that a newly activated oncogene could convert an E_2-responsive tumor cell to one that is hormonally unresponsive; this change could provide a selective growth advantage to the E_2-unresponsive cell and eventually result in the grossly apparent autonomy of the tumor phenotype. An analogous situation may have been described in a melanoma patient, where one metastatic tumor had an activated ras^N gene, but the other metastases did not (*21*). We do not propose that *ras* activation is the only mechanism by which a breast cancer might alter its hormone dependent phenotype. We conclude, however, that amplification or mutation in a single gene may radically alter the growth regulatory and tumorigenic behavior of a cell that already has malignant potential.

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Gene for Human Insulin Receptor: Localization to Site on Chromosome 19 Involved in Pre-B-Cell Leukemia

Abstract. *Consistent chromosomal translocations in neoplastic cells may alter the expression of proto-oncogenes that are located near the breakpoints. The complementary DNA sequence of the human insulin receptor is similar to those of the EGF receptor (*erbB *oncogene) and products of the* src *family of oncogenes. With in situ hybridization and Southern blot analysis of somatic cell hybrid DNA, the human insulin receptor gene was mapped to the distal short arm of chromosome 19 (bands p13.2→p13.3), a site involved in a nonrandom translocation in pre-B-cell acute leukemia.*

Insulin initiates its biological effects by binding to specific membrane receptors on target cells (*1*). The mature human insulin receptor is a transmembrane glycoprotein comprised of two α subunits (molecular weight of ~130,000) and two β subunits (molecular weight of ~90,000) (*2*). The α subunit contains the insulin binding site (*3*). The β subunit, bearing an adenosine triphosphate (ATP)–binding site, possesses a tyrosine protein kinase activity (*4*) that catalyzes both the autophosphorylation of the β subunit and the insulin-dependent phosphorylation of exogenous protein substrates (*5*). The α and β subunits of the mature insulin receptor are synthesized as a single polypeptide chain (*6*). A complementary DNA (cDNA) for the precursor of the human insulin receptor has been isolated and characterized (*7*). We have now mapped the insulin receptor gene (*INSR*) by in situ hybridization and Southern blot analysis of rodent × human somatic cell hybrid lines.

In situ hybridization to human chromosome preparations was carried out with a ^{3}H-labeled subclone (pHP/HIR 12.1) containing a 4.2-kilobase (kb) insert fragment that includes the entire β- and

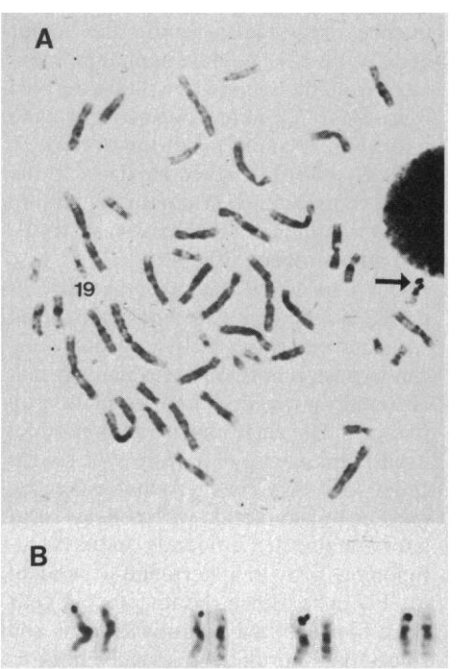

Fig. 1. G-banded human chromosomes after in situ hybridization with ^{3}H-labeled *INSR* probe. Human metaphase and prometaphase chromosomes were prepared from methotrexate-synchronized peripheral blood cell cultures (*25*) of two normal individuals. The pHP/HIR 12.1 was ^{3}H-labeled to a specific activity of 1.4×10^7 cpm/μg by nick-translation with [^{3}H]dCTP, [^{3}H]dATP, and [^{3}H]dTTP. In situ hybridization was carried out according to the method of Harper and Saunders (*26*). The probe was hybridized to chromosome preparations at a concentration of 25 ng/ml and 50 ng/ml for 15 hours at 37°C. The slides were exposed to Kodak NTB-2 emulsion for 10 to 14 days at 4°C. After autoradiographic development, the chromosomes were stained with quinacrine mustard dihydrochloride and photographed under a fluorescence microscope. The chromosomes were then G-banded with Wright's stain and a second photograph was taken of the previously selected cells. G-banded chromosomes were analyzed for silver grain localization. (A) Representative normal female metaphase spread with silver grains on the short arm of chromosome 19 (arrow) and low background labeling. The number 19 is directly below the unlabeled chromosome 19. (B) Pairs of chromosome 19 from 4 prometaphase cells, illustrating typical labeling at the distal short arm (bands p13.2→p13.3).

most of the α-subunit coding regions and 3′ untranslated sequences of the precursor gene for the human insulin receptor (*7*). A high percentage of metaphase cells (49 percent) had silver grains on bands 19p13.2→p13.3 of at least one chromosome 19 (Fig. 1). Of 70 grains observed on chromosome 19, 63 (90 percent) were located on this region, with a peak in the most distal sub-band 19p13.3 (Fig. 2A). Furthermore, grains over this specific site (19p13.2→p13.3) represented 26 percent (63/243) of all chromosomal label, and no other sites were labeled above background (Fig. 2B). We conclude that *INSR* can be assigned to the distal short arm of chromosome 19, sub-bands 19p13.2→p13.3.

Table 1. Human chromosome content and human *INSR* restriction fragments in five selected Chinese hamster × human hybrids. The 3′-probe contained 857 base pairs of 3′-untranslated sequence of pHP/HIR 12.1. L, chromosome present at a frequency less than 10 percent; data excluded from analysis. R, chromosome rearranged; data excluded from analysis.

Somatic cell hybrids	Human chromosomes																								Hybridization to pHP/HIR 12.1 3′-probe	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y		
XVIII-13A-2d	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	-	+	-	+	+	+	+	-	-	-
XVIII-54A-1a	-	-	+	-	-	-	-	-	-	-	+	+	L	+	-	-	-	-	+	+	+	-	+	-	+	+
XV-15A-4a	R	+	+	+	-	R	+	+	-	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	-	-
XVII-10A-12a	-	-	+	R	-	+	+	-	-	+	+	+	-	-	+	-	-	-	-	+	+	+	+	-	-	-
XVIII-23H-a	-	-	-	+	-	+	-	R	-	-	-	-	+	+	-	+	-	-	+	-	+	+	+	-	+	+
Discordancy ratio	3/4	4/5	4/5	3/4	3/5	3/4	4/5	3/4	3/5	5/5	4/5	4/5	2/4	2/5	3/5	3/5	3/5	4/5	0/5	4/5	3/5	4/5	3/5	3/5		

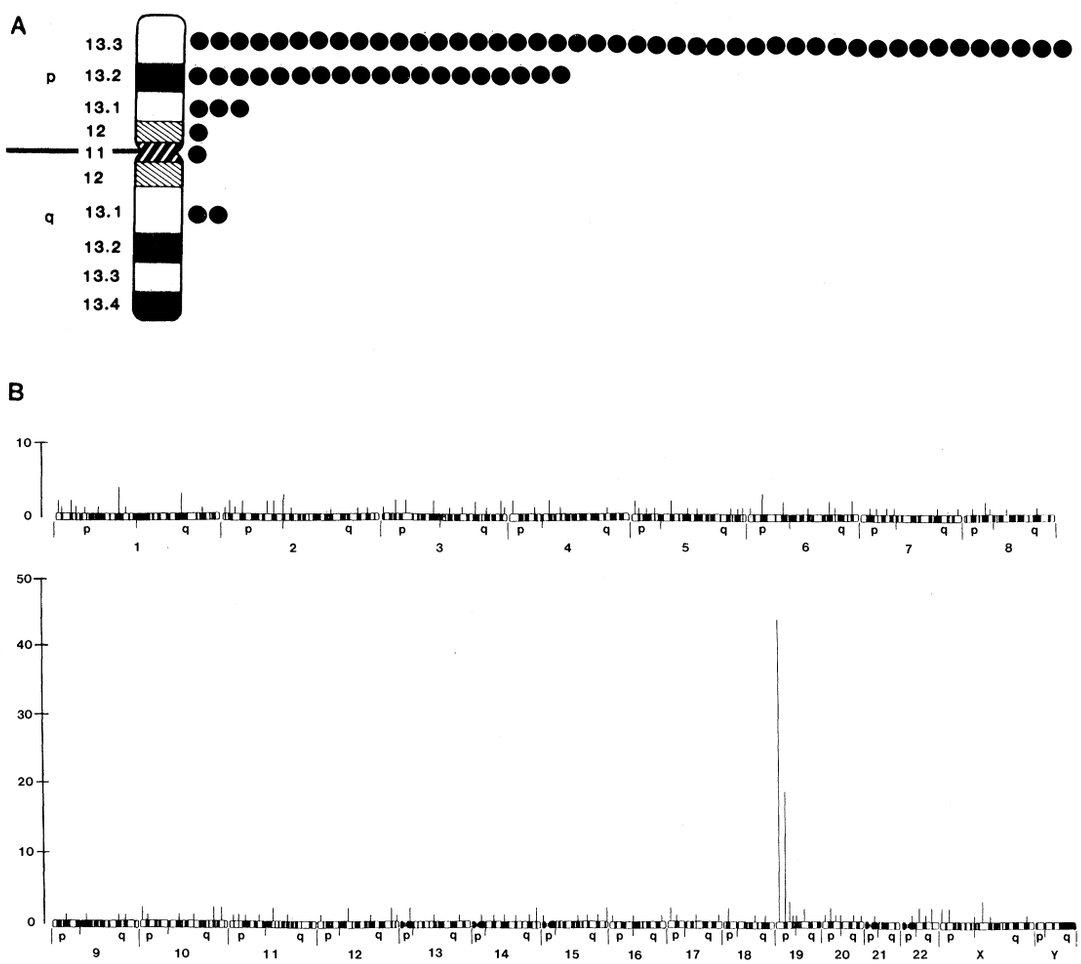

Fig. 2. (A) Silver grain distribution along chromosome 19 [ideogram according to ISCN 1981 (*27*)]. (B) Histogram showing the distribution of silver grains over the human chromosome complement. Of 243 grains observed in 108 metaphase cells, 63 (26 percent) were located at 19p13.2→p13.3.

The chromosomal assignment of *INSR* made by in situ hybridization was confirmed by Southern blot analysis of DNA extracted from five human × Chinese hamster somatic cell lines selected from a large collection of hybrids. The ^{32}P-labeled human insulin receptor sequence (pHP/HIR 12.1) was hybridized to DNA samples that had been digested with Hind III and Eco RI. Eight hybridizing bands were detected in human DNA (Fig. 3A, lane 1) and one major cross-hybridizing band was present in Chinese hamster DNA (Fig. 3A, lane 2). All seven human DNA bands that could clearly be distinguished from the Chinese hamster DNA bands were present in hybrids containing human chromosome 19 (Fig. 3A, lanes 4 and 7) and absent in hybrids lacking this chromosome (Fig. 3A, lanes 3, 5, and 6). To determine the number of the sequences recognized by pHP/HIR 12.1, we hybridized a Pst I–Eco RI subfragment of the probe, consisting of 857 base pairs of 3′ untranslated sequence to the same set of DNA's. This subfragment hybridized to a single 5.9-kb band in human DNA (Fig. 3B, lane 1) and in hybrids containing human chromosome 19 (Fig. 3B, lanes 4 and 7), consistent with the presence of a single gene for human insulin receptor. Table 1 summarizes the chromosome content of the five hybrids and the discordancy analysis; chromosome 19 is the only chromosome with no discordancies observed. These data provide independent confirmation for the mapping of the single human insulin receptor gene to chromosome 19.

Insulin exerts its biological effects by interacting with its specific cell surface receptors, which generate a signal that activates a cascade of cellular events (*1*). Defects in any of these processes could affect carbohydrate homeostasis. A receptor defect in diabetes was first suggested by Vinik *et al.* (*8*). Insulin resistance-associated clinical conditions, resulting from prereceptor, receptor, or postreceptor defects, have been identified (*9*). Receptor defects that alter the responsiveness to insulin because of a decrease in the number of receptors, have been observed in obesity (*10*) and type A acanthosis nigricans (*9*). Postreceptor abnormalities may be due to defects in the generation of a second messenger or messengers involved in the complex events of insulin action in target tissues and have been reported in obesity and leprechaunism (*10, 11*).

As the direct metabolic consequences of the insulin-receptor interaction are not well established, it is difficult to dissect the exact causes of insulin-resistant clinical conditions. Multiple etiologies and genetic heterogeneity are likely to play a role (*12*). The precise localization of the human insulin receptor gene should prove valuable in the diagnosis of specific disorders associated with receptor defects and in elucidating the role of the receptor in the pathogenesis of insulin resistance in clinical conditions. Furthermore, DNA restriction fragment length polymorphisms, if detected with the insulin receptor sequence, will be useful markers for genetic linkage analysis. They could add valuable information to the genetic map of chromosome 19, particularly as part of the large chromosome 19 linkage group which includes the complement component C3, apolipoproteins CII and E, and the LDL receptor loci (*13–16*).

Our assignment of *INSR* places this locus near the breakpoint of a specific chromosome rearrangement, translocation t(1;19)(q23;p13.3) found in pre-B-cell leukemia (*17*). This form of acute lymphoblastic leukemia in childhood involves cells of the B-cell lineage that are capable of producing cytoplasmic, but not cell-surface, immunoglobulin heavy chains (*18*). Pre-B-cell acute lymphoblastic leukemia responds poorly to treatment (*19*). The t(1;19) translocation was discovered independently by several groups within the past year. Two of the reports suggest that the distal long arm of chromosome 19 is involved, but there was difficulty in distinguishing between the chromosome arms given the symmetry of this small chromosome (*20*). We agree with Williams and colleagues (*17*) that the specific breakpoint is located in 19p13. Moreover, the t(11;19) translocation found in a patient with acute monocytic leukemia type M5a also involved a breakpoint in the region 19p13 (*21*).

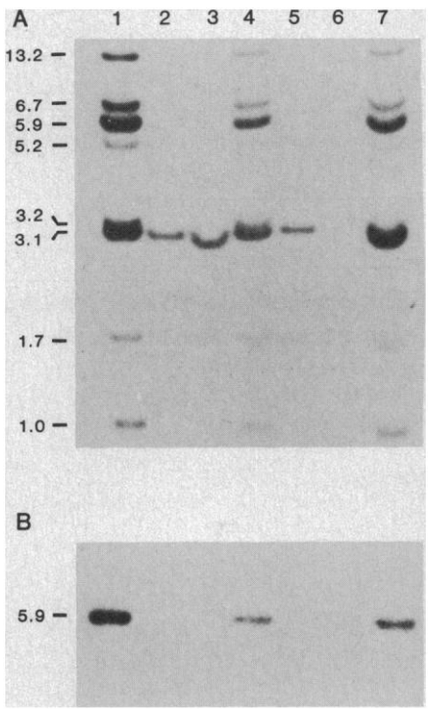

Fig. 3. Hybridization of human insulin receptor cDNA to human × Chinese hamster hybrid cell DNA's. Cell lines were derived from three different fusions as described (*28*). DNA (10 μg) that had been digested with Hind III and Eco RI was applied to all lanes except lane 6 which contained approximately 5 μg. Southern blots were performed according to standard procedures (*29*). (Lane 1) human cell line; (lane 2) Chinese hamster cell line; (lanes 4 and 7) hybrid cell lines containing human chromosome 19; (lanes 3, 5, and 6) hybrid cell lines missing human chromosome 19. Chinese hamster bands became visible in lane 6 on longer exposure. (A) Hybridization of ^{32}P-labeled nick-translated (*30*) pHP/HIR 12.1 to human × Chinese hamster hybrid cell DNA's. (B) Hybridization of ^{32}P-oligolabeled (*31*) subfragment of pHP/HIR 12.1 (3′-untranslated sequence) to a filter containing the same hybrids as Fig. 3A.

In other forms of human hematologic malignancies, consistent translocations can lead to the activation of cellular proto-oncogenes, such as c-*abl* in chronic myelocytic leukemia (*22*) and c-*myc* in Burkitt lymphoma (*23*). Furthermore, the discovery of consistent chromosome translocations in certain types of leukemia has led some investigators to postulate the existence of as yet unidentified cellular proto-oncogenes at the sites of the breakpoints (*bcl-1* on chromosome 11, *bcl-2* on chromosome 18, and *tcl-1* on chromosome 14) (*24*). The possibility that the insulin receptor could function as an oncogene is suggested by the structural and sequence homologies between the *INSR* β-subunit cytoplasmic domain and other tyrosine-specific protein kinases coded for by *src* gene family members (*7*). Chromosome rearrangements such as the t(1;19) that involve *INSR* may alter its expression and contribute to malignant transformation or cell proliferation. This hypothesis can be tested by looking for *INSR* sequence rearrangements in pre-B-cell leukemic cells that contain the t(1;19) translocation.

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Recurring Origins of Allopolyploid Species in *Asplenium*

Abstract. *A large proportion of plant species has originated through allopolyploidy: interspecific hybridization followed by chromosome doubling. Heterozygosity remains fixed in allopolyploids because of nonsegregation of parental chromosomes. Two allotetraploid species of the fern genus* Asplenium *show allozyme polymorphisms at loci that are polymorphic in their diploid progenitors, indicating that each has originated more than once and implicating continued gene flow from diploids to tetraploids.*

Allopolyploidization is a prevalent mode of speciation in plants (*1*). In contrast to speciation through divergence at a diploid level, which requires numerous generations (*2*), allopolyploids form abruptly through interspecific hybridizations and subsequent doubling of chromosomes (*3*). Reproductive isolation from progenitors is effected instantaneously through differences in ploidy. If well-differentiated species hybridize, resulting allopolyploids have fixed heterozygous genotypes at many loci because of nonsegregation of nonhomologous chromosomes (*4, 5*). In this respect the population genetic structure of allopolyploids is similar to that of apomictic species, which are often of hybrid origin as well. Apomixis is an alternative reproductive strategy for a genetically favorable but meiotically impaired hybrid (*6*). The retention of sexuality in an allopolyploid, however, is important in that it favors increased diversity in the gene pool of the allopolyploid species. Our alloyzme data on the Appalachian *Asplenium* fern complex show that a major source of genetic diversity in allopolyploids can be the recurrence of allopolyploidization events.

The hybridization that launches an allopolyploid species is often recognizable many generations later by the fixed genotype that results. Parentages of numerous allopolyploids have been described throughout the world's floras. Perhaps there are no firmer data than those on the Appalachian-Ozarkian species of *Asplenium* in which the presumed parentages of three allotetraploid species, as originally proposed on morphological grounds (*7*), have been corroborated by data from flavonoids (*8*) and allozymes (*5*). One of the allotetraploids, *A. ebenoides* (*platyneuron* × *rhizophyllum*) is known from a single locality in Alabama, although its analogous sterile F_1 allodiploid is found at numerous localities where it occurs with its relatively common parents. In contrast, the other two allotetraploids, *A. bradleyi* (*montanum* × *platyneuron*) and *A. pinnatifidum* (*montanum* × *rhizophyllum*), are wide ranging and roughly coterminous although somewhat local. Their ranges extend considerably westward of that of their mutual diploid progenitor *A. montanum*, which is nearly restricted to the Appalachian provinces. Both of these allotetraploids occur in habitats quite similar to that of *A. montanum*—that is, in siliceous, partially shaded rock outcrops. Only a single sterile allodiploid *A. bradleyi* plant has been reported (*9*), and *A. pinnatifidum* is known only as an allotetraploid. Thus it would appear that these hybrids are less readily formed than *A. ebenoides*. However, our allozyme data indicate that both *A. bradleyi* and *A. pinnatifidum* have originated repeatedly.

Electrophoretic data phenotypes for nine enzyme loci from several populations of all six species of the complex (Tables 1, 2, and 3) (*10*) show that two loci (*GDH* and *6-PGDH*) are invariable for all species; two others (*ACPH* and *LAP*) vary among, but not within, diploid species; and the other five (*GOT-1, IDH, PGI-2, PGM-2*, and *SKDH*) show intraspecific variability in the diploids (Table 1) (*11*). *Asplenium montanum* has a duplicated *PGM-2* locus, as judged from populations in which all individuals have two bands for *PGM-2* (*12*). Other populations contain individuals with single bands, indicating either a lack of this duplication or, more probably, possession of electrophoretically identical alleles at both loci.

The allotetraploids *A. bradleyi* and *A. pinnatifidum* are generally heterozygous for alleles present in their respective diploid progenitors (Tables 2 and 3) (*13*). Two types of variation in genotype are evident. Some individuals, or entire populations, express only one parental allele at one or two loci (for example, *LAP* in *A. pinnatifidum* from Chester County, Pennsylvania), whereas the rest of the loci conform to the expected hybrid genotype. This loss of gene expression may be a derived condition resulting from mutation (*14*), rare recombinational events (*15*), or regulational silencing (*16*).

Alleles for which the allopolyploids are heterozygous show another type of variation. For loci that are invariant within the diploid progenitors, the allotetraploids uniformly express the same alleles as are fixed in the diploids (*17*). However, four of the five loci that are variable in the diploids are also variable in both allotetraploids (Tables 2 and 3). Genotypic differences exist within some and between most allotetraploid populations. The alleles that show this variation are electrophoretically identical to those detected in the diploids with the follow-