# Tissue-Specific Expression of Two Alternatively Spliced Insulin Receptor mRNAs in Man

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**Two previously reported insulin receptor cDNA sequences differ by 36 base pairs (bp) in the distal $\alpha$-subunit, suggesting that alternative mRNA splicing within the coding region may occur (two insulin receptor isoforms). We developed a quantitative modification of the polymerase chain reaction technique in order to detect and characterize differential mRNA splicing at this site within the distal $\alpha$-subunit. Using RNA derived from a variety of human cell types, we detected two polymerase chain reaction-amplified cDNA species reflecting the presence or absence of the above 36 nucleotides. Identity of the two cDNA species was confirmed by Southern blots, the use of a *BANI* restriction site present only in the 36 base pair segment and dideoxy sequencing. The relative expression of the two mRNA forms varied markedly in a tissue-specific manner. Buffy coat leukocytes and Epstein-Barr virus-transformed lymphocytes express only the shorter mRNA. Placenta expresses both species equally; muscle, isolated adipocytes and cultured fibroblasts express somewhat more of the longer mRNA (relative ratios of mRNA abundance of 1.51, 3.18, and 2.77, respectively); liver expresses mostly the longer mRNA (relative ratio of 9.8). In RNA derived from cultured and fresh cells from patients with several states of insulin resistance, the relative expression of the two mRNA species was similar to results obtained with comparable normal tissues. Although the functional significance of alternative splicing of the insulin receptor mRNA is unknown, differential expression of these two receptor mRNAs may provide a structural basis for previously observed tissue-specific differences in insulin binding and action. (Molecular Endocrinology 3: 1263–1269, 1989)**

## INTRODUCTION

The insulin receptor is composed of two extra-cellular $\alpha$-subunits ($M_r$ = 135,000) which confer the ability to bind insulin, and two membrane-spanning $\beta$-subunits ($M_r$ = 95,000) which contain the tyrosine kinase domain (1, 2). Formation of the mature $\alpha 2\beta 2$ insulin receptor tetramer requires proteolytic cleavage of a colinear 1,382 amino acid $\alpha/\beta$ proreceptor which is encoded by insulin receptor mRNA. Two insulin receptor cDNAs cloned from human placenta (3, 4) differ by a block of 36 nucleotides within the coding region (amino acid codons 718–729)[1] near the carboxy-terminal end of the $\alpha$-subunit, resulting in predicted proreceptors of either 1,382 or 1,370 amino acids. Since insulin receptor mRNA is transcribed from a single gene located on human chromosome 19 (3–5), the two mRNA species are likely the result of alternative mRNA splicing. Other possible explanations, such as allelic polymorphism in the normal population or cloning artifacts are excluded by the genomic sequence data of Seino *et al.* (6), which reveals that the 36 nucleotides are encoded by a mini-exon (exon 11) of the insulin receptor gene. Thus, insulin receptor mRNA in human placenta may exist as a longer (Ex11+) or shorter (Ex11−) transcript on the basis of alternative mRNA splicing. To what extent

[1] The nucleotide and amino acid numbering system of Ebina *et al.* (4) is used hereafter.

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these two mRNAs are expressed in other tissues including those involved in insulin action *in vivo* is currently unknown. To detect and quantify these species we developed a modification of the polymerase chain reaction (PCR) technique (7, 8). Starting with RNA obtained from several tissues of normal and diabetic individuals, we amplified a region of insulin receptor cDNA encompassing the 36 nucleotides encoded by exon 11 (6). We demonstrate that the two insulin receptor mRNA species are equally expressed in placenta. Importantly, we have determined that there is marked tissue specificity to the relative expression of the two alternate insulin receptor mRNA forms, providing a possible structural basis for differences in insulin action in different tissues. Preliminary data suggests that in fresh and cultured cells of patients with several states of insulin resistance, the relative abundance of the two mRNA species is not changed.

## RESULTS AND DISCUSSION

Previous studies have noted the existence of multiple mRNA species that range in size from 4.6 to 11.0 kilobases (3, 4, 9, 10). The relative abundance of some of these mRNAs, the size variation of which appears to be due to differences in the length of the 3′-untranslated region (2), may be tissue specific (9). Here, we have used the PCR technique to demonstrate distinct insulin receptor mRNA species which are due to alternative mRNA processing. These mRNAs vary within the coding region and direct the synthesis of different receptor isoforms.

Two oligonucleotide primers (A and B) were used to amplify insulin receptor cDNA by the PCR reaction. Based on the inclusion or exclusion of the 36 nucleotides encoded by exon 11, the predicted size of the fragments would be 721 or 685 bp as indicated by the schematic shown in Fig. 1. As seen in Fig. 2, one or both of these cDNA species are efficiently amplified, and their relative abundance varies markedly depending upon the tissue from which the RNA was isolated. The two species are nearly equivalent when amplified from placental RNA. Liver demonstrates predominantly the Ex11+ form and Epstein-Barr virus (EBV) lymphoblasts show only the Ex11− form on ethidium stained gels of the PCR reaction products. RNA from buffy-coat leukocytes of eight normal subjects was amplified and only the Ex11− cDNA fragment was detected by ethidium staining as well as by Southern blotting, which would detect even small amounts of the Ex11+ species if it were present (data not shown). Thus, this pattern of differential splicing of exon 11 is a consistent finding in this tissue. Other tissues including muscle, adipocytes, and cultured fibroblasts show both species (see below). Southern blotting of the PCR-product DNA gels with a nearly full-length insulin receptor cDNA probe confirmed that both cDNA fragments were insulin receptor sequence (data not shown). To verify that the larger cDNA contains exon 11, we took advantage of the fact that only the Ex11+ cDNA contains a site for the restriction enzyme *BANI* (Fig. 2). Subcloning and dideoxy sequencing of both the 721 and 685 base pairs (bp) DNA fragments amplified from human fibroblast RNA revealed the normal receptor sequence in this region with or without exon 11, respectively.

In amplifying 677–938 bp fragments from five other regions of the insulin receptor mRNA sequence (the remainder of the coding region) with placental, fibroblast, or leukocyte RNA as the initial template, we detected only the single predicted size fragment with each primer set used (11, unpublished results). Since exon 11 is the smallest of the 22 insulin receptor exons (6), we would expect to detect variation in the size or number of these PCR-product cDNA fragments if differential mRNA splicing occurred elsewhere within the coding region in these tissues.

To quantify the expression of the Ex11+ *vs.* Ex11− mRNA species in tissues where both were expressed, we developed a quantitative PCR assay. To validate this, we used RNA from cell lines CHO-IR and CHO-HIRC, which overexpress the Ex11+ and Ex11− forms, respectively. RNAs from these cells were mixed at several fixed ratios of insulin receptor mRNA abundance and then coamplified. The abundance of each amplified cDNA was quantified by measuring the incorporation of [$^{32}$P]dCTP into the reaction products. There was a linear relationship between the ratio of Ex11+/Ex11− mRNA templates present in the initial mixture and the ratio of counts per min incorporated into the two respective PCR-product cDNA fragments at ratios of template mRNA ranging from 1–10 (Fig. 3). At ratios of 20:1 or greater, the less abundant template appeared not to amplify. Given the range over which a linear relationship between the two mRNA templates is preserved after PCR amplification, it is possible that leukocytes or EBV lymphoblasts do express Ex11+ message (but in very small amounts relative to the Ex11− form).

When these methods were applied to RNA from several human tissues, characteristic but divergent tissue-specific ratios of the two mRNAs were observed (Fig. 4). Placenta expressed both mRNAs in nearly equal amounts. Muscle, adipocytes, and fibroblasts expressed both mRNAs, but somewhat more of the Ex11+ form (mean count per min ratios of 1.51, 3.18, and 2.77, respectively). As suggested by ethidium stained gels of the PCR products (Fig. 2), liver expressed predominantly the Ex11+ mRNA (mean counts per min ratio 9.8). Where RNA samples from several subjects were available from a given tissue and in repeated amplifications performed with individual RNA samples, there was little variation in the resultant ratios (Fig. 4). The expression of both mRNA species by placenta explains the 36 bp discrepancy between the two originally reported insulin receptor cDNA sequences which were both cloned from human placental cDNA libraries (3, 4).

Tissue-specific, developmental, or physiological reg-

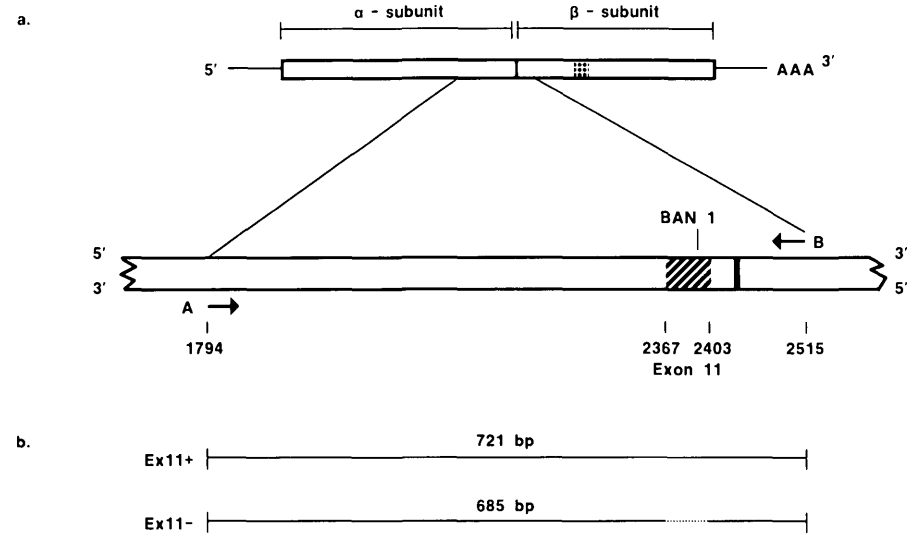


**Fig. 1.** Location of the Region Encoded br Exon II within the Insulin Receptor cDNA Sequence

a, Schematic representation of the human insulin receptor cDNA sequence which encodes a single $\alpha/\beta$ proreceptor protein. The $\alpha/\beta$ cleavage site (*dark vertical bar*) and transmembrane domain (*stippled area*) are shown. The expanded portion shows a 721-bp region beginning at nucleotide 1794 (amino acid codon 526) in the distal $\alpha$-subunit and extending to nucleotide 2515 (codon 766) in the extracellular $\beta$-subunit. This region was amplified with oligonucleotide primers A and B (*horizontal arrows*) and includes the 36 bp alternatively spliced exon 11 (*hatched area*). The *BAN*I cleavage site within the exon 11 is noted. b, The predicted size of cDNAs amplified with primers A and B. A 721 bp fragment (Ex11+) would be generated if exon 11 were present in the template mRNA; a 685 bp fragment (Ex11−) would be generated if exon 11 were absent.

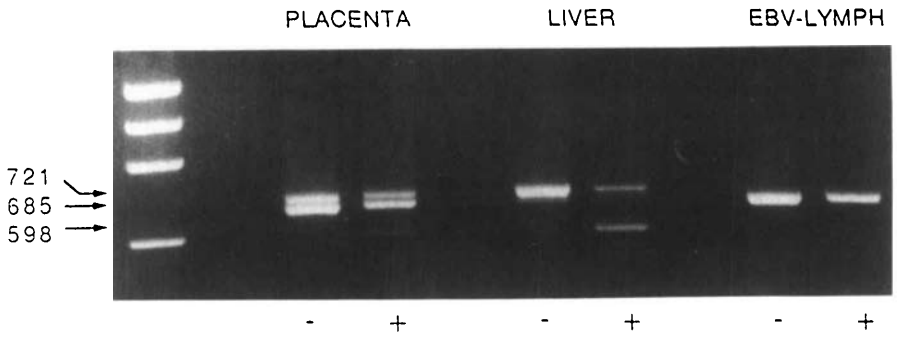


**Fig. 2.** PCR Amplification of a Region of Human Insulin Receptor cDNA Sequence

RNA was prepared from placenta, liver and EBV-transformed lymphocytes. First strand cDNA was synthesized from 10 $\mu$g total RNA; subsequent PCR amplification was performed for 40 cycles with primers A and B. This ethidium bromide stained 1.8% agarose gel reveals the expected 721 and 685 bp PCR-product DNA fragments corresponding to the two (Ex11+ or Ex11−) insulin receptor cDNA species. Each sample was partially digested with *BAN*I (+ sign lanes) which cuts only within the 36 bp alternately spliced region (Ex11+) generating 598 and 123 (not visible) bp fragments as seen with liver and (faintly seen 598 band) with placenta. Lanes are not loaded for equivalent DNA amount. EBV-lymphocytes express only the Ex11− form and is therefore not cut by *BAN*I. *Hae*III-digested ΦX174 RF DNA molecular size markers are shown in the *far left lane*.

ulation of differential RNA splicing can be assessed by a variety of techniques (12). These include $S_1$-nuclease mapping (13), Northern blotting, and *in situ* hybridization with exon-specific probes (14, 15), or simply comparing different cDNA clones derived from different tissues or conditions. The PCR technique is extremely sensitive and can detect and quantitate rare transcripts present in small RNA samples (16–18). The PCR technique as applied here may be the method of choice for detection and quantitation of alternatively spliced mRNAs in large numbers of samples.

There are several examples of mammalian genes encoding membrane receptors which undergo alternative mRNA splicing (12). These include the Drosophila epidermal growth factor receptor (15), chicken c-Erb B (14), the asialoglycoprotein receptor (19), and the T cell receptor $\beta$-chain (20). Different Drosophila epidermal growth factor receptor isoforms result from the use of alternative 5′-exons (15) which differs from the alternative splicing of an internal cassette exon seen with c-Erb B in avian leukemia virus-induced erythroblastosis (14) or with the insulin receptor as shown here. The differential splicing of cassette exon 11 containing translated sequences, should result in the synthesis of

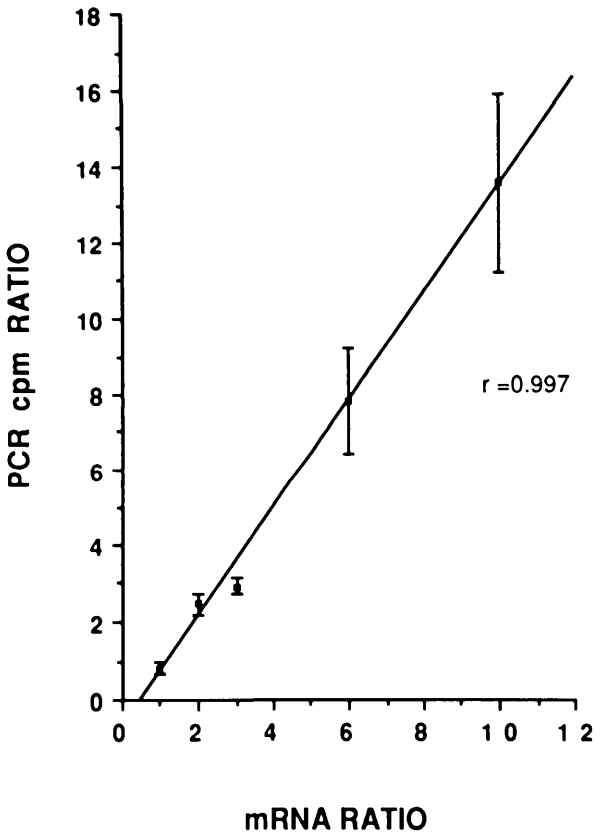


**Fig. 3.** Standard Curve for Relative Abundance of Two Insulin Receptor mRNA Species

RNA was derived from two cell lines, CHO-IR and CHO-HIRC, each expressing exclusively the Ex11+ or Ex11− alternatively spliced mRNA species, respectively. After determining the relative abundance of insulin receptor mRNA in CHO-IR *vs.* CHO-HIRC, several fixed ratios of CHO-IR insulin receptor mRNA to CHO-HIRC insulin receptor mRNA were combined (total 5 μg RNA) and coamplified after cosynthesis of first strand cDNA. [$\alpha$-$^{32}$P]dCTP was added to the PCR reaction mix. PCR-product DNA gel slices were excised and counted. Insulin receptor mRNA ratios (CHO-IR:CHO-HIRC) of 1:1 to 10:1 and the corresponding ratio of counts per min in each band after 40 cycles of PCR are shown.

more than one insulin receptor isoform (12). In cases such as rat troponin T (21), rat thyroid hormone receptor-$\alpha$ (22), and others (12), such protein isoforms display important functional differences. The fact that alternative processing of insulin receptor mRNA is tissue specific is further evidence that these two isoforms will be shown to differ functionally. Although both receptor isoforms have been over-expressed in mammalian cell systems (23–26) and can clearly function as insulin receptors, they have not as yet been functionally compared in parallel.

Abnormal function of the insulin receptor has been described in noninsulin dependent diabetic subjects (NIDDM) (1, 2, 27–29) as well as in rare syndromes of extreme insulin resistance such as the type A syndrome (2, 29, 30). Primary defects in insulin receptor genetic sequence have been identified in certain insulin resistant patients (11, 31, 32), and mutations causing altered exon splicing could exist (33). Tissue-specific differential splicing of exon 11 implies the existence of one or more *trans*-acting factor(s) which regulate posttranscriptional mRNA processing in different cell types (12). Little is known about the mechanism for such regulation, and abnormal expression of splice variants could potentially result from some consequence of the *in vivo* diabetic metabolic milieu. Although the functional consequences Ex11+ and Ex11− isoforms are unknown, we have quantified expression of the two mRNA species in several patients with insulin resistant disorders. As in normal subjects, lymphoblast cell lines established from 3 Pima Indians (34) with NIDDM expressed only the Ex11− mRNA species (by both ethidium staining and Southern blotting) (Figs. 2 and 4). Two fibroblast cell lines from patients with the type A syndrome of insulin resistance ($A_2$, $A_3$, Ref. 30) demonstrated a mean ratio (2.25 ) of Ex11+ to Ex11− mRNAs very similar to two normal fibroblast lines (3.39) (Fig. 4). Amplification of liver-derived RNA from obese nondiabetic (n = 4) or obese with NIDDM (n = 3) subjects (28) demonstrated mean PCR-product cpm ratios (6.89 and 6.88) similar to values noted with lean normal subjects (mean 3.8, n = 6) (Fig. 4). Thus, preliminary studies of cultured and fresh cells from patients with several disorders characterized by insulin resistance do not reveal clear differences in the expression of exon 11 splice variants.

The possibility that insulin receptor structure varies in a tissue-specific manner, and that insulin receptor affinity and/or function may differ in different cell types has been supported by previous studies (35–40). Differential expression of alternatively spliced insulin receptor mRNAs provides a potential structural basis for tissue-specific differences in insulin binding and action. Additional studies will need to address potential functional differences between the two receptor isoforms and the possible role of altered exon 11 splicing in disease states.[2]

## MATERIALS AND METHODS

### RNA Sources and Preparation

Cultured fibroblasts and Epstein-Barr virus EBV transformed lymphocyte cell lines were established from forearm skin biopsies and peripheral blood lymphocytes, respectively, by standard techniques (41, 42). Buffy coat leukocytes were isolated from heparinized whole blood after sedimenting erythrocytes in the presence of one fifth volume of 6% Dextran 70 (Macrodex, Pharmacia, Piscataway, NJ). Three diabetic Pima Indian lymphoblast cell lines, GM02417, GM02566, and GM02575 were obtained from the National Institute of General Medical

[2] Since the completion of this study, a paper by Seino and Bell was published which describes results similar to ours (Biochem Biophys Res Commun **159**:312–316, 1989). Using the PCR technique, they note tissue specific expression of alternatively spliced insulin receptor mRNA with patterns similar to those described in the present study.

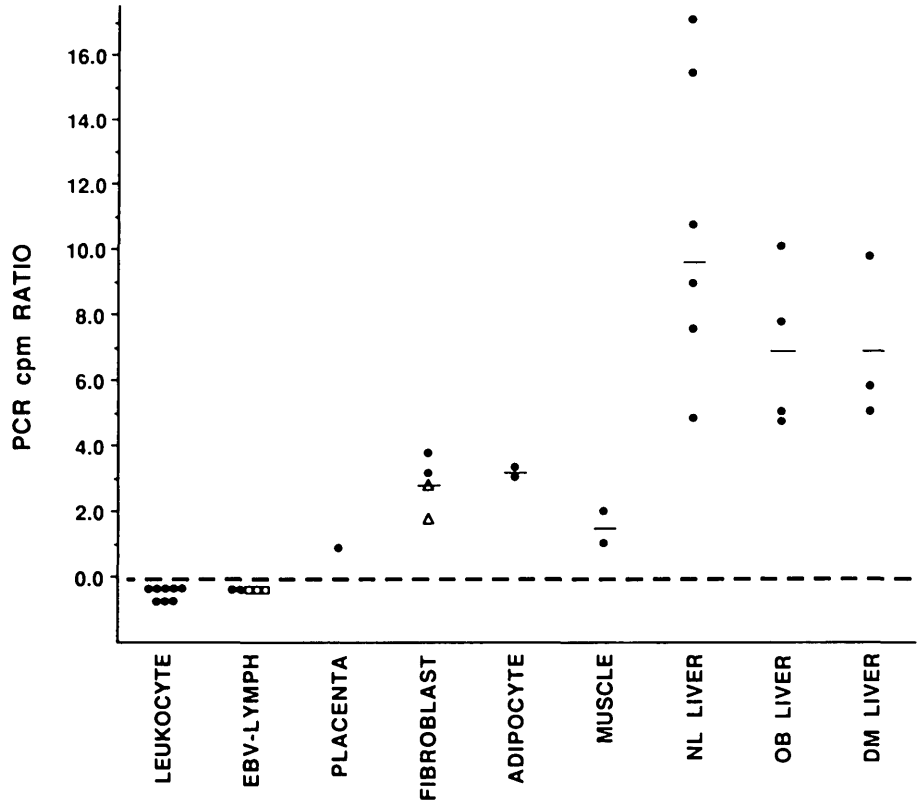


**Fig. 4.** Expression of Two Alternatively Spliced Insulin Receptor mRNA Species in Human Tissues

The relative abundance of the Ex11+ *vs.* the Ex11− cDNA fragments after PCR is shown for each tissue. RNA was prepared with tissue from one (placenta) or more subjects. Five to ten micrograms of total RNA were used for cDNA and PCR reactions. Results with buffy coat leukocytes (eight normal subjects) and EBV-transformed lymphocytes [two normal subjects (●) and 3 Pima Indian diabetic subjects (□)] are depicted as ratios of less than or equal to 0 to indicate that only the Ex11− species was detected (by ethidium staining and Southern blotting). For tissues where both the Ex11+ and Ex11− species were detected, incorporation of [$\alpha^{32}$P]dCTP into the two cDNA PCR-product fragments was measured (each point is the mean PCR count per min ratio of three separate cDNA/PCR reactions for each sample). Mean values for each group are noted by *horizontal lines*. For fibroblast there are four subjects, two normals (●) and two type A patients (△). Liver samples are from three subject groups, lean normals (NL), obese nondiabetics (OB), and obese diabetics (DM).

Sciences Human Genetic Mutant Cell Respository (Camden, NJ). Isolated human adipocytes were provided by Donald C. Simonson (Joslin Diabetes Center, Boston, MA) after preparation by standard methods (43). Cultured fibroblasts from one insulin-resistant patient ($A_2$) were provided by Fredda Ginsberg-Fellner (Mount Sinai School of Medicine, New York, NY). Human placenta was obtained after caesarian section in a normal subject. Normal human skeletal muscle (abdominal wall) and human liver from lean normal, obese nondiabetic and obese NIDDM subjects was obtained during elective surgery as previously described (27, 28) and frozen at −70° C until analyzed. A Chinese hamster ovary (CHO) cell line (CHO-IR) overexpressing the normal exon 11+ insulin receptor cDNA sequence of Ebina *et al.* (4, 23) was provided by William J. Rutter (University of California, San Francisco, CA). A cell line (CHO-HIRC) overexpressing the normal exon 11− insulin receptor cDNA sequence of Ullrich *et al.* (3, 24) was prepared and provided by Morris F. White (Joslin Diabetes Center) using plasmid cDNA provided by Axel Ullrich (Genentech, Inc., San Francisco, CA). CHO cell lines were maintained in culture with G418-sulfate (Geneticin, GIBCO, Grand Island, NY). Total cellular RNA was prepared by solubilizing cultured cells or rapid homogenization of fresh and frozen tissues in 4 M guanidine isothiocyanate containing 25 mM sodium citrate (pH 7.0), 100 mM β-mercaptoethanol and 17 mM *N*-laurylsarcosine followed by centrifugation through a 5.7 M CsCl cushion and ethanol precipitation (44).

### Oligonucleotide Primers and cDNA Synthesis

Two 25-base primers, 5′-TCCTGCAGTTGGACGGTGGTAGACA -3′(A) and 5′-CCGAATTCGTGGGCACGCTGGTCGA -3′(B), complementary to the normal insulin receptor cDNA sequence, were synthesized on an Applied Biosystems 381A DNA synthesizer by the methoxyphosphormadite method (45). Primers A and B flank a 721 (Ex11+) or 685 (Ex11−) base region of insulin receptor mRNA/cDNA as depicted in Fig. 1. Specific first strand cDNA copies of insulin receptor mRNA in this region were synthesized in a 10-μl reaction volume containing 5–10 μg total RNA, 0.5 μM oligonucleotide primer B, 10 U RNAsin (Promega Biotec, Madison, WI), and 200 U M-MLV reverse transcriptase (Bethesda Research Laboratories, Gaithersburg, MD), under conditions suggested by the manufacturer (except that actinomycin D was omitted).

### Amplification of Insulin Receptor cDNA

The entire 10-μl cDNA synthesis reaction volume was combined in a 50-μl final reaction volume for PCR amplification containing 0.25 μM each oligonucleotide primer (A and B) and

1.5 U *Thermus acquaticus* DNA polymerase (Taq Polymerase, Perkin-Elmer Cetus). In experiments where radioactive dCTP incorporation was analyzed, 30 $\mu$Ci [$\alpha$-$^{32}$P]dCTP (3000 Ci/mmol, DuPont-New England Nuclear, Boston, MA) was added to the reaction mixture. Other conditions are as previously described (7). Forty cycles of PCR amplification were performed using a DNA thermal cycler (Perkin-Elmer Cetus). Each cycle consisted of 30-sec denaturation at 95 C, 30-sec annealing at 60 C, and 60 sec at 72 C for enzymatic extension. After DNA amplification the PCR mixture was digested with RNAse A (Sigma, St. Louis, MO) at 0.1 mg/ml for 15 min at 37 C followed by phenol-chloroform extraction and ethanol precipitation. Insulin receptor PCR-product DNA (50–75% of each sample) was then electrophoresed in 1.8% agarose gels under nondenaturing conditions and visualized after ethidium bromide staining by UV fluorescence (13). In one experiment the PCR-product DNA was digested with the restriction enzyme *BAN*1 (New England Biolabs, Beverly, MA) before gel electrophoresis.

### Subcloning and Sequencing of PCR Product

Amplified DNA fragments were recovered from agarose gels by electroelution, precipitated with ethanol, digested with *PST*I (site in primer A) and *ECO*RI (site in primer B) before subcloning into M13 (mp 18 and mp 19). Single stranded phage DNA was sequenced by the dideoxy-chain termination method (13).

### Quantitation of PCR-Amplified Insulin Receptor cDNA Species

Coamplification of RNA derived from cell lines CHO-HIRC and CHO-IR was performed as a control for experiments with human tissues. The abundance of insulin receptor mRNA expressed by each of the two control cell lines was assessed by Northern blotting and slot-blot analysis (Hybri-slot manifold, Bethesda Research Laboratories) of RNA samples performed at several dilutions by standard methods (13). These RNAs were then combined together at several fixed ratios of insulin receptor mRNA abundance and coamplified after cosynthesis of cDNA. PCR-product gels were Southern blotted using standard methods (13). A 4187 bp *ECO*RI insulin receptor cDNA fragment (plasmid provided by Ora Rosen, Memorial-Sloan Kettering Cancer Center, New York, NY) was labeled with $^{32}$P by random priming and used to hybridize with both RNA and DNA blots with stringent hybridization (42 C, 50% formamide) and washing (0.25 ×SSC, 68 C) conditions (13) followed by autoradiography. Quantification of [$\alpha$-$^{32}$P]dCTP incorporation into Ex11+ *vs.* Ex11− insulin receptor cDNA PCR-products was accomplished in the following way for both control cell line samples and human tissue samples: both bands were visualized and carefully excised after adequate electrophoretic separation; radioactivity was determined after subtraction of background counts present in comparably sized gel slices.

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## REFERENCES

1. Goldfine ID 1987 The insulin receptor: molecular biology and transmembrane signaling. Endocr Rev 8:235–255
2. Kahn CR and White MF 1988 The insulin receptor and the molecular mechanism of insulin action. J Clin Invest 82:1151–1156
3. Ullrich A, Bell JR, Chen EY, Herrera R, Petruzzelli LM, Dull TJ, Gray A, Coussens L, Liao YC, Tsubokawa M, Mason M, Seeburg PH, Grunfeld C, Rosen OM, Ramachandran J 1985 Human insulin receptor and its relationship to the tyrosine kinase family of oncogenes. Nature 313:756–761
4. Ebina Y, Ellis L, Jarnagin K, Edery M, Graf L, Clauser E, Ou J-H, Masiarz F, Kan YW, Goldfine ID, Roth RA, Rutter WJ 1985 The human insulin receptor cDNA: the structural basis for hormone-activated transmembrane signaling. Cell 40:747–758
5. Yang-Feng TL, Francke U, Ullrich A 1985 Gene for human insulin receptor: localization to a site on chromosome 19 involved in pre-B-cell leukemia. Science 228:728–730
6. Seino S, Seino M, Nishi S, Bell GI 1989 Structure of the human insulin receptor gene and characterization of its promoter. Proc Natl Acad Sci USA, 86:114–118
7. Veres G, Gibbs RA, Scherer SE, Caskey TC 1987 The molecular basis of the sparse fur mouse mutation. Science 237:415–417
8. Saiki RK, Gelfand DW, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, Erlich HA 1988 Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239:487–494
9. Goldstein BJ, Muller-Wieland D, Kahn CR 1987 Variation in insulin receptor messenger ribonucleic acid expression in human and rodent tissues. Mol Endocrinol 1:759–766
10. MacDonald AR, Maddux BA, Okabayashi Y, Wong KY, Hawley DM, Logsdon CD, Goldfine ID 1987 Regulation of insulin-receptor mRNA levels by glucocorticoids. Diabetes 36:779–781
11. Moller DE, Flier JS 1988 Detection of an alteration in the insulin-receptor gene in a patient with insulin resistance, acanthosis nigricans, and the polycystic ovary syndrome (type A insulin resistance). New Engl J Med 319:1526–1530
12. Andreadis A, Gallego ME, Nadal-Ginard B 1987 Generation of protein isoform diversity by alternative splicing: mechanistic and biological implications. Annu Rev Cell Biol 3:207–242
13. Maniatis T, Fritsch EF, Sambrook J 1982 Molecular Cloning, A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York
14. Goodwin RG, Rottman FM, Callaghan T, Kung HJ, Maroney PA, Nilsen TW 1986 C-erbB activation in avian leukosis virus-induced erythroblastosis: multiple epidermal growth factor receptor mRNAs are generated by alternative RNA processing. Mol Cell Biol 6:3128–3133
15. Schejter ED, Segal D, Glazer L, Shilo BZ 1986 Alternative

5′ exons and tissue-specific expression of the Drosophila EGF receptor homolog transcripts. Cell 46:1091–1101
16. Rappolee DA, Mark D, Banda MJ, Werb Z 1988 Wound macrophages express TGF-$\alpha$ and other growth factors *in vivo*: analysis by mRNA phenotyping. Science 241:708–712
17. Chelly J, Kaplan JC, Marie P, Gautron S, Kahn A 1988 Transcription of the dystrophin gene in human muscle and non-muscle tissues. Nature 333:858–860
18. Rappolee DA, Brenner CA, Schultz R, Mark D, Werb Z 1988 Developmental expression of PDGF, TGF-$\alpha$, and TGF-$\beta$ genes in preimplantation mouse embryos. Science 241:1823–1825
19. McPhaul M, Berg P 1987 Identification and characterization of cDNA clones encoding two homologous proteins that are part of the asialoglyprotein receptor. Mol Cell Biol 7:1841–1847
20. Behlke MA, Loh DY 1986 Alternative splicing of murine T-cell receptor beta-chain transcripts. Nature 322:379–382
21. Breitbart RE, Nguyen HT, Medford RM, Destree AT, Mahdavi V, Nadal-Ginard B 1985 Intricate combinatorial patterns of exon splicing generate multiple regulated troponin T isoforms from a single gene. Cell 41:67–82
22. Izumo S, Mahdavi V 1988 Thyroid hormone receptor $\alpha$ isoforms generated by alternative splicing differentially activate myosin HC gene transcription. Nature 334:593–595
23. Ebina Y, Araki E, Taira M, Shimada F, Masataka M, Craik CS, Siddle K, Pierce SB, Roth RA, Rutter WJ 1987 Replacement of lysine residue 1030 in the putative ATP-binding region of the insulin receptor abolishes insulin- and antibody-stimulated glucose uptake and receptor kinase activity. Proc Natl Acad Sci USA 84:704–708
24. Chou CK, Dull TJ, Russell DS, Gherzi R, Lebwohl D, Ullrich A, Rosen OM 1887 Human insulin receptors mutated at the ATP-binding site lack protein tyrosine activity and fail to mediate postreceptor effects of insulin. J Biol Chem 262:1842–1847
25. Ebina Y, Edery M, Ellis L, Standring D, Beaudoin J, Roth RA, Rutter WJ 1985 Expression of a functional human insulin receptor from a cloned cDNA in Chinese hamster ovary cells. Proc Natl Acad Sci USA 2:8014–8018
26. Whittaker J, Okamoto AK, Thys R, Bell GI, Steiner DF, Hofmann CA 1987 High-level expression of human insulin receptor cDNA in mouse NIH 3T3 cells. Proc Natl Acad Sci USA 84:5237–5241
27. Caro JF, Sinha MK, Raju SM, Itoop O, Pories WJ, Flickinger EG, Meelheim D, Dohm GL 1987 Insulin receptor kinase in human skeletal muscle from obese subjects with and without noninsulin dependent diabetes. J Clin Invest 79:1330–1337
28. Caro JF, Itoop O, Pries WJ, Meelheim D, Flickinger EG, Thomas F, Jenquin M, Silverman JF, Khazanie PG, Sinha MK 1986 Studies on the mechanism of insulin resistance in the liver from humans with noninsulin dependent diabetes. J Clin Invest 78:249–258
29. Flier JS 1983 Insulin receptors and insulin resistance. Annu Rev Med 34:145–160
30. Grigorescu F, Flier JS, Kahn CR 1986 Characterization of binding and phosphorylation defects of erythrocyte insulin receptors in the type A syndrome of insulin resistance. Diabetes 35:127–128
31. Yoshimasa Y, Seino S, Whittaker J, Kakehi T, Kosaki A, Kuzuya H, Imura H, Bell GI, Steiner DF 1988 Insulin-resistant diabetes due to a point mutation that prevents insulin proreceptor processing. Science 240:784–787
32. Kadowaki T, Bevins CL, Cama A, Ojamaa K, Marcus-Samuels B, Kadowaki H, Beitz L, McKeon C, Taylor SI 1988 Two mutant alleles of the insulin receptor gene in a patient with extreme insulin resistance. Science 240:787–790
33. Myerowitz R 1988 Splice junction mutation in some Ashkenazi Jews with Tay-Sachs disease: evidence against a single defect within this ethnic group. Proc Natl Acad Sci USA 85:3955–3959
34. Mott DM, Howard BV, Savage PJ, Nagulesparan M 1983 Altered insulin binding to monocytes and diploid fibroblasts from obese donors. J Clin Endocrinol Metab 57:1–7
35. Heidenreich KA, Zahniser NR, Berhanu P, Brandenburg D, Olefsky JM 1983 Structural differences between insulin receptors in the brain and peripheral target tissues. J Biol Chem 258:8527–8530
36. Caro JF, Raju SM, Sinha MK, Goldfine ID, Dohm GL 1988 Heterogeneity of human liver, muscle, and adipose tissue insulin receptor. Biochem Biophys Res Comm 151:123–129
37. Burant CF, Treutelaar MK, Block NE, Buse MG 1986 Structural differences between liver and muscle-derived insulin receptors in rats. J Biol Chem 261:14361–14364
38. James DE, Zorzano A, Bon-Schnetzler M, Nemenoff RA, Powers A, Pilch PF, Ruderman NB 1986 Intrinsic differences of insulin receptor kinase activity in red and white muscle. J Biol Chem 261:14939–14944
39. Jonas HA, Newman JD, Harrison LC 1986 An atypical insulin receptor with high affinity for insulin-like growth factors copurified with placental insulin receptors. Proc Natl Acad Sci USA 83:4124–4128
40. Gammeltoft S, Kristensen LO, Sestoft L 1978 Insulin receptors in isolated rat hepatocytes: reassessment of binding properties and observations on the inactivation of insulin at 37C. J Biol Chem 253:8406–8413
41. King GL, Kahn CR, Rechler MM, Nissley SP 1980 Direct demonstration of separate receptors for growth and metabolic activities of insulin and multiplication-stimulating activity (an insulinlike growth factor) using antibodies to the insulin receptor. J Clin Invest 66:130–140
42. Taylor SI, Roth RA, Blizzard RM, Elders MJ 1981 Qualitative abnormalities in insulin binding in a patient with extreme insulin resistance: decreased sensitivity to alterations in temperature and pH. Proc Natl Acad Sci USA 78:7157–7161
43. Rodbell M 1964 Metabolism of isolated fat cells. J Biol Chem 239:375–380
44. Chirgwin JM, Przybyla AW, MacDonald RJ, Rutter WJ 1979 Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry 18:5294–5299
45. Beaucage SL, Caruthers MH 1981 Deoxynucleoside phosphormidites—a new class of key intermediates for deoxypolynucleotide synthesis. Tetrahedron Lett 22: 1859–1862