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Activation of glucose transport by a natural mutation in the human insulin receptor

(insulin resistance/insulin binding/cell growth/leprechaunism)

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ABSTRACT **Naturally occurring mutations in the insulin receptor gene cause heritable severe insulin resistance. These mutations usually impair insulin receptor signaling in cells cultured from affected individuals. However, fibroblasts cultured from a patient with intrauterine growth restriction and severe insulin resistance (leprechaun Atl-1) had normal amounts of insulin receptor protein and defective insulin binding but constitutive activation of insulin-receptor autophosphorylation and kinase activity and of glucose transport. In the same fibroblasts, growth was impaired. Homozygosity for a mutation in the insulin receptor gene was suspected, since he inherited identical DNA haplotypes for this gene from both related parents. Here we report that the proband was homozygous and both parents were heterozygous for a point mutation in the insulin receptor gene converting the Arg^{86} codon (CGA) to Pro (CCA) (R86P). The R86P substitution is contiguous to the hydrophobic β-sheet of the receptor α subunit implicated by DeMeyts *et al.* [DeMeyts, P., Gu, J.-L., Shymko, R. M., Kaplan, B. E., Bell, G. I. & Whittaker, J. (1990) *Mol. Endocrinol.* 4, 409–416] in the binding of aromatic residues of the insulin molecule. The R86P mutant insulin receptor cDNA was inserted into a plasmid under control of a simian virus 40 promoter and transfected into Chinese hamster ovary (CHO) cells. In contrast with fibroblasts from patient Atl-1, which had normal insulin receptor processing, CHO cells stably transfected with the R86P mutant cDNA (CHO-R86P) had altered posttranslational processing. The R86P mutant receptor failed to bind insulin but caused a significant increase in basal glucose transport in CHO cells. As in fibroblasts cultured from the patient, the R86P mutant insulin receptor did not stimulate growth in transfected CHO cells. These results suggest that the R86P mutation in the insulin receptor activates glucose transport without promoting cell growth and that distinct cell types process this mutant insulin receptor differently.**

The insulin receptor is a heterotetramer composed of two α and two β subunits, linked by disulfide bonds (1, 2). Insulin binding to the α subunit activates β-subunit autophosphorylation and kinase activity. Insulin receptor kinase activity is essential for transmembrane signaling of glucose transport (3). Both subunits of the insulin receptor are encoded by a single gene located on chromosome 19 (4). Mutations in this gene have been identified in patients with the severe insulin-resistant syndrome leprechaunism (5–10). This autosomal recessive phenotype is characterized by intrauterine and postnatal growth restriction, loss of glucose homeostasis, and very high concentrations of circulating insulin (11). To date, the mutations identified in the insulin receptor gene of these patients decrease insulin-stimulated kinase activity of the receptor as a result of decreased receptor number or of structural mutations that impair insulin binding. Reduced insulin receptor kinase activity is associated with a reduced ability of insulin to stimulate glucose transport in fibroblasts obtained from patients with these syndromes (11, 12). Defective insulin receptors may also play a role in the retarded growth that is observed in patients with leprechaunism and their cultured fibroblasts (13, 14), but a direct effect of the mutated insulin receptor on cellular growth has not been defined.

Fibroblasts cultured from a unique patient with leprechaunism, Atl-1, had markedly decreased insulin binding (15). A partial binding defect was present in cells cultured from his phenotypically normal parents (15). Since this patient inherited the same restriction fragment length polymorphism haplotype from each parent and his parents were related, we proposed that he was homozygous for a mutation in his insulin receptor gene (16). Fibroblasts from patient Atl-1 had normal amounts of insulin receptor mRNA (15) and membrane-bound immunoreactive receptors (17, 18). In contrast to cells cultured from other patients with leprechaunism, glucose transport and insulin receptor kinase activity in patient Atl-1's fibroblasts were constitutively elevated and insulin-insensitive (15, 19), suggesting that the structural alteration produced by the mutation not only abolished insulin binding but also activated receptor signaling of glucose transport.

Only two types of artificial manipulations of the insulin receptor activate its signaling. The first type involves truncation of the extracellular α subunit of the insulin receptor by cDNA deletion, which constitutively increases glucose transport (20). At the protein level, proteolysis of the extracellular α subunit also activates receptor phosphorylation (21). These results suggest that the ligand-binding site of the insulin receptor has an inhibitory influence on the intracellular kinase domain, as proposed for other tyrosine kinase receptors (22). A gross deletion was unlikely in our patient because the α subunit of the insulin receptor was of normal size by immunoblot analysis (18) and after cross-linking to ^{125}I-labeled insulin (23). A single amino acid substitution in the transmembrane domain also activates insulin receptor signaling (24). This second type of mutation confirms the importance of the transmembrane domain in the activation of tyrosine kinase receptors (25). However, this transmembrane mutation does not affect insulin binding and stimulates rather than reduces cell growth (24), making it an unlikely site for the Atl-1 mutation.

In another tyrosine kinase receptor, the colony-stimulating factor 1 (CSF-1) receptor, a point mutation in the extracellular domain activates receptor signaling in the absence of ligand (26). This mutation dose not affect CSF-1 binding although it mimics receptor occupancy by the ligand (26). In contrast, the Atl-1 mutation ablates insulin binding while activating receptor kinase activity and glucose transport (15, 19).

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Abbreviations: OMG, 3-*O*-methyl-D-glucose.

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Here we report that patient Atl-1 was homozygous for a G → C transversion at base pair (bp) 476 of the insulin receptor cDNA converting Arg^{86} to Pro. Expression of this mutation in CHO cells duplicated the natural mutation by activating glucose transport without increasing insulin binding or insulin-stimulated cellular growth.

MATERIALS AND METHODS

Materials. Sera, growth media, antibiotics, and trypsin were obtained from Whittaker Bioproducts. [^{32}P]dCTP was from Amersham; 3-*O*-[*methyl*-^{3}H]-D-glucose (OMG) and Na^{125}I were from New England Nuclear. Porcine insulin (sodium, 25 units/mg) was from Calbiochem. Restriction enzymes and other products for molecular biology were from Boehringer Mannheim. Geneticin (G418 sulfate) was from GIBCO. Sigma was the source of other chemicals.

Cell Culture. Human fibroblasts were grown in Dulbecco–Vogt medium supplemented with 10% (vol/vol) fetal bovine serum. Fibroblasts were used between 15 and 25 population doublings. CHO-K1 cells, from the American Type Culture Collection, were grown in Ham's F12 medium supplemented with 6% fetal bovine serum in the presence of penicillin and streptomycin. Culture conditions were as follows: pH 7.4; temperature, 37°C; 5% CO_2/95% air. CHO cell growth was measured in a defined medium containing 0.1% bovine serum albumin, transferrin (1 μg/ml), and the indicated concentrations of insulin (0, 1, 200 nM) or 6% serum.

Sequence of the Insulin Receptor Gene and cDNA. Genomic DNA was extracted from cultured cells by the "salting out" procedure (27). DNA was amplified by a PCR using primers flanking the exons of the insulin receptor gene (28). cDNA synthesized from fibroblast RNA was amplified by PCR using internal primers (10). Amplified double-stranded DNA was purified by low-melting-temperature agarose gel electrophoresis and sequenced directly by adding one of the two end primers or an internal primer labeled with [^{32}P]ATP (for details, see ref. 10).

Construction of the R86P Insulin Receptor cDNA. The pNEO-IR expression vector was constructed from plasmids pcDV1 and pL2 (29) and from the insulin receptor cDNA (1) as described (24). It contained both the neomycin-resistance gene and the insulin receptor cDNA under control of two simian virus 40 early promoters. PCR-amplified exon 2 of the insulin receptor gene from patient Atl-1 was digested with *Eco*RV and *Xma* I and exchanged for the corresponding fragment in the pNEO-IR expression vector. The presence and uniqueness of the R86P mutation in the resulting insulin receptor cDNA was confirmed by sequencing. This construct was linearized by *Hin*dIII digestion and transfected into CHO cells by electroporation. Transfected cells were selected for 3 weeks in Ham's F12 containing Geneticin (0.8 mg/ml) and assayed for insulin binding and insulin receptor mRNA levels by standard procedures (15, 24).

Structural Analysis of the Insulin Receptor. The structure of the insulin receptor encoded by the transfected cDNA was evaluated by immunoblot analysis using a rabbit polyclonal antibody raised against the human placental insulin receptor (18, 23). Confluent cells were scraped from flasks and solubilized in 2% (vol/vol) Triton X-100/50 mM Tris·HCl, pH 7.4/0.2 mM phenylmethylsulfonyl fluoride/aprotinin (1 mg/ml). Alternatively, cells were mechanically disrupted and membranes were separated by differential centrifugation (23). Membranes were then solubilized in Triton X-100, as above. Protein concentration was then determined in all samples using the Bio-Rad protein assay. Proteins were separated by SDS/PAGE, blotted to a poly(vinylidene difluoride) membrane, and incubated overnight with a 1:10,000 dilution of a rabbit polyclonal anti-insulin receptor antibody (23), and then for 20 min with a second antibody (1:20,000 dilution) conjugated with alkaline phosphatase. Insulin receptors were visualized by autoradiography after incubating the blot with the chemiluminescent substrate disodium 3-(4-methoxyspiro{1,2-dioxetane-3,2′-tricyclo[3.3.1.1^{3,7}]decan}-4-yl)phenyl phosphate (Tropix, Bedford, MA).

Insulin Binding and Glucose Transport. Cells were plated in 24-well plates and grown to confluence. ^{125}I-labeled insulin (0.9 ng/ml) binding was measured for 2 h at 20°C as described (24). For glucose transport, cells were washed and incubated for 3 h at 37°C in Earle's balanced salt solution supplemented with 0.2% bovine serum albumin in the absence of serum. Increasing concentrations of insulin were then added for 1 h (24). The initial rate of entry of OMG (1 mM) was then measured for 10–15 s (30). Cells were washed three times with ice-cold 0.1 M $MgCl_2$. Intracellular radioactivity was extracted in 0.5 ml of ethanol and measured in a Beckman LS 7500 scintillation spectrometer. The cell monolayer inside the well was solubilized in 200 μl of 1 M NaOH and evaluated for protein (31). OMG uptake was corrected for cellular protein and normalized to intracellular water, calculated from the equilibrium distribution of OMG (32).

RESULTS

Identification of the Mutation in the Insulin Receptor Gene. Both cDNA, synthesized from fibroblast mRNA, and genomic DNA were amplified using a PCR and insulin receptor cDNA primers (10) or primers in the flanking region of each exon of the insulin receptor gene (28). Amplified DNA was sequenced directly without subcloning using a ^{32}P-labeled end primer or internal primer. Patient Atl-1 was homozygous for a G → C transversion in exon 2 of the insulin receptor gene converting Arg^{86} to Pro (R86P) (Fig. 1). This change was also found at bp 476 of his insulin receptor cDNA. Both parents were heterozygous and had both the normal guanine and the mutant cytosine in exon 2 of the insulin receptor genes (Fig. 1 *Lower*). Except for the G → C transversion, the proband had no other abnormal variations in the remaining insulin receptor cDNA and the first 12 exons of his gene.

Expression of the R86P Mutant Insulin Receptor cDNA in CHO Cells. Expression of normal and mutant insulin receptor

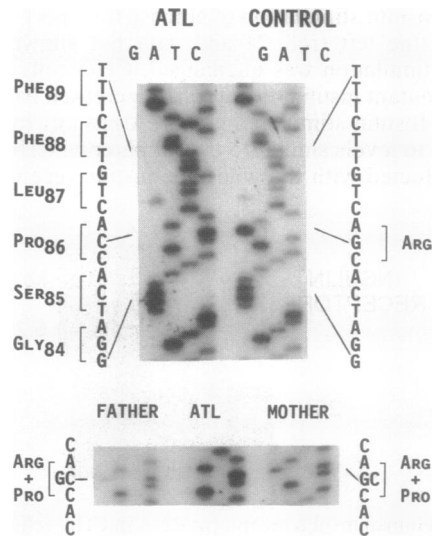

FIG. 1. Partial sequence of the insulin receptor gene in patient Atl-1. Genomic DNA was amplified using primers specific for exon 2 of the insulin receptor gene (28). Both alleles were sequenced directly in both directions without subcloning. (*Upper*) The patient was homozygous for a mutation at bp 476 converting Arg^{86} to Pro. (*Lower*) His parents were heterozygous for this mutation and had both a guanine and a cytosine at position 476.

cDNA was evaluated by measuring the levels of human insulin receptor mRNA (Fig. 2). As expected, CHO cells transfected with the resistance marker alone (CHO-NEO, lanes 1 and 2) had no human insulin receptor mRNA. In contrast, two independent clones of CHO cells transfected with the wild-type insulin receptor cDNA (24) had human insulin receptor mRNA (CHO-WT-IR-Low and CHO-WT-IR-High, lanes 3 and 4). The levels of insulin receptor mRNA in CHO-IR-R86P cells (lane 5) were intermediate between those of CHO-WT-IR-Low and CHO-WT-IR-High cells.

The structure and subcellular localization of the insulin receptor encoded by normal and mutant cDNAs were investigated by incubating total cell extracts (lanes 1–3) and enriched membrane preparations (lanes 4–6) with a polyclonal antibody directed against the human insulin receptor (Fig. 3). Total cell extract and plasma membranes enriched from CHO-NEO cells had no detectable levels of human insulin receptor protein (lanes 1 and 4). Cell extracts of CHO-WT-IR-High cells exhibited a band of 200 kDa and two additional bands of 130 and 95 kDa (lane 2). The size of these bands corresponded to that of the uncleaved $\alpha\beta$ proreceptor (200 kDa) and to the mature α (130 kDa) and β (95 kDa) subunits (33). As expected, the relative intensity of the mature α and β subunits increased in the enriched membrane fraction (lane 5). CHO-IR-R86P cells had increased amounts of 200-kDa proreceptor protein both in total cell extracts and in the membrane fraction (lanes 3 and 6). Mature α and β subunits were not evident in the total cell extract and were barely visible in the membrane fraction.

We next assayed transfected cells for insulin binding and glucose transport (Table 1). Insulin binding increased up to 100 times in cells expressing the normal insulin receptor cDNA (CHO-WT-IR-High) but not in cells expressing the resistance marker alone (CHO-NEO). Cells transfected with the R86P mutant insulin receptor had only a slight increase in insulin binding, which was below that measured in CHO-WT-IR-Low and accounted for <2% of that observed in CHO-WT-IR-High cells.

The initial rate of entry of the nonmetabolizable sugar OMG was stimulated ≈2-fold by insulin in CHO-NEO cells (Table 1). In CHO cells transfected with the normal insulin receptor cDNA (CHO-WT-IR-High), the dose–response curve for insulin stimulation of glucose transport was shifted 40-fold to the left (ref. 24 and data not shown), but the maximal stimulation was unchanged. CHO cells expressing the R86P mutant insulin receptor had increased basal glucose transport. Insulin stimulated glucose transport by CHO-IR-R86P cells to levels similar to that of parental CHO cells and cells transfected with the wild-type insulin receptor cDNA.

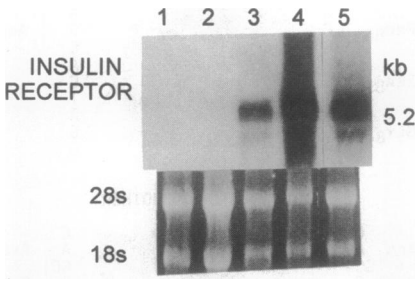

FIG. 2. Human insulin receptor mRNA in CHO cells transfected with normal and mutant insulin receptor cDNA. Total RNA from CHO cells (10 μg in lanes 1 and 3–5; 20 μg in lane 2) was separated by formaldehyde/agarose gel electrophoresis, transferred to nylon, and hybridized in high-stringency conditions to the human insulin receptor cDNA. The ethidium bromide-stained gel is also shown for comparison. Lanes: 1, CHO-NEO; 2, CHO-NEO (20 μg); 3, CHO-WT-IR-Low; 4, CHO-WT-IR-High; 5, CHO-IR-R86P. kb, Kilobase(s).

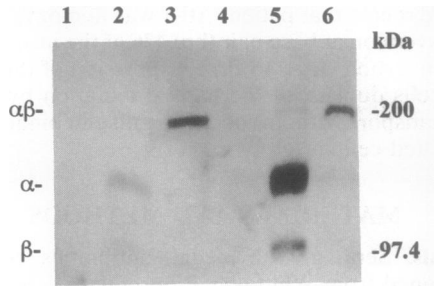

FIG. 3. Insulin receptor protein in CHO cells transfected with normal and mutant insulin receptor cDNA. CHO cells (100 μg per lane, lanes 1–3) and their membranes (30 μg per lane, lanes 4–6) were solubilized in 2% Triton X-100, separated by SDS/PAGE, and blotted to poly(vinylidene difluoride). Insulin receptors were identified by a rabbit polyclonal antibody directed against the human insulin receptor (23). The experiment was repeated twice with identical results. Size markers, whose migration is indicated on the right, included myosin (200 kDa), phosphorylase *b* (97.4 kDa), and bovine serum albumin (69 kDa). Lanes: 1, CHO-NEO; 2, CHO-WT-IR-High; 3, CHO-IR-R86P; 4, CHO-NEO (membranes); 5, CHO-WT-IR-High (membranes); 6, CHO-IR-R86P (membranes).

Effect of the R86P Mutation on Cellular Growth. A consistent observation in patients with leprechaunism is intrauterine and postnatal growth restriction (10, 11, 15). Fibroblasts from patients with leprechaunism have defective growth *in vitro* that correlates with the intrauterine and postnatal growth restriction observed *in vivo* (13, 14). Fig. 4 describes the *in vitro* growth of fibroblasts cultured from patient Atl-1 as compared to that of fibroblasts obtained from patient Mount Sinai, whose cells have reduced levels of insulin receptor mRNA (10). Fibroblasts obtained from both patients with leprechaunism grew more slowly than cells obtained from matched controls.

To test the hypothesis that defective insulin receptors were responsible for the delayed growth, we measured the growth of CHO cells expressing the normal and the R86P mutant insulin receptor (Fig. 5). Cells were incubated in a defined medium in which insulin was the only growth factor. Insulin (200 nM) stimulated the growth of CHO-NEO cells to 60% of that observed in 6% serum. Insulin at 1 nM was one-third as effective as 200 nM insulin in stimulating growth (Fig. 5 *A* and *D*). In CHO-WT-IR-High cells, 1 nM insulin was sufficient to

Table 1. Insulin binding and glucose transport in CHO cells stably transfected with normal and mutant insulin receptor cDNA

Cells	Specific insulin binding, fmol/mg of cell protein	OMG uptake, nmol per ml of cell water per s	
		Basal	Insulin
Parental CHO	1.3 ± 0.2	7.3 ± 0.6	17.7 ± 1.5
CHO-NEO	1.4 ± 0.1	7.4 ± 0.3	18.1 ± 0.8
CHO-WT-IR-Low	28.6 ± 0.5*	8.8 ± 0.8	17.1 ± 0.6
CHO-WT-IR-High	186.8 ± 4.3*	9.5 ± 0.3*	18.7 ± 0.2
CHO-IR-R86P	2.7 ± 0.3*	14.0 ± 1.0**	21.8 ± 1.0

CHO cells were transfected with vectors containing the neomycin-resistance gene and the insulin receptor cDNA under control of the simian virus 40 early promoter. Cells were selected for 3 weeks in the presence of geneticin (G418; 0.8 mg/ml). Cells were assayed for insulin (0.9 ng/ml) binding for 2 h at 20°C. Nonspecific binding was measured in the presence of unlabeled insulin (5 μg/ml) and was subtracted from each point. Each point is the mean ± SEM of triplicates. OMG (1 mM) transport was measured for 15 s in cells incubated for 1 h in the absence (basal) or in the presence of 200 nM insulin (insulin). Points are the mean ± SD of triplicates. In other experiments, insulin-stimulated glucose transport was identical in CHO-IR-R86P, CHO-NEO, and CHO-WT-IR-High cells (data not shown). *, $P < 0.01$ vs. parental CHO cells; **, $P < 0.01$ vs. CHO-WT-IR-High, using analysis of variance.

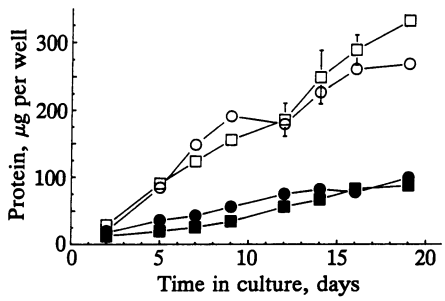

FIG. 4. Growth curve of fibroblasts cultured from controls and patients with leprechaunism. Fibroblasts were grown in Dulbecco–Vogt medium supplemented with 10% fetal bovine serum, which was renewed every 3 days. After the indicated time in culture, cells were washed three times with ice-cold saline and cellular proteins were determined (31). Each point is the mean ± SD of triplicates. The experiment was repeated twice with similar results. □ and ○, Controls; ●, fibroblasts from patient Mount Sinai; ■, fibroblasts from patient Alt-1.

fully stimulate growth (Fig. 5 *B* and *E*). In contrast, 200 nM insulin was required to stimulate growth in CHO-IR-R86P cells (Fig. 5 *C* and *F*) comparable to CHO-NEO cells. These results indicated that, under these experimental conditions, the R86P mutant insulin receptor was unable to enhance growth in response to 1 nM insulin.

DISCUSSION

The proband Atl-1 had severe intrauterine and postnatal growth restriction, postprandial hyperglycemia, fasting hypoglycemia, and markedly elevated insulin levels (15), a syndrome of severe insulin resistance known as leprechaunism. Our previous studies suggested the presence of a mutation in his insulin receptor gene that both impaired insulin binding and activated some insulin signals such as

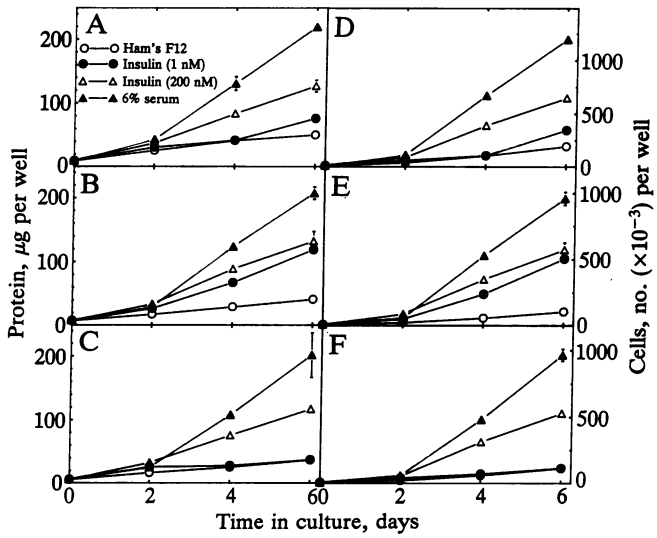

FIG. 5. Growth curve of CHO cells expressing normal and mutant insulin receptors. (*A* and *D*) CHO-NEO. (*B* and *E*) CHO-WT-IR-High. (*C* and *F*) CHO-IR-R86P. Cells were trypsinized and seeded in 24-well plates in Ham's F12 containing 6% fetal bovine serum. The day after seeding, cells were washed twice and incubated in serum-free medium for an additional 24 h. At that point (day 0), cells were incubated in Ham's F12 containing 0.1% bovine serum albumin, transferrin (1 μg/ml), and insulin (0, 1, or 200 nM) or serum (6%). At the indicated intervals, parallel trays of cells were assayed for cellular protein (*A–C*) or resuspended with trypsin, and counted (*D–F*). Data are means of duplicates, with the range of variation indicated.

glucose transport but not growth (refs. 15 and 19 and Fig. 4). Here we identify a single base pair change that converted the codon for Arg^{86} to Pro (R86P) in the α subunit of the insulin receptor. Patient Atl-1 was homozygous and both related parents were heterozygous for this mutation (Fig. 1).

The R86P mutation caused paradoxical impairment of insulin binding and the constitutive activation of glucose transport. CHO cells transfected with the R86P mutant insulin receptor cDNA produced human insulin receptor mRNA (Fig. 2) but did not increase insulin binding as compared to CHO cells transfected with the normal human insulin receptor cDNA (Table 1). This indicated that the mutation interfered with insulin binding either by inducing a structural change in the receptor molecule or by affecting posttranscriptional receptor processing. Western blot analysis of insulin receptors in CHO cells indicated that the R86P mutation impaired posttranslational receptor processing and increased the amount of αβ proreceptor both in total cell extracts and in the plasma membrane (Fig. 3). Thus, insulin receptor processing in CHO cells differed from that of human fibroblasts homozygous for the R86P mutation that have normal amounts of insulin receptors (17) with normal structure as determined using the same polyclonal antibody (18). The antibody used in this study could immunoprecipitate phosphorylated β subunits of normal size from fibroblasts homozygous for the R86P mutation (19). The reason for the different posttranslational insulin receptor processing between human fibroblasts and CHO cells is unclear. It is possible that the presence of normal receptors in CHO cells affects processing of the mutant insulin receptor. Alternatively, cell-specific factors involved in protein trafficking or targeting may be involved. This point should be clarified by expressing the R86P mutant insulin receptor cDNA in other cell types that more closely resemble human fibroblasts. Discrepancy between results in human fibroblasts and transfected CHO cells may occur with other natural mutations in the insulin receptor. In human fibroblasts homozygous for a Leu → Pro substitution at position 233 (L233P) of the insulin receptor, the α subunit remained localized in the cytoplasm and could be cross-linked to insulin after cell solubilization (34). In contrast, CHO cells expressing the L233P mutation had only the αβ proreceptor protein (33), suggesting, like our R86P mutation, different processing of mutant insulin receptors in CHO and fibroblast cells.

CHO cells expressing the R86P mutant human insulin receptor had increased basal glucose transport (Table 1) that remained insulin-sensitive, probably reflecting responsivity of endogenous normal rodent receptors. In contrast, human fibroblasts homozygous for the same mutation had extremely high basal glucose transport that was not further stimulated by insulin (15). Fibroblasts from both parents, who are heterozygous for the R86P mutation (Fig. 1) and express equal amounts of normal and mutant receptor, had normal basal and insulin-stimulated glucose uptake (15). These data indicate that activation of glucose transport by the R86P mutation is recessive and that a limited number of normal insulin receptors, such as those encountered in parental fibroblasts (15), is sufficient to prevent constitutive activation of glucose transport. In CHO cells, the high number of transfected mutant receptors could partially overcome the regulatory effects of endogenous rodent receptors and cause the partial increase in basal glucose transport. Patient Atl-1 had uncontrollable hypoglycemia *in vivo*, a phenotype consistent with the effects on glucose transport of the R86P mutation *in vitro*.

Cellular growth was not stimulated by the R86P mutation in fibroblasts from the proband (Fig. 4) or in CHO cells expressing the R86P mutant insulin receptor cDNA (Fig. 5). Patients with leprechaunism have intrauterine growth restriction and fail to grow after birth. Defective growth may be

related to the impaired growth-promoting ability of mutant insulin receptors.

Dissociation between the growth-promoting and metabolic effects of insulin is observed with other mutated insulin receptor cDNAs. Replacement of Tyr^{1162} and Tyr^{1163} of the insulin receptor impairs stimulation of glucose transport (35) but does not alter the mitogenic response to insulin (36). An insulin receptor lacking the 43 C-terminal amino acids is defective in signaling metabolic responses but exhibits augmented mitogenic activity compared to normal receptors (37). The same phenotype is observed when the two C-terminal tyrosines (Tyr^{1316} and Tyr^{1322}) are replaced by phenylalanines (38). The above reported dissociation between metabolic and mitogenic effects of insulin is caused by mutations in the cDNA coding for the intracellular domain of the receptor. Conformational changes in the extracellular domain of the insulin receptor also dissociate receptor signaling. In intact cells, a polyclonal antibody stimulates metabolic effects without affecting mitogenesis (39). These results as well as the natural R86P mutation indicate that the insulin receptor stimulates metabolic functions and cell growth through distinct pathways and that these functions can be separated by mutations in the receptor.

Based on the proposed tertiary structure of the insulin receptor, the R86P mutation is located in a β-turn between two β-strands (40). The β-strand C-terminal to this mutation, comprising amino acids Leu^{87}-Phe-Phe-Asn-Tyr-Ala-Leu-Val^{94}, is highly conserved between the insulin and insulin-like growth factor I receptors and is thought to be one of the major insulin binding sites (41). This domain of the receptor may interact with another site, comprising amino acids Leu^{426}-Phe-Phe-His-Tyr^{430} (42), to form a cleft or pocket within which insulin binds (42). Both domains of the receptor are also very similar to the C-terminal amino acids of the B-chain of the insulin molecule (41). The R86P substitution may change the configuration of the contiguous β-sheet and prevent insulin binding. Our results in human fibroblasts indicate that this domain not only is involved in insulin binding but also may play an essential role in the initiation of some insulin receptor functions, such as receptor phosphorylation, kinase activity (19), and glucose transport (15), but not insulin-sensitive growth.

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