both the normal insulin receptor and IRS-1 have been stably expressed in cells reveal a delicate balance between these two proteins and their ability to enhance or inhibit insulin-stimulated DNA synthesis (30). Thus, when CHO cells are produced that overexpress either the insulin receptor or IRS-1, insulin signaling is increased. In contrast, when both are simultaneously expressed, insulin-stimulated DNA synthesis decreases (30). Thus, the down-regulation of IRS-1 seen in cells expressing IR/TM$^{erbV\rightarrow E}$ may actually allow for the relatively increased basal levels of DNA synthesis—i.e., a persistent insulin-like effect—rather than diminish this response because the low level of IRS-1 present in these cells is constantly in a phosphorylated state. However, interpretation of the DNA-synthesis data from the cells expressing IR/TM$^{erbV\rightarrow E}$ is complicated because other factors involved in the serum/insulin signaling pathway may also be down-regulated.

The substitution of the *erbB-2*$^{V\rightarrow E}$-encoded TM domain into the insulin receptor results in constitutive kinase activation. This chimeric receptor provides a specific tool for the study of downstream components of the insulin-action pathway. Similar substitutions in other tyrosine kinase receptors should demonstrate chronic ligand-independent activation as well.

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1. Kahn, C. R. & White, M. F. (1988) *J. Clin. Invest.* **82,** 1151–1156.
2. Rosen, O. M. (1987) *Science* **237,** 1452–1458.
3. Ullrich, A. & Schlessinger, J. (1990) *Cell* **61,** 203–212.
4. Yarden, Y. & Schlessinger, J. (1987) *Biochemistry* **26,** 1443–1451.
5. Cochet, C., Kashles, O., Chambaz, E. M., Borello, I., King, C. R. & Schlessinger, J. (1988) *J. Biol. Chem.* **263,** 3290–3295.
6. Seifert, R. A., Hart, C. E., Phillips, P. E., Forstrom, J. W., Ross, R., Murray, M. J. & Bowen-Pope, D. F. (1989) *J. Biol. Chem.* **264,** 8771–8778.
7. Hammacher, A., Mellstrom, K., Heldin, C.-H. & Westermark, B. (1989) *EMBO J.* **8,** 2489–2495.
8. Yamada, K., Goncalves, E., Kahn, C. R. & Shoelson, S. E. (1992) *J. Biol. Chem.* **267,** 12452–12461.
9. Frattali, A. L., Treadway, J. L. & Pessin, J. E. (1991) *J. Biol. Chem.* **266,** 9829–9834.
10. Longo, N., Shuster, R. C., Griffin, L. D., Langley, S. D. & Elsas, L. J. (1992) *J. Biol. Chem.* **267,** 12416–12419.
11. Bargmann, C. I., Hung, M. & Weinberg, R. A. (1986) *Cell* **45,** 649–657.
12. Bargmann, C. I. & Weinberg, R. A. (1988) *EMBO J.* **7,** 2043–2052.
13. Akiyama, T., Matsuda, S., Namba, Y., Saito, T., Toyoshima, K. & Yamamoto, T. (1991) *Mol. Cell. Biol.* **11,** 833–842.
14. Cao, H., Bangalore, L., Bormann, B. J. & Stern, D. F. (1992) *EMBO J.* **11,** 923–932.
15. Cheatham, B. & Kahn, C. R. (1992) *J. Biol. Chem.* **267,** 7108–7115.
16. White, M. F. (1990) in *Peptide Hormone Action: A Practical Approach*, eds. Siddle, K. & Hutton, J. C. (IRL, Oxford), pp. 223–250.
17. Shoelson, S. E., Chatterjee, S., Chaudhuri, M. & White, M. F. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 2027–2031.
18. Sun, X. J., Rothenberg, P. L., Kahn, C. R., Backer, J. M., Araki, E., Wilden, P. A., Cahill, D. A., Goldstein, B. J. & White, M. F. (1991) *Nature (London)* **352,** 73–77.
19. Rothenberg, P. L., Lane, W. S., Karasik, A., Backer, J. M., White, M. F. & Kahn, C. R. (1991) *J. Biol. Chem.* **266,** 8302–8311.
20. Backer, J. M., Myers, M. G., Jr., Shoelson, S. E., Chin, D. J., Sun, X. J., Miralpeix, M., Hu, P., Margolis, B., Skolnik, E. Y., Schlessinger, J. & White, M. F. (1992) *EMBO J.* **11,** 3469–3479.
21. Wilden, P. A., Backer, J. M., Kahn, C. R., Cahill, D. A., Schroeder, G. J. & White, M. F. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 3358–3362.
22. Wiener, D. B., Liu, J., Cohen, J. A., Williams, W. V. & Greene, M. I. (1989) *Nature (London)* **339,** 230–231.
23. Sternberg, M. J. E. & Gullick, W. J. (1989) *Nature (London)* **339,** 587.
24. Frattali, A. L., Treadway, J. L. & Pessin, J. E. (1992) *J. Biol. Chem.* **267,** 19521–19528.
25. Kashles, O., Szapary, D., Bellot, F., Ullrich, A., Schlessinger, J. & Schmidt, A. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 9567–9571.
26. Carpenter, C. D., Ingraham, A., Cochet, C., Walton, G. M., Lazar, C. S., Sowadski, J. M., Rosenfeld, M. G. & Gill, G. N. (1991) *J. Biol. Chem.* **266,** 5750–5755.
27. Crettaz, M. & Kahn, C. R. (1984) *Diabetes* **33,** 477–485.
28. Garvey, W. T., Olefsky, J. M. & Marshall, S. (1986) *Diabetes* **35,** 258–267.
29. Rice, M. K., Lienhard, G. E. & Garner, C. W. (1992) *J. Biol. Chem.* **267,** 10163–10167.
30. Sun, X. J., Miralpeix, M., Myers, M. G., Jr., Glasheen, E. M., Backer, J. M., Kahn, C. R. & White, M. F. (1992) *J. Biol. Chem.* **267,** 22662–22672.