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36. The conclusions of Doyle and Donoghue (*8*) can be reconciled with our analysis by noting that they took into account that most diversity in the Magnoliales is confined to one large family, Annonaceae (2300 species), nested within that clade, which appears to have radiated more recently. They therefore concluded that the ancestral diversification rate in magnoliales was likely to be low.
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44. We thank P. Crane, J. Doyle, M. Frohlich, and J. Hayes for helpful comments.

18 January 1994; accepted 10 May 1994

G_h: A GTP-Binding Protein with Transglutaminase Activity and Receptor Signaling Function

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The α_1-adrenergic receptors activate a phospholipase C enzyme by coupling to members of the large molecular size (approximately 74 to 80 kilodaltons) $G\alpha_h$ family of guanosine triphosphate (GTP)–binding proteins. Rat liver $G\alpha_h$ is now shown to be a tissue transglutaminase type II (TGase II). The transglutaminase activity of rat liver TGase II expressed in COS-1 cells was inhibited by the nonhydrolyzable GTP analog guanosine 5′-*O*-(3-thiotriphosphate) or by α_1-adrenergic receptor activation. Rat liver TGase II also mediated α_1-adrenergic receptor stimulation of phospholipase C activity. Thus, $G\alpha_h$ represents a new class of GTP-binding proteins that participate in receptor signaling and may be a component of a complex regulatory network in which receptor-stimulated GTP binding switches the function of $G\alpha_h$ from transglutamination to receptor signaling.

We have shown previously that a GTP-binding protein, termed G_h, copurifies with rat liver α_1-adrenergic receptors in a ternary complex containing α_1-agonist, the receptor, and G_h (*1*). De novo purification of G_h revealed that the 74-kD α subunit ($G\alpha_h$) is associated with an ~50-kD β subunit ($G\beta_h$) (*2*). $G\beta_h$ modulates the GTP binding and guanosine triphosphatase (GTPase) activity of $G\alpha_h$. G_h proteins with α subunits of 74 to 80 kD exist in various species, including humans and cows (G_{h7}); G_{h7} couples to α_1-adrenergic receptors and activates a 69-kD phospholipase C (PLC) (*2*).

Microsequencing of endoproteinase lysine C–generated peptide fragments of $G\alpha_h$ purified from rat liver (*3*) yielded four sequences—SVXRDXREDITYTYK, YPEXXPE, SVEVSDPVPAGDXVKXRVXLFP, and SVKGYXN (the identity of the residues designated X is uncertain)—all of which are highly similar to sequences contained in guinea pig, mouse, human, and bovine tissue transglutaminase type II (TGase II or TGase C; *R*-glutaminyl-peptide:amino-γ-glutamyltransferase, E.C. 2.3.2.13). These enzymes are Ca^{2+}- and thiol-dependent acyl transferases that catalyze the formation of an amide bond between the γ-carboxamide groups of peptide-bound glutamine residues and the primary amino groups in various

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compounds, including the ε-amino group of lysines in certain proteins (*4*). Although TGase II has not been implicated in signal transduction, it specifically binds guanine nucleotides and hydrolyzes GTP, even when TGase activity has been eliminated by mutation of the active site cysteine (*5*). However, TGase II sequences show no similarity to the nucleotide-binding consensus sequences of heterotrimeric G proteins and small molecular size GTP-binding proteins (*6*).

The identity of $G\alpha_h$ and TGase II was further indicated by the observation that monoclonal antibodies to guinea pig liver TGase II and antibodies to $G\alpha_{h7}$ recognized both purified rat liver $G\alpha_h$ and purified guinea pig liver TGase II (*7*) (Fig. 1A). Moreover, purified guinea pig liver TGase II was photolabeled with [α-^{32}P]GTP (Fig. 1A). Purified rat liver $G\alpha_h$ was not recognized by antibodies to $G\alpha_q$ (Fig. 1B), $G\alpha_i$, $G\alpha_s$, or $G\alpha_{common}$ (*8*).

A full-length complementary DNA (cDNA) clone isolated from rat liver RNA by reverse transcription and the polymerase chain reaction (PCR) (*9*) revealed that the deduced amino acid sequence of rat liver TGase II was highly similar to that of guinea pig, human, mouse, and bovine TGase II (Fig. 2). The sequences of all four $G\alpha_h$ peptides were identical to sequences of rat liver TGase II. The molecular size of rat liver TGase II was calculated to be 75,460 daltons, which is consistent with the ~74-kD size determined for $G\alpha_h$ (*1*, *2*).

To investigate whether rat liver TGase II functions in receptor-coupled signaling, we transfected COS-1 cells with both the rat liver TGase II cDNA and an α_{1B}-adrenergic receptor cDNA. The expressed TGase II was recognized with the expected molecular size by antibodies to $G\alpha_{h7}$, as well as by monoclonal antibodies to guinea pig liver TGase II (Fig. 1B). The expressed protein also demonstrated TGase activity that was blocked either by the TGase inhibitor monodansylcadaverine, by guanosine 5′-*O*-(3-thiotriphosphate) (GTP-γ-S) alone, or by receptor activation in the presence of GTP-γ-S (Fig. 3, A and B). Stimulation of inositol phosphate accumulation by α_1-adrenergic agonists was enhanced in buffalo rat liver cells after cotransfection with both an α_{1B}-adrenergic receptor cDNA and the rat liver TGase II cDNA as compared to that in cells transfected with only the receptor cDNA (Fig. 3C). This enhancement of inositol phosphate accumulation was similar to that observed in cells cotransfected with both the receptor cDNA and a $G\alpha_q$ cDNA (*10*) and was inhibited by the selective α_1-adrenergic antagonist prazosin. Rat liver TGase II and $G\alpha_q$ also enhanced specific α_1-adrenergic receptor–induced hydrolysis of phosphatidylinositol-4,5-bisphosphate (PIP_2) in the membranes of COS-1 cell transfectants (Fig. 3D), and this response was blocked by prazosin (*8*). A functional interaction between the α_{1B}-adrenergic receptor and $G\alpha_h$ was further demonstrated by the observation that α_{1B}-adrenergic receptors present in a ternary complex of α_1-agonist, receptor, and $G\alpha_h$ could be immunoadsorbed with antibodies to TGase II that were bound to a protein A–agarose gel; the complex was induced by the treatment of rat liver membranes with the α_1-

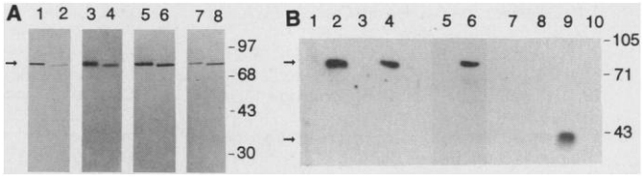

Fig. 1. (**A**) Identification of purified guinea pig liver TGase II (odd-numbered lanes) and rat liver $G\alpha_h$ (even-numbered lanes). Lanes 1 and 2, silver stain; lanes 3 and 4, photolabeling of the purified proteins with [α-^{32}P]GTP; lanes 5 and 6, immunoblot with an antiserum to $G\alpha_{h7}$ (*2*); lanes 7 and 8, immunoblot with monoclonal antibodies to guinea pig liver TGase II (*14*). The slight difference in size between the rat and guinea pig proteins has been noted (*2*). (**B**) Identification of rat liver TGase II and $G\alpha_q$ expressed in COS-1 cells with antibodies to TGase II (lanes 1 to 4), to $G\alpha_{h7}$ (lanes 5 and 6), or to $G\alpha_q$ (antiserum ×384) (*15*) (lanes 7 to 10). Lanes 1 and 7, untransfected COS-1 cell membranes. Although no ~74-kD band is seen in lane 1, with longer exposure of this immunoblot, and in repeat analyses with larger amounts of COS-1 membranes, a faint band at this position was apparent. Lanes 2 and 8, membranes from COS-1 cells transfected with a rat liver TGase II complementary DNA (cDNA). Lanes 3, 5, and 9, membranes from COS-1 cells transfected with a hamster ovary $G\alpha_q$ cDNA. Lanes 4, 6, and 10, purified rat liver $G\alpha_h$. The positions of molecular size markers (in kilodaltons) are shown on the right. Arrows indicate the positions of proteins of ~74 and ~42 kD.

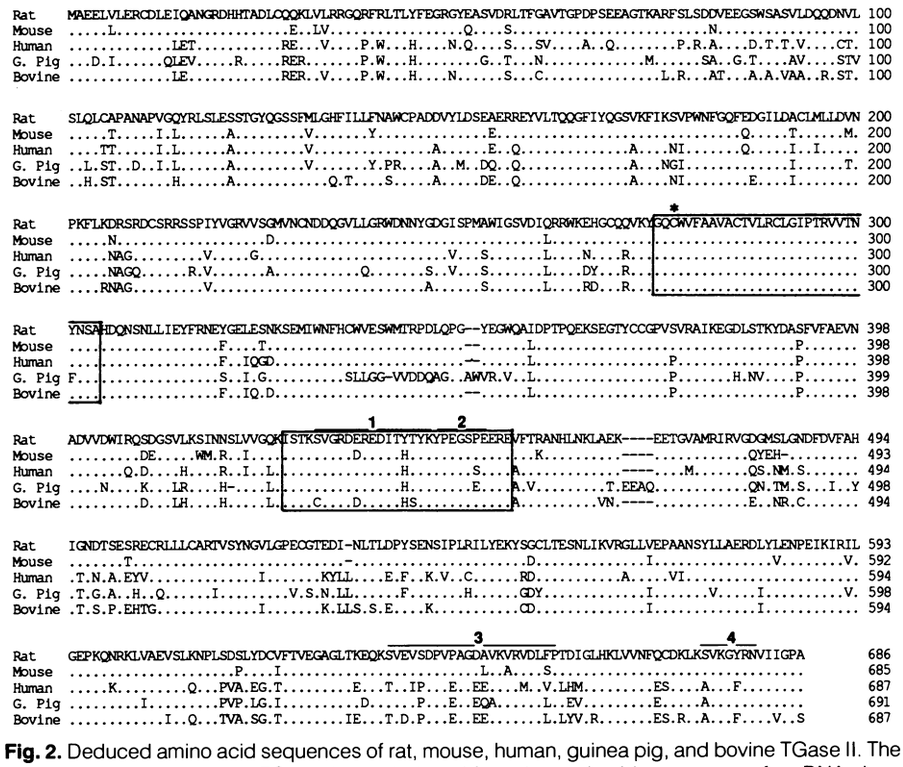

Fig. 2. Deduced amino acid sequences of rat, mouse, human, guinea pig, and bovine TGase II. The amino acid sequence of rat TGase II was deduced from the nucleotide sequence of a cDNA clone isolated from rat liver RNA. The amino acid sequences of mouse, human, guinea pig, and bovine TGase II were determined previously (*16*). Dashes indicate gaps introduced to maximize the alignment. Dots indicate identical residues to those shown at the same aligned position for the rat sequence. The four peptide sequences determined from purified rat liver $G\alpha_h$ are overlined and indicated by Arabic numerals. The active site (residues 275 to 304, rat TGase II) and putative Ca^{2+}-binding region (residues 426 to 454, rat TGase II) are boxed (*17*). The active site cysteine (Cys^{277}) (*17*) is indicated by the asterisk. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

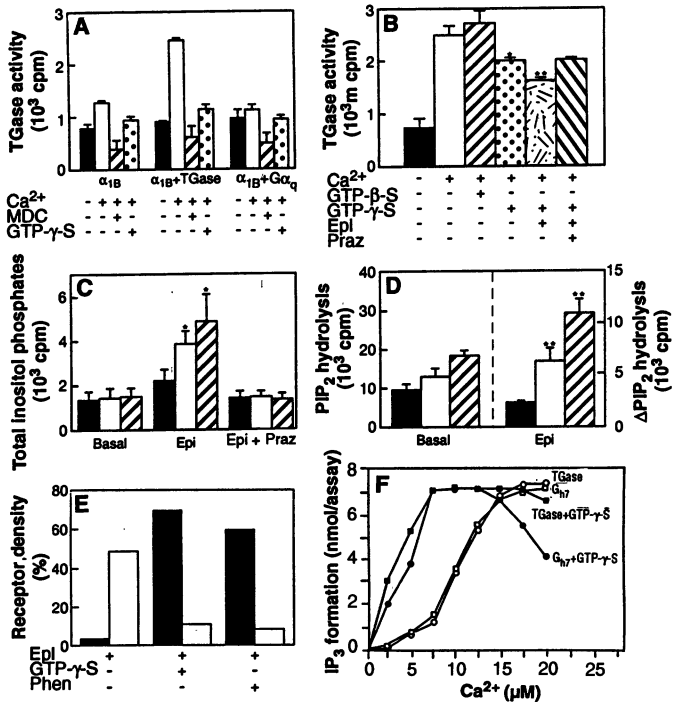

Fig. 3. (**A**) Transglutaminase activity in membranes from COS-1 cells transfected with either a plasmid containing a hamster α_{1B}-adrenergic receptor cDNA, pMT2′hamα_{1B} (α_{1B}); pMT2′hamα_{1B} and a plasmid containing rat TGase II cDNA, pMT2′rl-TGase (α_{1B} + TGase); or pMT2′hamα_{1B} and the plasmid pCMV5 containing a $G\alpha_q$ cDNA (α_{1B} + $G\alpha_q$). Activity was measured in the absence or presence of 0.4 mM $CaCl_2$ (Ca^{2+}), 0.4 mM monodansylcadaverine (MDC), or 20 μM GTP-γ-S [plus 100 μM guanosine 5′-*O*-(2-thiodiphosphate) (GDP-β-S)] (*18*). (**B**) TGase activity in membranes from COS-1 cells transfected with pMT2′hamα_{1B} and pMT2′rlTGase measured in the absence or presence of 0.8 mM $CaCl_2$, 20 μM GTP-β-S, 20 μM GTP-γ-S, 1 μM (−)epinephrine (Epi), or 1 μM prazosin (Praz). $^*P < 0.05$ versus Ca^{2+} alone; $^{**}P < 0.05$ versus Ca^{2+} plus GTP-γ-S. (**C** and **D**) Effect of epinephrine on inositol phosphate accumulation (C) and PIP_2 hydrolysis (D) in transfected buffalo rat liver cells (C) or membranes from transfected COS-1 cells (D). Both types of cells were transfected with pMT2′hamα_{1B} (closed bars), pMT2′hamα_{1B} and pMT2′rlTGase (open bars), or pMT2′hamα_{1B} and pCMV5 containing the $G\alpha_q$ cDNA (hatched bars) (*19*). ΔPIP_2 hydrolysis is the difference in [^{3}H]PIP_2 hydrolysis between membranes stimulated with (−)epinephrine and GTP-γ-S, and membranes treated with GTP-γ-S alone. $^*P < 0.05$ versus cells transfected with only pMT2′hamα_{1B} (C). $^{**}P < 0.05$ versus membranes from cells transfected with only pMT2′hamα_{1B} (D). Results are means ± SEM of at least three independent experiments performed in duplicate, and statistical differences determined by analysis of variance for multiple comparisons (A to D). (**E**) Immunoadsorption of a ternary complex of α_1-adrenergic agonist, receptor, and $G\alpha_h$ by an anti–TGase II antibody affinity resin (*20*). The ternary complex was incubated with the resin in the presence of 5 μM (−)epinephrine, 5 μM (−)epinephrine plus 5 μM GTP-γ-S, or 5 μM (−)epinephrine plus 0.1 mM phentolamine (Phen). α_1-Adrenergic receptor density in the flow-through (solid bars) and eluted (open bars) fractions is expressed as a percentage of the receptor in the ternary complex that was applied to the column. (**F**) Effect of TGase II and G_{h7} on the Ca^{2+} concentration dependence of the 69-kD PLC. Purified TGase II and G_{h7} were incubated in the absence or presence of 5 μM GTP-γ-S before assay of PLC activity (IP_3 formation) (*21*). Values are means of duplicate measurements and are representative of three independent experiments with different preparations.

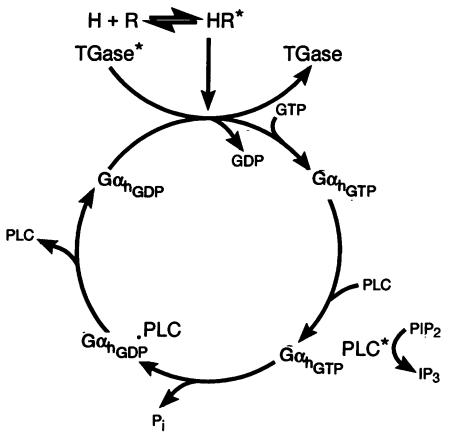

Fig. 4. Model for the regulation of the TGase and signaling functions of $G\alpha_h$ by hormone-activated receptors. H, hormone; R, receptor; Pi, inorganic phosphate. Asterisks indicate the activated state.

agonist (−)epinephrine (Fig. 3E). The receptor was dissociated from the adsorbed ternary complex by treatment with GTP-γ-S. Moreover, immunoadsorption of α_{1B}-adrenergic receptors was not observed if the ternary complex was dissociated by treatment with either (−)epinephrine and GTP-γ-S or (−)epinephrine and the α_1-antagonist phentolamine (Fig. 3E). Finally, both guinea pig liver TGase II and G_{h7} that had been activated by exposure to GTP-γ-S stimulated the 69-kD PLC (*2*) by enhancing its sensitivity to Ca^{2+} without altering maximal inositol-1,4,5-trisphosphate (IP_3) formation (Fig. 3F).

Our results indicate that $G\alpha_h$ is a multifunctional GTP-binding protein that can mediate both receptor-stimulated PLC activation and transglutamination. The relation between these two functions is unclear. Micromolar concentrations of GTP-γ-S inhibit the TGase activity of $G\alpha_h$ and this effect is augmented by receptor activation, suggesting that GTP may be a negative regulator of TGase activity. Because receptor activation stimulates the binding of GTP to $G\alpha_h$, it may act as a switch to allow $G\alpha_h$ to function as a signaling molecule rather than as a TGase (Fig. 4). According to this model, hydrolysis of GTP would terminate signal transduction and restore the TGase function of $G\alpha_h$, because guanosine diphosphate (GDP) is a less potent inhibitor of TGase activity (Fig. 3B). The binding of GTP-γ-S induces dissociation of G_h into its α and β subunits (*2*). However, it is unclear whether $G\beta_h$ also directly modulates TGase activity.

α_1-Adrenergic receptors, as well as other Ca^{2+}-mobilizing receptors, also activate PLC via G_q (*11*). Both G_h and G_q are expressed in liver (*1, 12*) and may also be coexpressed in other tissues. Whereas $G\alpha_h$ hydrolyzes GTP rapidly (*2*), the GTPase activity of $G\alpha_q$ is slow, although it is increased by interaction with its effector, PLC-β (*13*). Thus, $G\alpha_h$ and $G\alpha_q$ are differentially regulated and may act in concert to enhance or sustain PLC activity. By virtue of its multifunctional nature, however, $G\alpha_h$ may also regulate cellular responses distinct from those controlled by heterotrimeric G proteins. Moreover, the TGase domain of $G\alpha_h$, because of its ability to bind Ca^{2+} and, thus, to inhibit GTP binding (*5*), may also serve to regulate input by Ca^{2+}-mobilizing receptors using this G protein pathway. The similarity between $G\alpha_h$ and the heterotrimeric G proteins, with respect to their GTP binding, GTPase activity, and signaling functions, appears to have resulted from convergent evolution, because these two classes of proteins have distinct primary structures.

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3. $G\alpha_h$ was purified to homogeneity from rat liver membranes by sequential column chromatography according to a slight modification of our previously described procedure (*2*), which will be detailed elsewhere (H. Nakaoka, K. Misono, D. M. Perez, A. Husain, R. M. Graham, in preparation).
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7. Guinea pig liver TGase II (Sigma) was purified as described (*5*), with the exception that the protein was eluted from the GTP-agarose column with a buffer containing 5 mM GTP and 1 M KCl. Purified TGase II and rat liver $G\alpha_h$ were photolabeled with [α-^{32}P]GTP as described (*1, 2*). COS-1 cells were transfected with a plasmid containing the rat liver

TGase II cDNA (pMT2′rl TGase) (Fig. 2) or with a plasmid (pCMV5) containing a $G\alpha_q$ cDNA (*10*) (6 to 8 μg per 5 × 10^6 cells) by the DEAE-dextran method; after 48 to 72 hours, cells were harvested and membranes prepared as described [D. M. Perez, M. B. DeYoung, R. M. Graham, *Mol. Pharmacol.* **44**, 784 (1993); R. R. Franke, T. P. Sakmar, R. M. Graham, H. G. Khorana, *J. Biol. Chem.* **267**, 14767 (1992)]. Membranes or purified $G\alpha_h$ were subjected to SDS–polyacrylamide gel electrophoresis (8% polyacrylamide). The proteins were transferred to nitrocellulose filters, which were then incubated in 5% (w/v) dried milk and probed with the various antibodies diluted 1:500. Bound antibodies were detected with ^{125}I-labeled protein A (ICN) (polyclonal antisera), or with rabbit antibodies to mouse immunoglobulin G followed by ^{125}I-labeled protein A (monoclonal antibodies). The blots were subjected to autoradiography with Kodak XAR-5 film and image intensifying screens (Dupont).

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18. TGase activity was determined by quantitating the incorporation of [^{3}H]putrescine into *N,N′*-dimethylcasein as described by Achyuthan and Greenberg (*5*). Membranes (25 to 30 μg of protein) in 50 μl of tris-HCl (pH 7.4) containing 1 mM $MgCl_2$, 20 mM dithiothreitol, and 20% (v/v) glycerol were incubated with 0.4% *N,N′*-dimethylcasein, 2 μCi of [^{3}H]putrescine, and various indicated additions for 1 hour at 37°C. The reactions were stopped and the radioactivity incorporated was determined as described (*5*).
19. Buffalo rat liver cells (American Type Culture Collection) or COS-1 cells were transfected with 6 to 8 μg of each plasmid per 5 × 10^6 cells. After 48 to 72 hours, the effects of (−)epinephrine and prazosin on [^{3}H]inositol phosphate accumulation were assayed in buffalo rat liver cells (*1*), and membranes were prepared from COS-1 cells. Membranes were assayed for [^{3}H]PIP_2 hydrolysis after treatment with (−)epinephrine (1 μM), or epinephrine plus prazosin (1 μM), essentially as described (*15*), except that the concentrations of GTP-γ-S and GDP-β-S were 1 and 50 μM, respectively.
20. An anti–TGase II antibody affinity column (50 μl) was prepared by incubation of protein A–agarose with antibodies to TGase II for 2 hours at 4°C. The gel was washed extensively with 20 mM Hepes (pH 7.4) containing 100 mM NaCl, 0.5 mM dithiothreitol, 5% (v/v) glycerol, and 0.05% sucrose monolaurate. A ternary complex of α_1-agonist, receptor, and $G\alpha_h$ (300 fmol of α_1-receptor, 330 fmol of $G\alpha_h$), prepared as previously described (*1*), was incubated with the resin at 4°C for 2 hours in the presence of 1 mM $MgCl_2$, and either 5 μM (−)epinephrine, 5 μM (−)epinephrine and 5 μM GTP-γ-S, or 5 μM (−)epinephrine and 0.1 mM phentolamine. Unbound material was eluted from the column by applying gentle pressure until the column was dry. After the resin was washed with the same buffer (3 to 5 ml) in the absence of ligand, adsorbed α_1-adrenergic receptor was eluted by incubating the gel with 2 μM (−)epinephrine, 5 μM GTP-γ-S, and 1 mM $MgCl_2$ for 30 to 60 min at 4°C. Receptor density in the flow-through and eluted fractions was determined after removing excess ligand on a dried Sephadex G-25 (Pharmacia) column (3 ml) as described (*1, 2*).
21. The 69-kD PLC (100 ng) and either guinea pig liver TGase II (500 ng) or G_{h7} (1 μg) were purified and reconstituted into phospholipid vesicles as described (*2, 3, 7*). The vesicles were incubated with 1 mM $MgCl_2$ in the absence or presence of 5 μM GTP-γ-S at 30°C for 30 min before evaluation of PLC activity. The samples were then incubated at 30°C for 10 min in the presence of [^{3}H]PIP_2 (500 cpm/nmol) and various concentrations of Ca^{2+}, and IP_3 formation was measured as described (*2*).
22. We thank P. J. Birckbichler for the antibodies to guinea pig liver TGase II; P. C. Sternweis for antibodies to $G\alpha_q$; G. L. Johnson for the $G\alpha_q$ cDNA clone; J. R. Shainoff for a sample of purified guinea pig liver TGase II; G. Matsueda for valuable discussions; R. Gaivin, T. Tang, and R. P. Riek for technical assistance; and S. Cox for typing the manuscript. Supported by NIH (A.H., M.-J.I., and R.M.G.), a grant-in-aid (D.M.P.) and postdoctoral fellowship (H.N.) from the American Heart Association (Northeast Ohio Affiliate), and an Eccles Award (R.M.G.) from the National Health and Medical Research Council of Australia.

31 January 1994; accepted 26 April 1994

Human Severe Combined Immunodeficiency Due to a Defect in ZAP-70, a T Cell Tyrosine Kinase

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A homozygous mutation in the kinase domain of ZAP-70, a T cell receptor–associated protein tyrosine kinase, produced a distinctive form of human severe combined immunodeficiency. Manifestations of this disorder included profound immunodeficiency, absence of peripheral $CD8^+$ T cells, and abundant peripheral $CD4^+$ T cells that were refractory to T cell receptor–mediated activation. These findings demonstrate that ZAP-70 is essential for human T cell function and suggest that $CD4^+$ and $CD8^+$ T cells depend on different intracellular signaling pathways to support their development or survival.

Severe combined immunodeficiencies (SCIDs) are a heterogeneous group of inherited disorders characterized by profound deficiency of both T cell and B cell immunity (*1*). In about 30% of cases, the underlying genetic defect is unknown. We recently identified a 1-year-old girl (the daughter of first cousins) in whom SCID was associated with a highly unusual T cell subset distribution in the blood (*2*). T cells are normally divided into two major subsets that express either CD4 or CD8 protein on their surfaces. In our patient, the overall T cell count was moderately elevated, but $CD8^+$ cells were virtually absent and nearly all circulating T cells were of the $CD4^+$ type (Fig. 1).

The patient's $CD4^+$ T cells were polyclonal, expressed other surface markers of mature lymphocytes, and proliferated normally when exposed to a combination of phorbol ester and calcium ionophore in vitro. However, they did not respond to mitogenic stimuli mediated through the surface T cell receptor (TCR)–CD3 complex, such as CD3-CD4 co-crosslinking, or to allogeneic stimulation in a culture of mixed lymphocytes. An earlier report (*3*) described similar findings in children from a Mennonite kindred and attributed the abnormalities to an undetermined defect in TCR signal transduction. As was consistent with this view, CD3-CD4 co-crosslinking of our patient's cells failed to trigger the cascade of cytoplasmic protein tyrosine kinase (PTK) reactions that is an early, obligatory step in T cell activation (*2, 4*). This implied a functional defect in coupling of the TCR to cytoplasmic PTKs.

We therefore used protein immunoblots to assay for individual PTKs that have been implicated in TCR signal transduction, including Lck, Fyn, and the T cell–specific kinase ZAP-70. ZAP-70 is a 70-kD cytosolic kinase that associates with the ζ chain of the TCR complex soon after this complex binds a ligand, and it has been proposed that it participates in T cell activation (*5–7*). Normal amounts of Lck and Fyn were present in our patient's cells, but no ZAP-70 protein was detectable (Fig. 2A).

A Northern (RNA) blot of the patient's T cell RNA showed ZAP-70 mRNA of

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