21), isolated hepatic membrane receptor studies (*5–7, 10, 22, 23*), and lipoprotein metabolism in cultured hepatic cells (*24*) point to an elaborate apolipoprotein recognition system in mammalian liver. The LDL receptor on canine hepatic membranes and the human fibroblast LDL receptor undergo rapid regulation by perturbations in cholesterol and bile acid metabolism (*5–7, 19*). Specific apoE recognition sites, however, did not appear to undergo similar modulation (*7, 19*). In addition, a pathway in liver that is distinct from the LDL and apoE receptor and that may have a broad specificity for lipoprotein recognition has been proposed (*10, 24, 25*). The mammalian liver, therefore, contains numerous pathways by which lipids and lipoproteins may be metabolized.

The genetic and physiologic roles of these different hepatic receptors were investigated in human subjects by studying isolated hepatic membranes from normal individuals, from a patient with FH who lacked the fibroblast LDL receptor, and from a patient with abetalipoproteinemia who had no circulating LDL lipoprotein particles. Despite the absence of the usual fibroblast LDL receptor, low levels of residual LDL-specific binding were observed in hepatic membranes from the FH patient (Fig. 1) (*10*). Of particular importance, however, was the observation that apoE binding was normal. The residual apoB binding to these membranes may reflect binding to receptors with broad specificity or a cross-recognition with the apoE receptor; this possibility is supported by the 47 percent increase in both apoE and apoB binding to membranes obtained from the FH patient after portacaval shunt surgery (Fig. 2). The apoE binding, however, was not only genetically distinct from LDL binding but was also regulated upward after portacaval shunt surgery.

Hepatic membrane studies in abetalipoproteinemia also support the concept of distinct LDL and apoE receptors in humans. The absence of plasma apoB lipoprotein particles in this disorder was associated with a reciprocal increase in LDL receptor expression and a reduction in the expression of the apoE receptors on isolated hepatic membranes (Fig. 1). Blum *et al.* (*26*) and Innerarity *et al.* (*27*) proposed that the increased apoE lipid-rich particles in abetalipoproteinemia lead to normalized lipid transport through an apoE-mediated uptake process. Our study supports the concept that the lipid-rich apoE particles are recognized by human hepatic membranes independent of an LDL receptor. Not only is the regulation of the LDL and apoE receptors independent, the apoE receptor pathway may in some way lead to normalization of cholesterol homeostasis in abetalipoproteinemia (*26–30*).

These combined results indicate that hepatic receptors for LDL and apoE are present in humans. The LDL receptor, as was reported in earlier mammalian studies, undergoes regulation. However, the hepatic apoE receptor in humans, unlike that in other species, can also be modulated. Therefore, human hepatic receptors for LDL and apoE-containing lipoproteins are physiologically and genetically distinct.

JEFFREY M. HOEG
STEPHEN J. DEMOSKY, JR.
RICHARD E. GREGG
ERNST J. SCHAEFER
H. BRYAN BREWER, JR.

Molecular Disease Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland 20205

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Immunoprecipitation of Insulin Receptors from Cultured Human Lymphocytes (IM-9 Cells) by Antibodies to pp60^{src}

Abstract. *The family of tyrosine-specific protein kinases includes proteins encoded by retroviral oncogenes as well as receptors for insulin and several growth factors. Antibodies to pp60^{src}, the protein encoded by the* src *oncogene of Rous sarcoma virus (RSV), can specifically immunoprecipitate affinity-labeled insulin receptors from cultured human lymphocytes (IM-9 cells). This precipitation is specifically inhibited by the* src *gene product purified from RSV-transformed rat cells. These observations provide evidence that there is structural homology between the insulin receptors and pp60^{src}.*

The product of the v-*erb* B oncogene of avian erythroblastosis virus is a truncated form of the epidermal growth factor (EGF) receptor (*1*). The v-*erb* B gene is a member of a family of retroviral oncogenes encoding tyrosine-specific protein kinases with structural homology to pp60^{src} (*2*), the protein encoded by the *src* oncogene of Rous sarcoma virus (RSV). Similarly, the EGF receptor and those for insulin, insulin-like growth factor I (IGF I), and platelet-derived growth factor belong to a family of cell surface receptors with ligand-stimulated, tyrosine-specific protein kinase activity (*3*). These observations have led to the hypothesis that the genes for this family of cell surface receptors may be proto-oncogenes corresponding to the retroviral oncogenes in the *src* family. We now show that antisera containing antibodies to pp60^{src} can immunoprecipitate affinity-labeled insulin receptors. Moreover, this precipitation can be competitively inhibited by the protein encoded by the *src* gene. These observations are consist-

ent with the hypothesis that there is structural homology between the insulin receptor and pp60^{src}.

Insulin receptors on human lymphoblasts (IM-9 cells) were labeled by the affinity–cross-linking technique of Pilch and Czech (*4*). After solubilization in Triton X-100, these insulin receptors (*5*) could be immunoprecipitated by tumor-bearing rabbit (TBR) serum (Fig. 1), a serum derived from newborn rabbits bearing tumors induced by injection with RSV (*6*) and which contains antibodies to pp60^{src} as well as other virally encoded proteins (*7, 8*). At dilutions of 1:10 and 1:20 (Fig. 1A, lanes a and b), TBR serum precipitated 17 percent and 11 percent, respectively, of the affinity-labeled insulin receptors in the detergent extract of the IM-9 cells (Fig. 1B) (*9*). This precipitation was specific in that normal rabbit serum did not precipitate the insulin receptor (Fig. 1A, lane e).

A highly purified preparation of the protein encoded by the *src* gene has been prepared from RSV-transformed rat cells (Schmidt-Ruppin normal rat kidney cells (*10*). This protein, p54, was used to assess the specificity of the immunoprecipitation reaction (Figs. 1 and 2). Half-maximum inhibition of precipitation was observed at a concentration of p54 of 10 to 30 ng per milliliter (Fig. 2), similar to the concentration reported earlier that inhibited precipitation of ^{32}P-labeled pp60^{src} by TBR serum (*10*). In contrast, the product of the *raf* oncogene (*11*) and several synthetic peptides related to the product (*12*) did not inhibit precipitation of the insulin receptor (Fig. 2). We confirmed that TBR serum could also specifically precipitate affinity-labeled insulin receptors that had been partially purified (~20-fold) by purification over wheat germ agglutinin-agarose. Finally, TBR serum did not precipitate ^{125}I-labeled insulin. This suggests that TBR serum precipitates receptors by binding to the receptor moiety rather than to the insulin moiety of the affinity-labeled receptor.

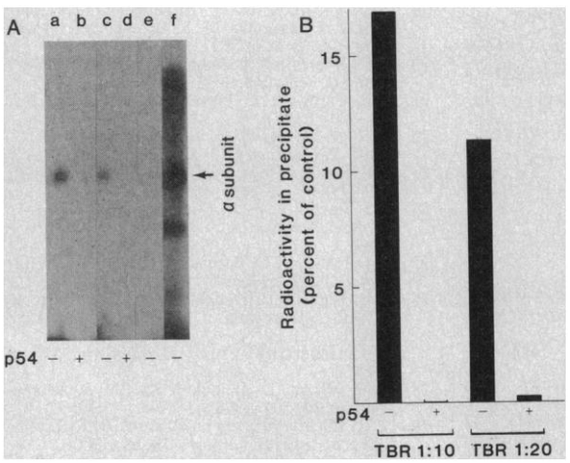

Fig. 1. Immunoprecipitation of insulin receptors by TBR serum. (A) Insulin receptors on IM-9 cells (~2 × 10^9 cells) were affinity-labeled with [^{125}I]insulin and disuccinimidyl suberate (*4*). Receptors were solubilized in 1 percent Triton X-100 (~5 ml) and precipitated with TBR serum (lot C-5) at a dilution of 1:10 (lanes a and b) or 1:20 (lanes c and d), with normal rabbit serum at a dilution of 1:10 (lane e), or with guinea pig antiserum to insulin (GP-625) at a dilution of 1:100 (lane f). Immunoprecipitations were carried out either in the absence (lanes a, c, e, and f) or presence (lanes b and d) of purified p54 (1.5 μg/ml) (*10*). Immune complexes were precipitated with Pansorbin (Calbiochem) and analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and autoradiography. (B) The regions of the gel corresponding to the 135-kilodalton (kD) subunit of the receptor were excised, and the quantity of ^{125}I associated with the band was determined by gamma counting (50 minutes). The amount of specifically precipitated ^{125}I was calculated by subtracting the radioactivity in the 135-kD region of lane e. Results are expressed as a percentage of the radioactivity (13,500 counts per 50 minutes) in the precipitate from the reaction with antiserum to insulin (lane f), which completely precipitates the affinity-labeled receptors under these conditions (*4*).

We investigated the ability of other antisera directed against oncogene products to immunoprecipitate insulin receptors. Whereas TBR serum (lot C-5) was able to precipitate affinity-labeled insulin receptors (Figs. 1 and 2), three other antibodies to pp60^{src} were not: monoclonal antibody ABsrc-1 (Oncor), TBR serum (lot C-8), and a polyclonal antiserum raised by immunization of rabbits with purified pp60^{src} (*13*). These observations suggest that pp60^{src} is unlikely to be identical to a truncated form of the human insulin receptor. Nevertheless, the existence of a cross-reacting antiserum (TBR serum lot C-5) provides experimental evidence in favor of structural similarity of one or more epitopes on the two proteins. In other studies, we have shown that antibodies to four other members of the *src* family of tyrosine-specific protein kinases (*raf*, *abl*, *fes*, and *fms*) did not precipitate the insulin receptor (*14*).

Tumor-bearing rabbit serum had been shown to contain immunoglobulins whose heavy chains were able to serve as substrates for the insulin receptor–associated protein kinase (*15*). However, in that study TBR serum did not precipitate the ^{32}P-labeled phosphoreceptor. Moreover, the previous studies did not show directly whether it was the antibodies to pp60^{src} that were responsible for the ability of the polyclonal TBR serum to serve as substrate for the insulin receptor–associated protein kinase. It is possible that the differences between these observations and ours derive from differences among preparations of TBR serum.

Alternatively, the differences may have resulted from differences in experimental technique (*16*). At present, we

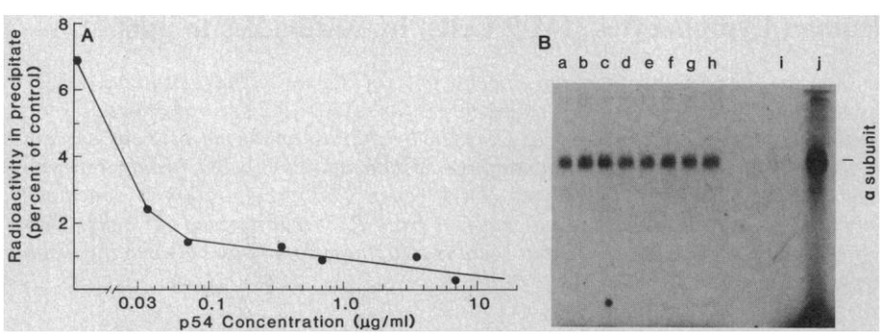

Fig. 2. Immunoprecipitation of insulin receptors by TBR serum: competition by p54. (A) TBR serum (0.04 ml) plus Pansorbin (0.1 ml) was incubated with purified p54 (0 to 7 μg/ml, as indicated) in a total volume of 0.44 ml for 30 minutes at 4°C. Affinity-labeled insulin receptors (0.2 ml) were added, and the incubation was continued for an additional 2 hours. The Pansorbin was separated by centrifugation and washed as described (*6*). The immune complexes were analyzed by SDS-PAGE, and the data are presented as described in the legend to Fig. 1. In this experiment, antiserum to insulin (GP-625) precipitated 16,200 counts per 50 minutes of ^{125}I in association with the α subunit of the insulin receptor. (B) Affinity-labeled insulin receptors were incubated at 4°C with TBR serum at a dilution of 1:10 (lanes a to h), normal rabbit serum at a dilution of 1:10 (lane i), or GP-625 at a dilution of 1:100 (lane j). In some lanes, either the product of the *raf* oncogene at a concentration of 70 μg/ml (lane b) or various synthetic peptides related to the product at a concentration of 25 μg per milliliter (SP-45, lane c; SP-46, lane d; SP-47, lane e; SP-67, lane f; SP-68, lane g; SP-63, lane h) were added (*11, 12*). Immune complexes were separated as described in the legend to Fig. 1, and the precipitates were analyzed by SDS-PAGE and subsequent autoradiography.

cannot rule out the possibility that TBR serum may have precipitated insulin receptors indirectly—for example, by virtue of pp60^{src} binding of insulin receptors and consequent bridging of the receptors to antibodies to pp60^{src}. However, this seems less likely since antibodies to the insulin receptor do not precipitate ^{35}S-labeled pp60^{src} (that is, a protein with an apparent molecular weight of 60,000 daltons) in association with insulin receptors labeled biosynthetically with [^{35}S]methionine (*17*).

NICOLA PERROTTI
SIMEON I. TAYLOR*
Diabetes Branch, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, Bethesda, Maryland 20205
NANCY D. RICHERT
Laboratory of Molecular Biology, National Cancer Institute, Bethesda, Maryland 20205
ULF R. RAPP
Laboratory of Viral Carcinogenesis, National Cancer Institute, Frederick, Maryland 21701
IRA H. PASTAN
Laboratory of Molecular Biology, National Cancer Institute
JESSE ROTH
Diabetes Branch, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases

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9. When the supernatant from the TBR serum immunoprecipitation was subjected to a second precipitation with TBR serum, it was possible to precipitate a similar fraction (10 to 20 percent) of the affinity-labeled insulin receptor. Thus, the precipitation of only a small amount (10 to 20 percent) of the insulin receptors by TBR serum probably results from the relatively low affinity of that antiserum for the insulin receptor. Moreover, these observations rule out the alternative possibility that the receptors precipitated by TBR serum make up a subpopulation that is different from the receptors not precipitated during the first immunoprecipitation.
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16. In this regard, Kasuga *et al.* (*15*) studied the ^{32}P-labeled insulin receptor whereas we studied the affinity-labeled insulin receptor. In our experiments, we showed that the precipitation of the insulin receptor occurs only when it is covalently cross-linked to insulin. The results of initial studies suggest that phosphorylation of the receptor with adenosine triphosphate substantially inhibits (by about 50 percent) the ability of TBR serum to precipitate the affinity-labeled insulin receptor. Cross-linking of insulin to its receptor may expose cryptic epitopes that enable TBR serum to bind to the receptor. Similarly, phosphorylation of the receptor may render those same epitopes less available for antibody binding.
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19. We thank R. L. Erikson for the polyclonal antiserum used in these studies.

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A Mutant of *Arabidopsis* Lacking a Chloroplast-Specific Lipid

Abstract. *In order to investigate the functional significance of membrane lipid unsaturation, we have isolated a series of mutants of* Arabidopsis thaliana *which are deficient in particular membrane fatty acids. The first of these mutants completely lacks* Δ*3-trans-hexadecenoate, an acyl group that until now has been thought to play an important role in the structure or function of thylakoid membranes in photosynthetic eukaryotes. The apparent absence of any marked physiological effect of the mutation illustrates the potential of this approach to the analysis of membrane structure and function.*

In most eukaryotes each lipid class has a characteristic fatty acyl composition defined by chain length and degree, position, and stereochemistry of unsaturation. However, with a few exceptions the functional significance of lipid acyl unsaturation remains uncertain in both animals (*1*) and plants (*2*). Much of the work on plants has been dominated by a hypothesis that suggested a causal relation between membrane lipid unsaturation and chilling sensitivity (*3*) and has frequently involved an attempt to correlate species differences in membrane composition with thermal tolerance. In addition, it is widely proposed that the unusual fatty acid composition that characterizes chloroplast membranes is critical to the maintenance of photosynthetic function (*4*). We have taken a novel approach to these questions by isolating a series of mutants of the crucifer *Arabidopsis thaliana* (L.) Heynh. with specific

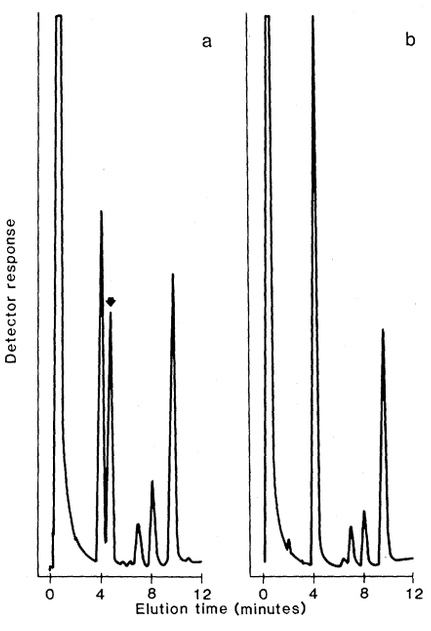

Fig. 1. Fatty acid composition of PG from wild-type (a) and mutant (b) *Arabidopsis*. PG was purified by preparative thin-layer chromatography of a chloroform-methanol extract of leaves (*20*). Lipid-containing zones were visualized with iodine vapor, the lipids were extracted from the silica gel, and fatty acid methyl esters were prepared as described in the legend to Table 1. Individual lipids were identified by comparison of Rf (relative mobility) values with those of known lipid standards. The major peaks (from left to right) and the percentages of total fatty acid in the wild-type sample were solvent, $C_{16:0}$ (34 percent); *trans*-$C_{16:1}$ (20 percent); $C_{18:1}$ (4 percent); $C_{18:2}$ (10 percent); and $C_{18:3}$ (32 percent).