MOLECULAR AND CELLULAR BIOLOGY, Jan. 1996, p. 309–317 Vol. 16, No. 1
0270-7306/96/$04.00+0

A Membrane-Proximal Region of the Interleukin-2 Receptor γ_c Chain Sufficient for Jak Kinase Activation and Induction of Proliferation in T Cells

BRAD H. NELSON,* JAMES D. LORD, AND PHILIP D. GREENBERG

Fred Hutchinson Cancer Research Center, Seattle, Washington 98104, and Departments of Immunology and Medicine, University of Washington, Seattle, Washington 98195

Received 14 August 1995/Returned for modification 14 September 1995/Accepted 17 October 1995

The interleukin-2 (IL-2) receptor (IL-2R) consists of three distinct subunits (α, β, and γ_c) and regulates proliferation of T lymphocytes. Intracellular signalling results from ligand-mediated heterodimerization of the cytoplasmic domains of the β and γ_c chains. To identify the residues of γ_c critical to this process, mutations were introduced into the cytoplasmic domain, and the effects on signalling were analyzed in the IL-2-dependent T-cell line CTLL2 and T-helper clone D10, using chimeric IL-2R chains that bind and are activated by granulocyte-macrophage colony-stimulating factor. Whereas previous studies of fibroblasts and transformed T cells have suggested that signalling by γ_c requires both membrane-proximal and C-terminal subdomains, our results for IL-2-dependent T cells demonstrate that the membrane-proximal 52 amino acids are sufficient to mediate a normal proliferative response, including induction of the proto-oncogenes c-*myc* and c-*fos*. Although γ_c is phosphorylated on tyrosine upon receptor activation and could potentially interact with downstream molecules containing SH2 domains, cytoplasmic tyrosine residues were dispensable for mitogenic signalling. However, deletion of a membrane-proximal region conserved among other cytokine receptors (cytoplasmic residues 5 to 37) or an adjacent region unique to γ_c (residues 40 to 52) abrogated functional interaction of the receptor chain with the tyrosine kinase Jak3. This correlated with a loss of all signalling events analyzed, including phosphorylation of the IL-2Rβ-associated kinase Jak1, expression of c-*myc* and c-*fos*, and induction of the proliferative response. Thus, it appears in T cells that Jak3 is a critical mediator of mitogenic signalling by the γ_c chain.

The recently described γ chain of the interleukin-2 (IL-2) receptor (IL-2R) has had a remarkable impact on the field of cytokine receptor biology. Its existence was first postulated to account for discrepancies in the ligand binding affinity of the IL-2R α and β chains when reconstituted in fibroblasts compared with lymphocytes (18). Shortly thereafter, the γ chain was detected as a lymphoid cell-specific 64-kDa species that precipitated with IL-2Rβ in the presence of IL-2 (1, 50, 51, 54). This biochemical characterization allowed purification of the IL-2Rγ polypeptide and ultimately molecular cloning of the corresponding cDNA (49). Subsequent reconstitution experiments in fibroblasts demonstrated that the α, β, and γ chains of the IL-2R mediate high-affinity binding of IL-2, whereas the β and γ chains constitute the intermediate-affinity receptor found on some lymphoid cell types.

Like the IL-2R β chain, the γ chain belongs to the hematopoietic receptor superfamily by virtue of conserved extracellular and intracellular sequence motifs. The suggestion that IL-2Rγ may be a component of other receptors in this superfamily came after the discovery that mutations in the IL-2Rγ gene were the genetic basis of X-linked severe combined immunodeficiency disease in humans (35, 39). This syndrome is characterized by an almost complete block in the development of T lymphocytes from immature precursors, a phenotype far more severe than that seen after targeted disruption of the IL-2 gene in mice (44). Indeed, the IL-2R γ chain was subsequently shown to also be a component of the receptors for IL-4, IL-7, IL-9, and IL-15 (IL-4R, IL-7R, IL-9R, and IL-15R) (6, 10, 13, 24, 25, 34, 41, 42) and for this reason is now referred to as γ_c, the common γ chain (34). Recent studies involving targeted deletion of the IL-7 and IL-7R genes suggest that the block in thymocyte development associated with γ_c mutations results primarily from disruption of IL-7 signalling (37, 53).

Signalling by the IL-2R is mediated by ligand-induced heterodimerization of the cytoplasmic domains of IL-2Rβ and γ_c (32, 33). Studies of IL-2Rβ have identified two subdomains that perform distinct yet partially overlapping signalling functions. A serine-rich subdomain located proximal to the membrane binds the tyrosine kinases Jak1 and Syk (28, 29, 41, 52) and is essential for cell proliferation and induction of the proto-oncogenes c-*myc*, c-*fos*, c-*jun*, and *bcl-2* (17, 27, 30, 45). Adjacent to this region is a subdomain rich in acidic residues that binds the tyrosine kinase $p56^{lck}$ (16) and is required for the induction of c-*fos* and c-*jun* but not c-*myc* or *bcl-2* (30, 43). The acidic subdomain is dispensable for proliferation in the pro-B-cell line BA/F3 (17) but not in normal T cells (26a).

The cytoplasmic domain of γ_c contains 86 amino acids and associates constitutively with the tyrosine kinase Jak3 (4, 29, 41). The membrane-proximal 38 residues have partial homology to the SH2 motif found in a variety of signalling molecules (49); however, this region is unlikely to function precisely as an SH2 domain, as it lacks many of the residues critical for binding phosphotyrosine (23). Although primary lymphocytes represent the most physiologically relevant cell type for studies of γ_c, the constitutive expression of the wild-type chain by these cells precludes standard mutational analysis of receptor domains. Therefore, to date studies of γ_c have been performed either in fibroblasts with reconstituted IL-2R complexes or in a transformed T-cell line that lacks endogenous γ_c (2, 3). These

* Corresponding author. Mailing address: Fred Hutchinson Cancer Research Center, 1124 Columbia St., M758, Seattle, WA 98104. Phone: (206) 667-4165. Fax: (206) 667-7983. Electronic mail address: bnelson@fred.fhcrc.org.

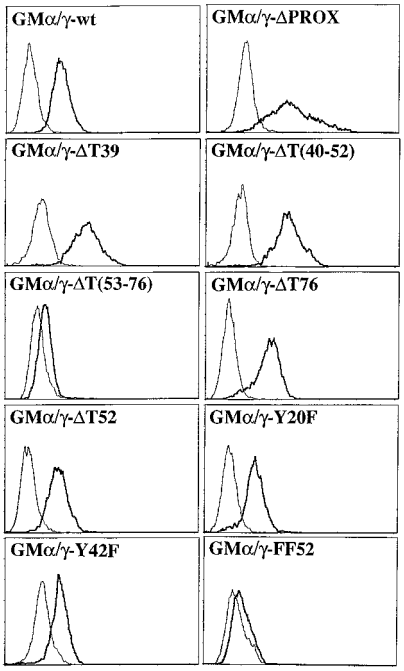

FIG. 1. Cell surface expression of GMα/γ-derived receptor chains on CTLL2 cells. CTLL2 cells expressing the chain GMβ/2β were transfected with vectors encoding wild-type GMα/γ (GMα/γ-wt) or a mutant version of GMα/γ. Hygromycin-resistant lines were stained with an antibody specific for the extracellular domain of human GM-CSFRα (thick line) or with medium alone (thin line) and analyzed by flow cytometry. Fluorescence intensity is plotted on the abscissa on a logarithmic scale, and cell number is plotted on the ordinate.

studies have suggested that the signalling domains of γ_c may be organized similarly to those of IL-2Rβ in that the membrane-proximal region is sufficient for interaction with Jak3, induction of c-*myc*, and cell proliferation, whereas distal residues are required for induction of c-*fos* and c-*jun*. This observation suggests that IL-2Rβ and γ_c may interact through their respective proximal and distal subdomains to activate distinct signalling pathways leading to the induction of c-*myc* or of c-*fos* and c-*jun*, respectively.

One potential limitation of this prior work is that fibroblasts and transformed T cells do not normally use the IL-2R and thus may lack relevant downstream components of the signalling pathway. In light of this, we have developed a chimeric granulocyte-macrophage colony-stimulating factor (GM-CSF)/IL-2 receptor that allows analysis of the signalling properties of modified IL-2Rβ and γ_c chains in IL-2-dependent T-cell lines and clones. The chimeric receptor consists of two chains: GMα/γ, with the extracellular region of the GM-CSF receptor (GM-CSFR) α chain fused to the transmembrane and cytoplasmic regions of γ_c, and GMβ/2β, with the extracellular region of the GM-CSFR β chain fused to the transmembrane and cytoplasmic regions of IL-2Rβ. In T cells coexpressing GMα/γ and GMβ/2β, GM-CSF induces proliferation, tyrosine phosphorylation, and STAT factor activation characteristic of the normal IL-2 response (15a, 33). Using this system, we have now analyzed a panel of mutants of γ_c and have found that the partial SH2 domain (referred to here as the PROX domain) and downstream 14 amino acids are necessary and sufficient for induction of Jak kinase activity, c-*myc* and c-*fos* expression, and proliferation in T cells. In contrast to previous studies in fibroblasts and transformed T cells, we found no evidence of proximal and distal subdomains of γ_c mediating distinct signals to the nucleus. Instead, our results suggest that Jak3, which associates with the membrane-proximal region of γ_c, is a critical mediator of multiple pathways associated with IL-2R signal transmission.

MATERIALS AND METHODS

Cell culture. CTLL2 cells were maintained in Click's medium (Altick Enterprises) containing 100 U of human IL-2 (Hoffmann-La Roche) per ml, 10 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), 0.14% sodium bicarbonate, 1% glutamine, 1% penicillin-streptomycin, and 10% fetal calf serum (FCS). The T-helper clone D10 was maintained in RPMI-HEPES containing 50 U of human IL-2 (Hoffmann-La Roche) per ml, 1% glutamine, 1% penicillin-streptomycin, and 10% FCS and were stimulated biweekly with the cognate antigen conalbumin presented by C3H splenocytes. Some CTLL2 and D10 cell lines expressing chimeric receptors were maintained with GM-CSF (125 to 250 ng/ml) (Immunex Corp.) in place of IL-2.

Plasmid constructions. The expression vectors encoding the wild-type forms of GMβ/2β and GMα/γ (formerly referred to as GMα/2γ) have been previously described (14, 33). To introduce mutations into the cytoplasmic domain of GMα/γ, mutant oligonucleotides were synthesized and incorporated into plasmid pBS/GMα/γ by standard PCR-based techniques, by splice-overlap extension PCR (20), or by second-strand synthesis after annealing to a single-stranded, uracil-containing template (Bio-Rad). Standard methods were used to confirm the sequences of all DNA fragments subjected to mutagenesis procedures.

Expression of chimeric receptor chains. Plasmids were introduced into CTLL2 and D10 cells by electroporation, and stably transfected lines or subclones were selected for resistance to G418 (1 mg/ml for CTLL2 transfectants and 0.5 mg/ml for D10 transfectants; Gibco/BRL) or hygromycin B (0.5 mg/ml for CTLL2 transfectants and 0.125 mg/ml for D10 transfectants; Sigma) or, in some cases, for growth in response to GM-CSF. Receptor expression was assessed by incubation of cells with antibodies anti-GM-CSFRα-M1 (Immunex) for GMα/γ and mutant derivatives and anti-GM-CSFRβ (Santa Cruz Biotechnology catalog no. SC-457) for GMβ/2β, followed by incubation with fluorescein isothiocyanate-conjugated secondary antibodies and flow cytometric analysis. For some experiments, cells were further selected for receptor expression by fluorescence-activated cell sorting after immunostaining.

Proliferative assays. CTLL2 or D10 cells were washed twice with phosphate-buffered saline (PBS) and incubated for 18 to 20 h in a 96-well plate at 4,000 (CTLL2) or 20,000 (D10) cells per well in complete medium with the indicated concentrations of cytokines. In the experiment shown in Fig. 3D, 0.5% bovine serum albumin (BSA) was substituted for 10% FCS where indicated. DNA synthesis was quantified by pulsing cells for 4 h with [^{3}H]thymidine (2.5 μCi) and then subjecting them to liquid scintillation counting.

GM-CSF binding assays. CTLL2 cells were washed twice with PBS and preblocked by incubation with phycoerythrin-conjugated streptavidin (R&D Systems) in PBS (4°C, 30 min). Cells were then incubated with various concentrations of phycoerythrin-conjugated human GM-CSF (PE*GM; R&D Systems) at 4°C for 1 h, washed twice with RDF1 buffer (R&D Systems), and fixed in 1% paraformaldehyde. The mean fluorescence intensity (MFI) of each sample was determined by flow cytometry. To correct for differences in MFI attributable to different receptor densities, relative fluorescence was calculated for each sample by assigning the MFI measured for the lowest concentration of PE*GM (1.44 ng/ml) an arbitrary value of 0 and the MFI for the highest concentration of PE*GM (3.14 μg/ml) a value of 1; relative values for intermediate concentrations of PE*GM were then calculated on the basis of a linear scale.

Analysis of proto-oncogene induction. CTLL2 or D10 cells were washed twice with PBS and incubated at a density of 10^6/ml in complete medium without added cytokines (37°C, 4 h). Cells were stimulated with human IL-2 (100 U/ml) or GM-CSF (100 ng/ml) at 37°C for 1 to 4 h. To prepare nuclear extracts, aliquots of 5×10^6 cells were washed once with PBS and once with buffer H [20 mM HEPES-NaOH (pH 7.9), 1 mM EDTA, 0.1 mM ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), 2 mM $MgCl_2$, 1 mM Na_3VO_4, 20 mM NaF, 1 mM dithiothreitol, 0.1 mM 4-(2-aminoethyl)-benzenesulfonyl fluoride (AEBSF), 1 μg of leupeptin per ml] and then lysed in 0.5 ml of buffer I (buffer H with 0.2% Nonidet P-40; 0°C, 10 min). Nuclei were pelleted by microcentrifugation (4°C, 2 min) and incubated in 20 μl of buffer K (buffer H with 0.42 M NaCl and 20% [vol/vol] glycerol; 4°C, 30 min) with occasional vortexing. Insoluble nuclear material was removed by microcentrifugation (4°C, 20 min), and extracts were boiled for 3 min in reducing sodium dodecyl sulfate (SDS) sample buffer. Extracts from 1.25×10^6 cells were run on 10% polyacrylamide gels and transferred to nitrocellulose. Western blotting (immunoblotting) was performed by blocking membranes in TTBS (0.1 M Tris, 0.9% NaCl, 0.05% Tween 20 [pH 7.5]) containing skim milk powder (5% [wt/vol]) and then incubating them with a rabbit antiserum specific for $p62^{c\text{-}fos}$ (55) (1:250 in blocking buffer; Santa Cruz Biotechnology catalog no. SC-52) or a mouse monoclonal antibody specific for $p67^{c\text{-}myc}$ (1:200; Santa Cruz Biotechnology catalog no. SC-42). Membranes were washed with TTBS and incubated with horseradish peroxidase-conjugated goat anti-rabbit or goat anti-mouse antibodies (Gibco/BRL) in blocking buffer. Bound antibodies were detected by enhanced chemiluminescence (Amersham).

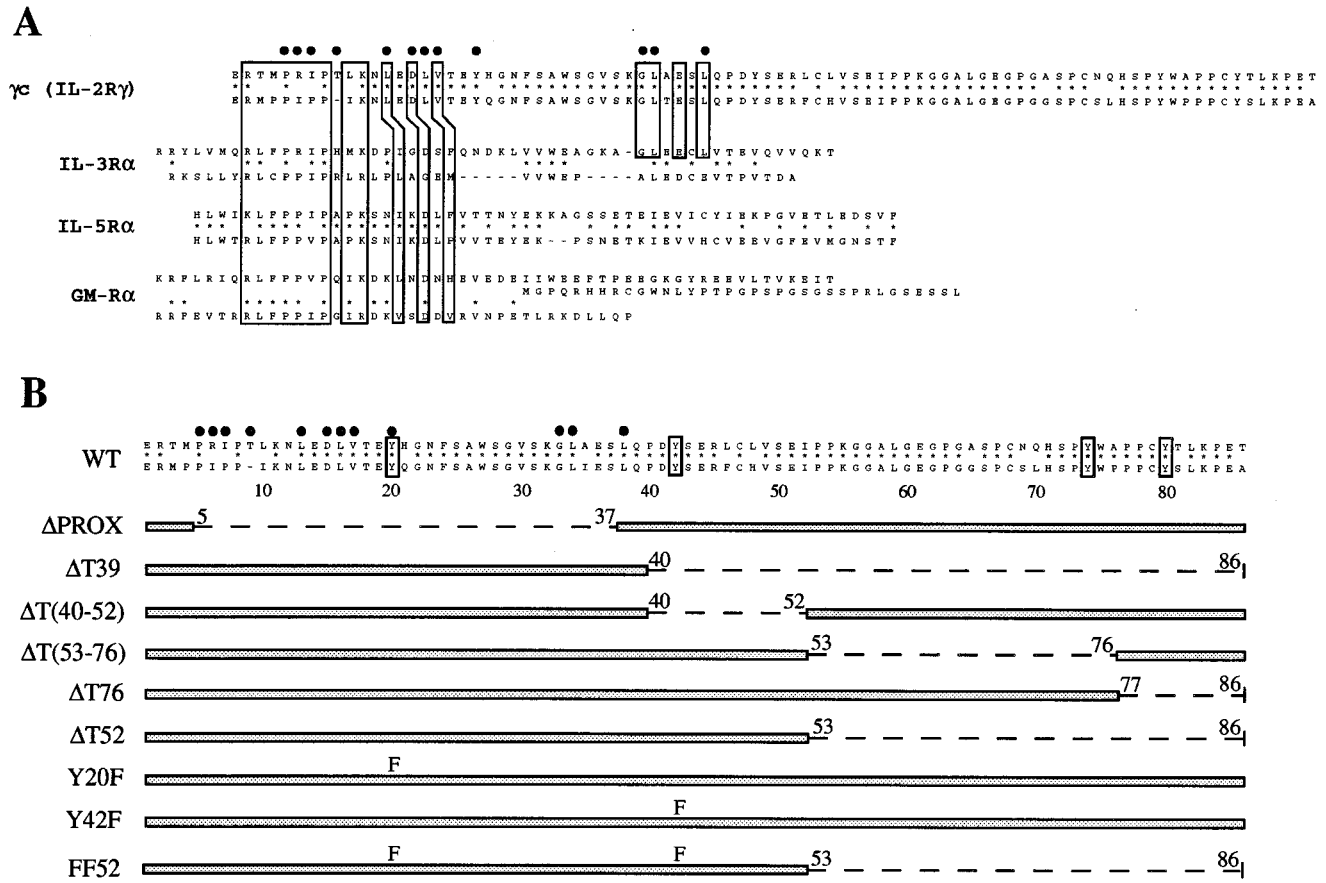

FIG. 2. Cytoplasmic signalling domains of γ_c. (A) Sequence alignment of the cytoplasmic domains of γ_c (5, 49), IL-3Rα (15, 22), IL-5Rα (31, 48), and GM-CSFRα (9, 12, 36) shown in single-letter amino acid code. For each receptor chain, the human sequence is shown above the murine sequence. Dots over the γ_c sequence denote residues characteristic of SH2 domains, asterisks indicate regions of identity between human and murine sequences, and boxes highlight conserved regions between heterologous receptor chains. As a result of alternative splicing, the C terminus of the human GM-CSFR α chain can have two different amino acid sequences. (B) Schematic representation of the cytoplasmic domains of the γ_c mutants analyzed in this study. The sequences of the cytoplasmic domains of human and murine γ_c are shown at the top, with SH2 domain-like residues highlighted by dots and tyrosine residues boxed. Nine mutants are shown below, with dashed lines indicating deleted regions and F denoting tyrosine-to-phenylalanine substitutions. WT, wild type.

Immunoprecipitation of Jak1 and Jak3. CTLL2 cells were washed twice with PBS, incubated at a density of 10^6/ml in complete medium without added cytokines (37°C, 4 h), and then stimulated with human IL-2 (100 U/ml) or GM-CSF (100 ng/ml) (37°C, 10 min). Aliquots of 1.5×10^7 cells were washed once with PBS and then lysed in 0.3 ml of buffer W (1% Triton X-100, 50 mM Tris-HCl [pH 7.6], 150 mM NaCl, 1 mM EDTA, 0.1 mM Na_3VO_4, 1 mM phenylmethylsulfonyl fluoride; 0°C, 10 min). Nuclei were removed by microcentrifugation (4°C, 10 min), and cytoplasmic fractions were precleared by addition of 1 μg of rabbit anti-rat immunoglobulin antiserum and 20 μl of protein A-agarose (Santa Cruz Biotechnology). Jak1 and Jak3 proteins were immunoprecipitated with specific antisera (Upstate Biotechnology Inc. catalog no. 06-272 and 06-342, respectively; 4°C, 1 h) and 20 μl of protein A-agarose (4°C, 1 h). Agarose-bound immune complexes were washed three times with buffer W and either boiled for 3 min in SDS sample buffer (for Western blotting) or subjected to an in vitro kinase assay.

Western blotting of Jak1 and Jak3. Immunoprecipitated proteins were run on 7% polyacrylamide gels and transferred to nitrocellulose. Membranes were incubated with TTBS containing either 3% BSA (Sigma; for antiphosphotyrosine blots) or 5% skim milk powder (Carnation; for anti-Jak blots), and then a mouse monoclonal antibody specific for phosphotyrosine (4G10; Upstate Biotechnology, Inc.) or rabbit antisera specific for Jak1 and Jak3 (Upstate Biotechnology Inc. cat 06-272 and 06-342, respectively) were added. Subsequent steps were as described above for proto-oncogene induction.

Jak3 in vitro kinase assay. Anti-Jak3 immune complexes on protein A-agarose were washed three times with cold kinase buffer (50 mM NaCl, 5 mM $MgCl_2$, 5 mM $MnCl_2$, 0.1 mM Na_3VO_4, 10 mM HEPES [pH 7.4]) and then incubated at 37°C for 35 min with 80 μl of kinase buffer containing 0.25 mCi of [γ-^{33}P]ATP (Dupont NEN) per ml. Complexes were washed three times with cold buffer W and boiled for 3 min in 40 μl of SDS sample buffer. ^{33}P-containing proteins were separated on 7% polyacrylamide gels and visualized by autoradiography.

RESULTS

Establishing a chimeric GM-CSF/IL-2 receptor system in T cells. To analyze the role of the cytoplasmic region of γ_c in T-cell proliferative responses, we modified a chimeric GM-CSF/IL-2 receptor consisting of two chains, GMα/γ and GMβ/2β, containing the intracellular regions of γ_c and IL-2Rβ, respectively (33). Experiments were performed in a murine IL-2-dependent T-cell line (CTLL2) as well as a $CD4^+$ T-helper clone (D10) that is representative of primary T cells in requiring stimulation with both antigen and IL-2 for sustained proliferation. An expression vector encoding GMβ/2β was introduced into CTLL2 and D10 cells, and stably transfected neomycin-resistant subclones were assessed for surface expression of GMβ/2β by flow cytometry. One subclone of each cell type, CTLL-GMβ/2β or D10-GMβ/2β, was used in all subsequent experiments to ensure a constant level of GMβ/2β expression. CTLL-GMβ/2β and D10-GMβ/2β cells were transfected with vectors encoding the wild-type or a mutant version of GMα/γ, selected for resistance to hygromycin, and evaluated by flow cytometry for expression of receptor chains. Results for CTLL2 cells presented in Fig. 1 are also representative of D10 cells and demonstrate that the wild-type and mutant forms of GMα/γ were stably expressed on the cell

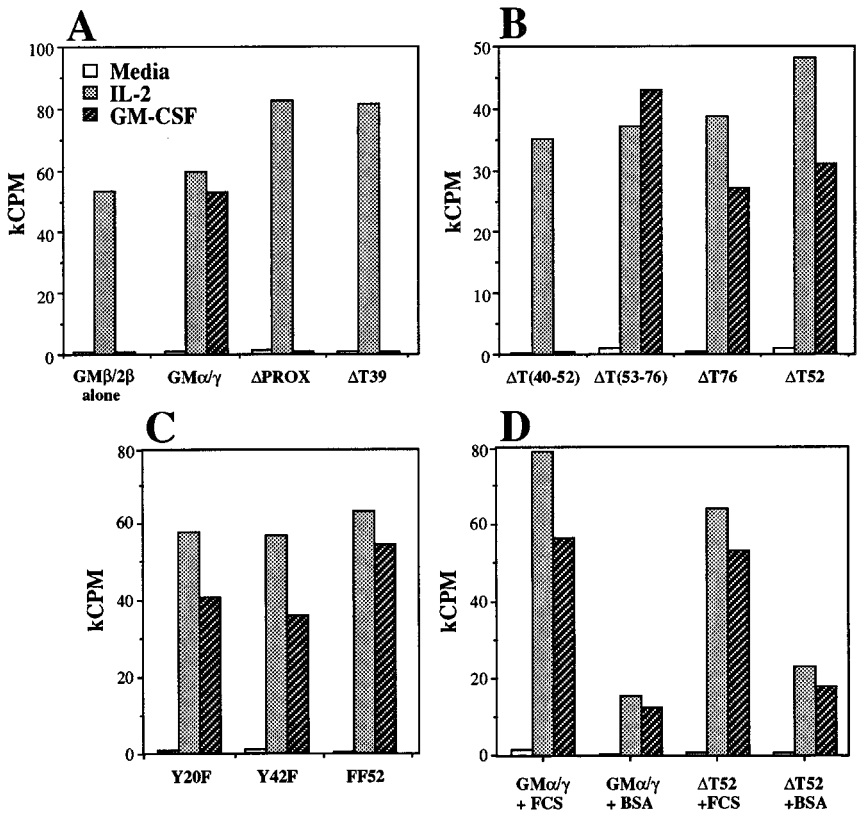

FIG. 3. Proliferative responses of CTLL2 and D10 T cells expressing chimeric GM-CSF/IL-2 receptor chains with mutations in the cytoplasmic domain of γ_c. Except for the leftmost sample in panel A, all cells expressed GMβ/2β in conjunction with the wild-type or a mutant form of GMα/γ. The data represent the mean counts per minute derived from triplicate wells; standard deviations ranged from 1 to 10% of the mean for positive responses and from 5 to 25% of the mean for responses at the background level (less than 1,000 cpm). (A to C) Proliferative responses of CTLL2 cells stimulated with medium alone, human IL-2 (5 U/ml, which induces an optimal proliferative response), or human GM-CSF (250 ng/ml). The cell lines used in these experiments were selected for expression of both chimeric receptor chains by fluorescence-activated cell sorting using antibodies to GMβ/2β and GMα/γ. For each mutant of GMα/γ, the results are representative of two to five experiments using cells derived from independent transfections. Additionally, the data shown for CTLL2 cells are representative of results obtained with the T-cell clone D10. (D) Proliferation of the T-cell clone D10 in response to medium alone, human IL-2 (20 U/ml), or human GM-CSF (100 ng/ml) in the presence (+FCS) or absence (+BSA) of FCS. The cell lines used in this experiment were selected for expression of chimeric receptor chains by growth on GM-CSF.

surface. The mutants GMα/γ-ΔT(53-76) and -FF52 were consistently expressed at low levels but nevertheless mediated normal proliferative responses (see below). All cells used for subsequent studies expressed GMα/γ-derived chains at levels within the range shown in Fig. 1 and had retained expression of GMβ/2β.

Role of γ_c subdomains in mediating T-cell proliferative signals. The membrane-proximal cytoplasmic region of γ_c, referred to as the PROX domain, is conserved in the α chains of the GM-CSFR, IL-3R, and IL-5R, related members of the hematopoietic superfamily (Fig. 2A). On the basis of this homology, we constructed two mutant chains: GMα/γ-ΔPROX, lacking the entire PROX domain; and GMα/γ-ΔT39, lacking all residues distal to this region (Fig. 2B). After transfection into CTLL-GMβ/2β or D10-GMβ/2β cells, neither the ΔPROX nor ΔT39 mutant chain mediated proliferation in response to GM-CSF, whereas a strong response was observed with wild-type GMα/γ (Fig. 3A). To identify the critical signalling residues absent in the ΔT39 mutant, we constructed three additional deletion mutants, GMα/γ-ΔT(40-52), -ΔT(53-76), and -ΔT76. CTLL-GMβ/2β or D10-GMβ/2β cells expressing the GMα/γ-ΔT(53-76) and -ΔT76 chains proliferated normally in response to GM-CSF, whereas cells expressing GMα/γ-ΔT(40-52) were completely nonresponsive (Fig. 3B). This finding suggested that the PROX domain and adjacent 14 amino acids are necessary for IL-2R signalling, whereas the distal 34 residues are dispensable. Therefore, we constructed an additional truncation mutant, GMα/γ-ΔT52, containing only the membrane-proximal 52 amino acids of γ_c (Fig. 2B). As predicted, cells expressing GMα/γ-ΔT52 proliferated normally in response to GM-CSF (Fig. 3B). GMα/γ-ΔT52 also induced short-term proliferation in serum-free medium, indicating that other growth factors in serum were not compensating for a signalling defect in this chain (Fig. 3D). The ability of mutant forms of GMα/γ to mediate short-term proliferation (Fig. 3) correlated with their abilities to promote long-term growth of T cells in culture (Fig. 4 and data not shown).

Role of tyrosine residues of γ_c in mediating T-cell proliferative signals. For receptor tyrosine kinases such as the platelet-derived growth factor receptor, cytoplasmic phosphotyrosine residues serve as binding sites for downstream signalling molecules (7). Since γ_c is phosphorylated on tyrosine in response to IL-2 (46), the requirement for the PROX domain and adjacent 14 amino acids could reflect critical signalling functions mediated by residue Y-20 or Y-42. To investigate this possibility, Y-20 and Y-42 were mutated to phenylalanine, generating the chains GMα/γ-Y20F and GMα/γ-Y42F, which were also recombined with the ΔT52 mutant to produce a chain completely devoid of cytoplasmic tyrosine residues (GMα/γ-FF52; Fig. 2B). GMα/γ-Y20F, -Y42F, and -FF52 mediated

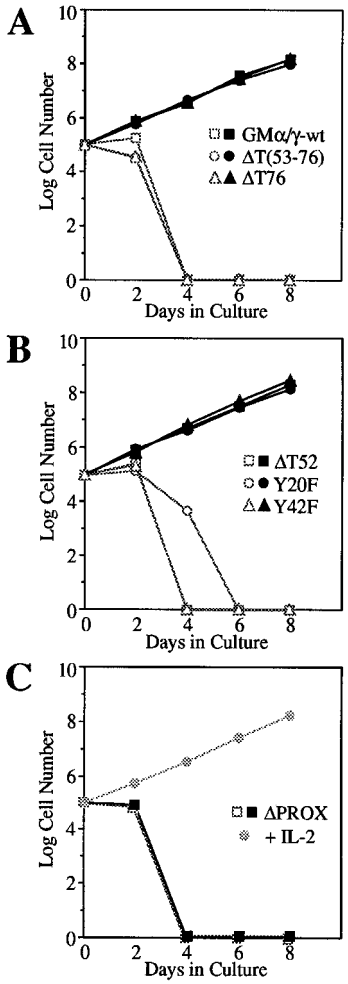

FIG. 4. In vitro growth of D10 T cells coexpressing GMβ/2β and wild-type GMα/γ (GMα/γ-wt) or a mutant form of GMα/γ. Cultures were established at a density of 10^5 cells per ml 1 week after antigen stimulation in the presence (solid symbols) or absence (open symbols) of human GM-CSF (250 ng/ml). In panel C, an aliquot of cells expressing GMα/γ-ΔPROX was cultured with human IL-2 (50 U/ml) as a positive control. The number of viable cells in each culture was determined every 2 days by staining with trypan blue, and cells were split as required to keep densities in the range of 1×10^5 to 5×10^5 cells per ml.

normal proliferation and growth in CTLL-GMβ/2β and/or D10-GMβ/2β cells (Fig. 3C and 4B), indicating that proliferative signalling by γ_c is not dependent on phosphotyrosine-mediated interactions with other molecules in the signalling complex.

Analysis of ligand binding. To confirm that the nonfunctional mutants of GMα/γ did not result from failure to bind ligand, cells expressing these chains were incubated with PE*GM and analyzed by flow cytometry. CTLL2 cells expressing GMβ/2β alone did not bind PE*GM (data not shown), consistent with the wild-type GM-CSFRβ chain requiring coexpression of GM-CSFRα for ligand binding (19). Importantly, functional and nonfunctional forms of GMα/γ, when coexpressed with GMβ/2β, demonstrated equivalent capacities for binding PE*GM over a 3-log-unit range of concentrations (Fig. 5).

With the functional mutants of GMα/γ, it was possible to assay the dose response to ligand binding by measuring proliferation of CTLL2 cells over a range of GM-CSF concentrations. All five mutant versions of GMα/γ, when coexpressed with GMβ/2β, induced proliferative responses equivalent to those found for wild-type GMα/γ at each concentration of GM-CSF, implying they have similar signalling thresholds and affinities for ligand (Fig. 6).

Induction of the proto-oncogenes c-*myc* and c-*fos*. Activation of the IL-2R induces a number of proto-oncogenes involved in cell growth, including c-*myc*, c-*fos*, and c-*jun*. Previous studies of IL-2Rβ in the pro-B-cell line BA/F3 and γ_c in transformed T cells have suggested that the acidic domain of IL-2Rβ and the C-terminal 30 amino acids of γ_c are required for induction of c-*fos* and c-*jun* but are dispensable for induction of c-*myc* and cell proliferation (43, 45). However, in IL-2-dependent T cells, we found a profound proliferative defect associated with deletion of the acidic region of IL-2Rβ (26a) but not deletion of the C terminus of γ_c (the GMα/γ-ΔT52 mutant in Fig. 3, 4, and 6). This discrepancy prompted us to reexamine the issue of proto-oncogene induction by mutant forms of γ_c. Nuclear extracts of GM-CSF-stimulated CTLL2 cells were probed with antibodies specific for $p67^{c\text{-}myc}$ and $p62^{c\text{-}fos}$, the latter also reflecting expression of the coordinately induced c-*jun* (43, 45). Similar to parental cells stimulated with IL-2, cells expressing GMβ/2β and wild-type GMα/γ had detectable expression of c-Myc and c-Fos proteins within 1 h of stimulation with GM-CSF, with maximal expression at 2 h for c-Myc and 4 h for c-Fos (Fig. 7A). The deletion mutants GMα/γ-ΔPROX, -ΔT39, and -ΔT(40-52) did not induce expression of either protein in response to GM-CSF. However, in contrast to predictions from previous studies (2, 3), GMα/γ-ΔT52 induced normal expression of both c-Myc and c-Fos (Fig. 7A). A similar pattern of proto-oncogene induction was observed in D10 T cells expressing the ΔT52 mutant, even in the absence of serum (Fig. 7B and data not shown). Thus, the membrane-proximal 52 amino acids of γ_c are necessary and sufficient for induction of c-*myc* and c-*fos*, and this correlates with generation of the proliferative response in T cells.

Activation of the tyrosine kinases Jak1 and Jak3. Recent studies have indicated that γ_c constitutively binds the tyrosine kinase Jak3, whereas a truncation mutant equivalent to ΔT39 does not (4, 29, 41). If activation of Jak3 is essential for proliferation, our data indicate that a γ_c chain containing the PROX domain and downstream 14 residues should be adequate for functional interaction with Jak3. Therefore, CTLL2 cells expressing chimeric receptor chains were stimulated with GM-CSF and assayed for Jak3 phosphorylation and catalytic activity. As predicted, cells coexpressing GMβ/2β and either

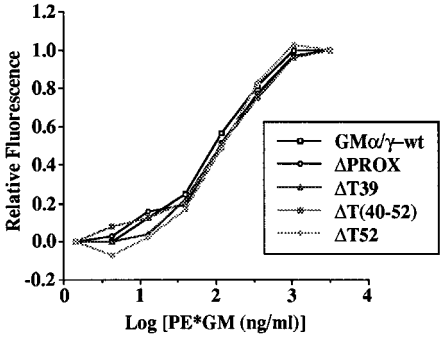

FIG. 5. Analysis of relative receptor affinities for GM-CSF. Subcloned CTLL2 cells expressing GMβ/2β in conjunction with wild-type GMα/γ (GMα/γ-wt) or a mutant form of GMα/γ were incubated with various concentrations of PE*GM. The fluorescence intensity of each sample was then measured by flow cytometry and converted to relative units.

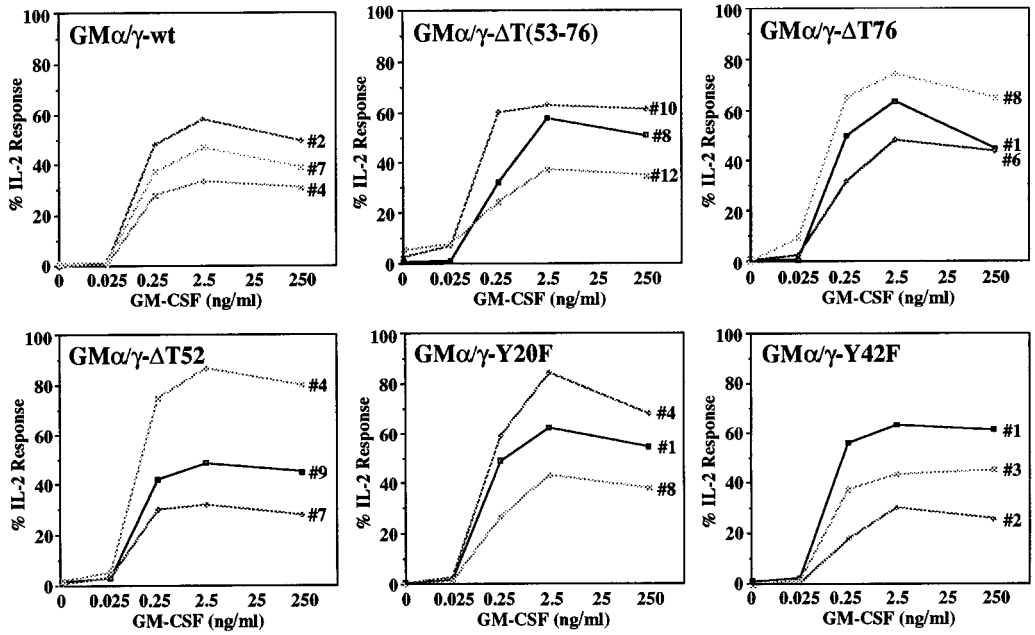

FIG. 6. Dose-response analysis of CTLL2 cells expressing GMβ/2β in conjunction with wild-type GMα/γ (GMα/γ-wt) or a mutant form of GMα/γ. Transfected cells were selected for growth on IL-2 in the presence of hygromycin and had not previously been exposed to GM-CSF. Cells were stimulated with medium alone, human IL-2 (10 U/ml), or human GM-CSF (0.025 to 250 ng/ml) and assayed for incorporation of tritiated thymidine. The response of each subclone to GM-CSF is plotted as a percentage of the response to IL-2, which ranged from 3×10^4 to 6×10^4 cpm.

wild-type GMα/γ or GMα/γ-ΔT52 demonstrated tyrosine phosphorylation and kinase activation of Jak3 in response to GM-CSF, whereas cells expressing GMα/γ-ΔPROX, -ΔT39, or -ΔT(40-52) did not (Fig. 8 and data not shown).

It has been suggested that the tyrosine kinase Jak1, which associates with the membrane-proximal region of IL-2Rβ (4, 29, 41, 52), may be a substrate for cross-phosphorylation by Jak3 in vivo. If this is the case, then phosphorylation of Jak1 should parallel the activation of Jak3 by mutant forms of γ_c. Indeed, CTLL2 cells coexpressing GMβ/2β and either wild-type GMα/γ or GMα/γ-ΔT52 demonstrated tyrosine phosphorylation of Jak1 in response to GM-CSF, whereas cells expressing GMα/γ-ΔPROX, -ΔT39, or -ΔT(40-52) did not (Fig. 8A). Together, our results support a model for IL-2R signalling in which ligand-mediated heterodimerization of IL-2Rβ and γ_c induces cross-phosphorylation and activation of Jak1 and Jak3, which contribute to activation of the mitogenic pathway in T cells.

DISCUSSION

The IL-2R signals by ligand-mediated heterodimerization of the cytoplasmic domains of IL-2Rβ and -γ_c (32, 33). The subdomains of the β and γ_c chains responsible for mitogenic signalling have not previously been investigated in IL-2-dependent T-cell lines or clones but have instead been defined through a variety of mutational studies in heterologous cell types. Here we report that of the 86 cytoplasmic residues of γ_c, the membrane-proximal 52 are sufficient for normal signalling in T cells, as assessed by proliferation, long-term growth, induction of the proto-oncogenes c-*myc* and c-*fos*, and activation of the receptor-associated tyrosine kinases Jak1 and Jak3.

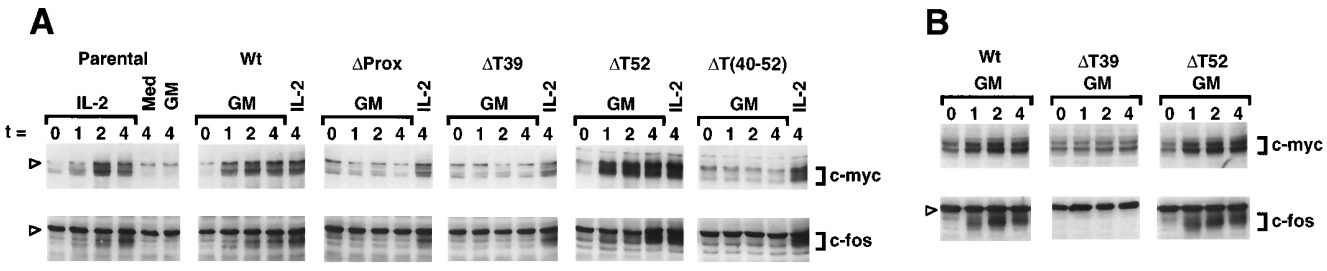

FIG. 7. Induction of c-Myc and c-Fos proteins expression in T cells stimulated with IL-2 or GM-CSF. CTLL2 and D10 cells deprived of exogenous cytokines were stimulated with medium alone (Med), human IL-2 (100 U/ml), or human GM-CSF (GM; 100 ng/ml) for 1 to 4 h. Nuclear extracts were analyzed by Western blot with antibodies specific for $p67^{c\text{-}myc}$ or $p62^{c\text{-}fos}$. (A) Parental CTLL2 cells and or subclones coexpressing GMβ/2β and the wild-type (Wt) or a mutant form of GMα/γ. Similar results were obtained when whole-cell lysates were analyzed, indicating that the observed increases in c-Myc and c-Fos expression reflect de novo synthesis rather than translocation of preexisting proteins from the cytoplasm to the nucleus (data not shown). (B) D10 cell lines coexpressing GMβ/2β and either wild-type GMα/γ or the ΔT52 or ΔT39 mutant. D10 cells generally display higher constitutive levels of c-Myc expression than CTLL2 cells. Arrowheads denote nonspecific staining of other nuclear proteins. Cell viability was assessed throughout the experiment by staining with trypan blue.

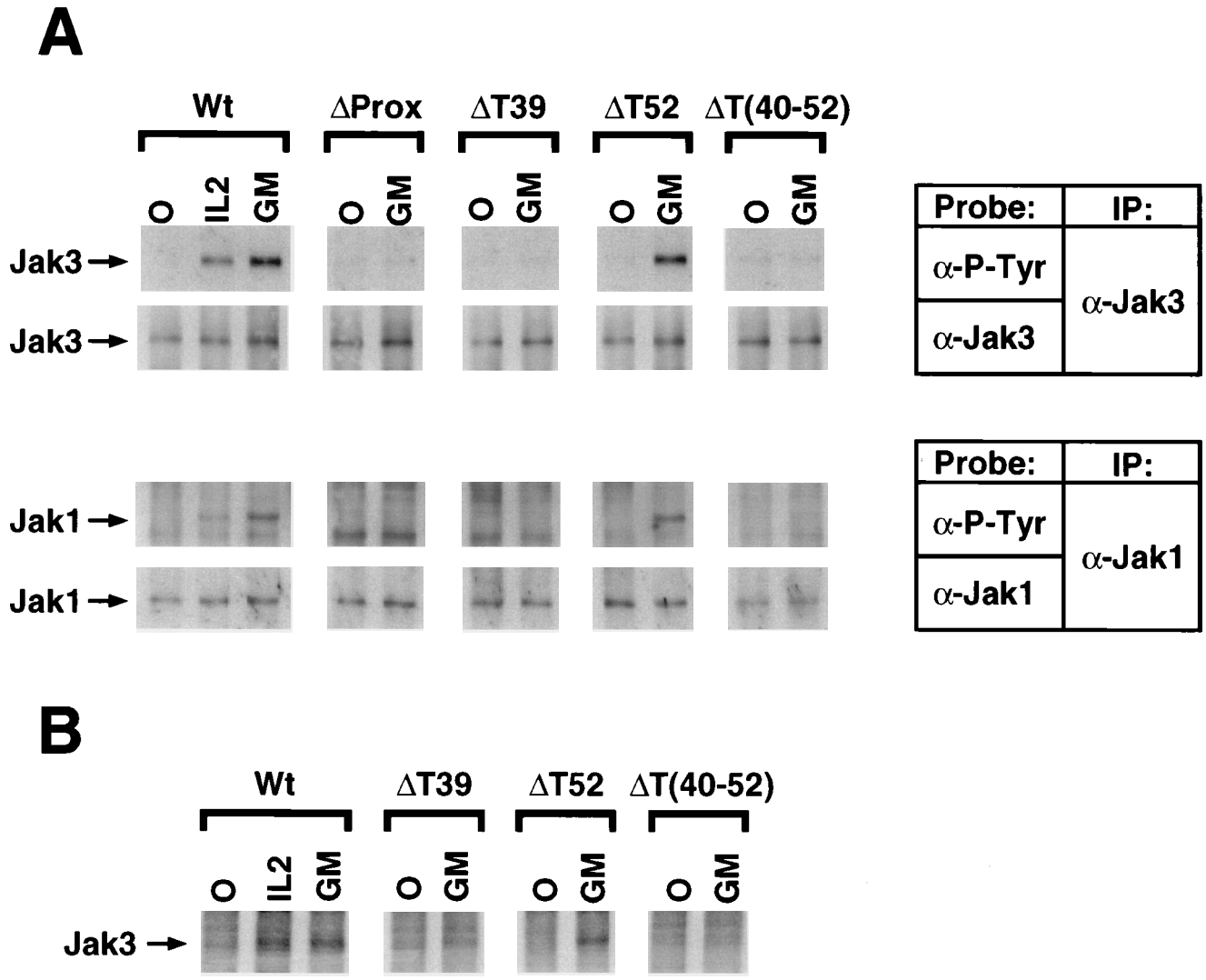

FIG. 8. GM-CSF-mediated phosphorylation and activation of the tyrosine kinases Jak1 and Jak3 in CTLL2 T cells expressing chimeric receptor chains. CTLL2-GMβ/2β subclones expressing the wild-type (Wt) or a mutant form of GMα/γ were stimulated with either medium alone, human IL-2 (100 U/ml), or GM-CSF (GM; 100 ng/ml), and Jak1 and Jak3 proteins were immunoprecipitated (IP) from cytoplasmic fractions. (A) Tyrosine phosphorylation of Jak1 and Jak3 assessed by Western blot with a phosphotyrosine-specific antibody (α-P-Tyr). Membranes were stripped and reprobed with an antiserum to Jak1 or Jak3 to ensure equivalent loading of proteins in each lane. (B) Induction of Jak3 catalytic activity assessed by in vitro autokinase assay. Immunoprecipitated Jak3 proteins were incubated with [γ-^{33}P]ATP, separated by SDS-polyacrylamide gel electrophoresis, and visualized by autoradiography.

These responses were lost coordinately with further truncation of γ_c to 39 cytoplasmic residues, or with an internal deletion of residues 40 to 52, indicating that the minimal signalling domain is between 40 and 52 amino acids in length. Notably, the mutant forms of γ_c analyzed in this study fell into only two phenotypic classes: those that induced normal proliferation and expression of both c-*myc* and c-*fos*, and those that failed to mediate any of these responses. This finding is in clear contrast to previous studies performed in fibroblasts and a leukemic T-cell line which suggested that two separable signalling pathways might emanate from γ_c. Specifically, Sugamura's group reported that the C-terminal 30 amino acids of γ_c are required for induction of c-*fos* and c-*jun*, but not expression of c-*myc* or cell proliferation, whereas the membrane-proximal region is required for all of these responses (2, 3, 21). The differences between our results and those of Sugamura's group can likely be attributed to their use of fibroblasts and transformed T cells instead of IL-2-dependent T cells. In cell types that do not normally utilize the IL-2R, signalling may be mediated by unconventional downstream molecules with different requirements of the IL-2R chains. For example, we have previously reported the different signalling properties of the IL-2Rβ cytoplasmic domain in the pro-B-cell line BA/F3 compared with T cells (33).

The results of Sugamura's group pertaining to γ_c, together with studies of IL-2Rβ in the pro-B-cell line BA/F3 (43, 45), have suggested that the membrane-proximal regions of IL-2Rβ and γ_c interact to generate a signal leading to cell proliferation and expression of c-*myc*, whereas the acidic region of IL-2Rβ and the distal region of γ_c interact to generate a signal required for expression of c-*fos* and c-*jun*. Our studies of IL-2Rβ and γ_c in CTLL2 and D10 T cells lead to an alternative model. As demonstrated here, the signalling functions of the γ_c chain all map to the same membrane-proximal region; however, we do find evidence of at least two distinct signalling domains on IL-2Rβ (26a), consistent with the prior studies in BA/F3 cells mentioned above. Therefore, we propose that the membrane-proximal 52 residues of γ_c interact with both the serine-rich

and acidic regions of IL-2Rβ to generate two distinct signals leading to induction of c-*myc* and of c-*fos* and c-*jun*. The two signals could result from direct interactions between the domains of the IL-2R β and γ_c chains and/or from indirect interactions involving receptor-associated molecules such as Jak1, Jak3, Syk, or Lck (4, 16, 28, 29, 41, 52).

The precise molecular function of the PROX domain and downstream 14 amino acids of γ_c remains undefined. Both regions contain tyrosine residues and therefore represent potential sites for phosphotyrosine-mediated interactions with other molecules, as has been demonstrated for other growth factor receptors such as the platelet-derived growth factor receptor (7). However, if such interactions occur with γ_c, they are unrelated to mitogenic signalling, as a mutant form of GMα/γ devoid of cytoplasmic tyrosine residues (FF52) mediated a normal proliferative response in CTLL2 cells. An alternative function of the membrane-proximal region is suggested by our observation that the PROX domain and downstream 14 residues of γ_c are necessary and sufficient for functional interaction with the tyrosine kinase Jak3. Prior reports demonstrated that a region distal to cytoplasmic residue Q-39 of γ_c is necessary for binding Jak3 (4, 29, 41); our functional studies suggest that this region is located between residues 40 to 52, as the remainder of the C terminus of γ_c is dispensable for activation of Jak3. Our data further suggest that Jak3 is a critical mediator of multiple signalling functions in T cells, as activation of Jak3 by mutants of γ_c correlated with phosphorylation of Jak1, expression of c-*myc* and c-*fos*, and induction of the proliferative response. These findings are consistent with a model in which the sole signalling function of γ_c is to bring Jak3 in contact with Jak1 and/or other molecules associated with IL-2Rβ after ligand-mediated receptor dimerization. Alternatively, in addition to Jak3, the membrane-proximal region of γ_c might interact with other, as yet unidentified molecules that are also critical for signalling. Indeed, the PROX domain of γ_c is conserved among the α chains of the IL-3R, IL-5R, and GM-CSFR (Fig. 2A), none of which have been reported to bind Jak kinases (8, 40). In the case of the IL-5R and GM-CSFR, residues within this region of homology have been shown to be essential for mitogenic signalling (38, 47). Thus, the membrane-proximal regions of all of these receptor chains may perform a common signalling function unrelated to the Jak/STAT pathway.

The finding that the C-terminal 34 amino acids of γ_c are dispensable for IL-2R signalling in T cells is remarkable given the high degree of sequence conservation in this region across species. This is reminiscent of the finding that the C-terminal portion of IL-2Rβ, which is also highly conserved, is dispensable for mitogenic signalling (17, 26a). The distal region of IL-2Rβ has recently been implicated in the activation of STAT transcription factors (11, 26), which raises the intriguing possibility that the distal region of γ_c contributes to nonmitogenic signalling functions mediated by the IL-2R, IL-4R, IL-7R, IL-9R, or IL-15R.

ACKNOWLEDGMENTS

This work was supported by grants from the NIH/NIAID (AI 36613) and the NIH/NCI (CA 18029 and CA 33084). B.H.N. was supported by a fellowship from the MRC of Canada, and J.D.L. was supported by a fellowship from the U.S. Department of Defense.

We thank Angel Lopez for providing antibody to the GM-CSFR β chain, Immunex Corp. for human GM-CSF, Brian Iritani and Bryan McIntosh for help in constructing receptor mutants, Lori Rosencrantz for technical assistance, and Jenny Michaels for help with preparation of the manuscript.

REFERENCES

1. **Arima, N., M. Kamio, K. Imada, T. Hori, T. Hattori, M. Tsudo, M. Okuma, and T. Uchiyama.** 1992. Pseudo-high affinity interleukin 2 (IL-2) receptor lacks the third component that is essential for functional IL-2 binding and signaling. J. Exp. Med. **176:**1265–1272.
2. **Asao, H., T. Takeshita, N. Ishii, S. Kumaki, M. Nakamura, and K. Sugamura.** 1993. Reconstitution of functional interleukin 2 receptor complexes on fibroblastoid cells: involvement of the cytoplasmic domain of the gamma chain in two distinct signaling pathways. Proc. Natl. Acad. Sci. USA **90:**4127–4131.
3. **Asao, H., N. Tanaka, N. Ishii, M. Higuchi, T. Takeshita, M. Nakamura, T. Shirasawa, and K. Sugamura.** 1994. Interleukin 2-induced activation of JAK3: possible involvement in signal transduction for c-myc induction and cell proliferation. FEBS Lett. **351:**201–206.
4. **Boussiotis, V. A., D. L. Barber, T. Nakarai, G. J. Freeman, J. G. Gribben, G. M. Bernstein, A. D. D'Andrea, J. Ritz, and L. M. Nadler.** 1994. Prevention of T cell anergy by signaling through the gamma c chain of the IL-2 receptor. Science **266:**1039–1042.
5. **Cao, X., C. A. Kozak, Y.-J. Liu, M. Noguchi, E. O'Connell, and W. J. Leonard.** 1993. Characterization of cDNAs encoding the murine interleukin 2 receptor (IL-2R) gamma chain: chromosomal mapping and tissue specificity of IL-2R gamma chain expression. Immunology **90:**8464–8468.
6. **Cao, X., E. W. Shores, J. Hu-Li, M. R. Anver, B. L. Kelsall, S. M. Russell, J. Drago, M. Noguchi, A. Grinberg, E. T. Bloom, W. E. Paul, S. I. Katz, P. E. Love, and W. J. Leonard.** 1995. Defective lymphoid development in mice lacking expression of the common cytokine receptor gamma chain. Immunity **2:**223–238.
7. **Claesson-Welsh, L.** 1994. Platelet-derived growth factor receptor signals. J. Biol. Chem. **269:**32023–32026.
8. **Cornelis, S., I. Fache, J. Van der Heyden, Y. Guisez, J. Tavernier, R. Devos, W. Fiers, and G. Plaetinck.** 1995. Characterization of critical residues in the cytoplasmic domain of the human interleukin-5 receptor alpha chain required for growth signal transduction. Eur. J. Immunol. **25:**1857–1864.
9. **Crosier, K. E., G. G. Wong, B. Mathey-Prevot, D. G. Nathan, and C. A. Sieff.** 1991. A functional isoform of the human granulocyte/macrophage colony-stimulating factor receptor has an unusual cytoplasmic domain. Proc. Natl. Acad. Sci. USA **88:**7744–7748.
10. **DiSanto, J. P., W. Muller, D. Guy-Grand, A. Fischer, and K. Rajewsky.** 1995. Lymphoid development in mice with a targeted deletion of the interleukin 2 receptor gamma chain. Proc. Natl. Acad. Sci. USA **92:**377–381.
11. **Fujii, H., Y. Nakagawa, U. Schindler, A. Kawahara, H. Mori, F. Gouilleux, B. Groner, J. Ihle, Y. Minami, T. Miyazaki, and T. Taniguchi.** 1995. Activation of stat5 by interleukin 2 requires a carboxyl-terminal region of the interleukin 2 receptor beta chain but is not essential for the proliferative signal transmission. Proc. Natl. Acad. Sci. USA **92:**5482–5486.
12. **Gearing, D. P., J. A. King, N. M. Gough, and N. A. Nicola.** 1989. Expression cloning of a receptor for human granulocyte-macrophage colony-stimulating factor. EMBO J. **8:**3667–3676.
13. **Giri, J. G., M. Ahdieh, J. Eisenman, K. Shanebeck, K. Grabstein, S. Kumaki, A. Namen, L. S. Park, D. Cosman, and K. Anderson.** 1994. Utilization of the beta and gamma chains of the IL-2 receptor by the novel cytokine IL-15. EMBO J. **13:**2822–2830.
14. **Gunning, P., J. Leavitt, G. Muscat, S. Ng, and L. Kedes.** 1987. A human beta-actin expression vector system directs high-level accumulation of antisense transcripts. Proc. Natl. Acad. Sci. USA **84:**4831–4835.
15. **Hara, T., and A. Miyajima.** 1992. Two distinct functional high affinity receptors for mouse interleukin-3 (IL-3). EMBO J. **11:**1875–1884.

15a. **Harada, N., and B. H. Nelson.** Unpublished data.

16. **Hatakeyama, M., T. Kono, N. Kobayashi, A. Kawahara, S. D. Levin, R. M. Perlmutter, and T. Taniguchi.** 1991. Interaction of the IL-2 receptor with the src-family kinase p56lck: identification of novel intermolecular association. Science **252:**1523–1528.
17. **Hatakeyama, M., H. Mori, T. Doi, and T. Taniguchi.** 1989. A restricted cytoplasmic region of IL-2 receptor beta chain is essential for growth signal transduction but not for ligand binding and internalization. Cell **59:**837–845.
18. **Hatakeyama, M., M. Tsudo, S. Minamoto, T. Kono, T. Doi, T. Miyata, M. Miyasaka, and T. Taniguchi.** 1989. Interleukin-2 receptor beta chain gene: generation of three receptor forms by cloned human alpha and beta chain cDNA's. Science **244:**551–556.
19. **Hayashida, K., T. Kitamura, D. M. Gorman, K.-I. Arai, T. Yokota, and A. Miyajima.** 1990. Molecular cloning of a second subunit of the receptor for human granulocyte-macrophage colony-stimulating factor (GM-CSF): reconstitution of a high-affinity GM-CSF receptor. Proc. Natl. Acad. Sci. USA **87:**9655–9659.
20. **Ho, S., H. Hunt, R. Horton, J. Pullen, and L. Pease.** 1989. Site-directed mutagenesis by overlap extension using the polymerase chain reaction. Gene **77:**51–59.
21. **Ishii, N., H. Asao, Y. Kimura, T. Takeshita, M. Nakamura, S. Tsuchiya, T. Konno, M. Maeda, T. Uchiyama, and K. Sugamura.** 1994. Impairment of ligand binding and growth signaling of mutant IL-2 receptor gamma chains in patients with X-linked severe combined immunodeficiency. J. Immunol. **153:**1310–1317.

22. **Kitamura, T., N. Sato, K. Arai, and A. Miyajima.** 1991. Expression cloning of the human IL-3 receptor cDNA reveals a shared beta subunit for the human IL-3 and GM-CSF receptors. Cell **66:**1165–1174.
23. **Koch, C. A., D. Anderson, M. F. Moran, C. Ellis, and T. Pawson.** 1991. SH2 and SH3 domains: elements that control interactions of cytoplasmic signaling proteins. Science **252:**668–674.
24. **Kondo, M., T. Takeshita, M. Higuchi, M. Nakamura, T. Sudo, S. Nishikawa, and K. Sugamura.** 1994. Functional participation of the IL-2 receptor gamma chain in IL-7 receptor complexes. Science **263:**1453–1454.
25. **Kondo, M., T. Takeshita, N. Ishii, M. Nakamura, S. Watanabe, K. Arai, and K. Sugamura.** 1993. Sharing of the interleukin-2 (IL-2) receptor gama chain between receptors for IL-2 and IL-4. Science **262:**1874–1877.
26. **Lin, J., T. Migone, M. Tsang, M. Friedmann, J. A. Wetherbee, L. Zhou, A. Yamauchi, E. T. Bloom, J. Mietz, S. John, and W. J. Leonard.** 1995. The role of shared receptor motifs and common stat proteins in the generation of cytokine pleiotropy and redundancy by IL-2, IL-4, IL-7, IL-13, and IL-15. Immunity **2:**331–339.

26a. **Lord, J. D., B. H. Nelson, and P. D. Greenberg.** Unpublished data.

27. **Merida, I., P. Williamson, W. A. Kuziel, W. C. Greene, and G. N. Gaulton.** 1993. The serine-rich cytoplasmic domain of the interleukin-2 receptor beta chain is essential for interleukin-2-dependent tyrosine protein kinase and phosphatidylinositol-3-kinase activation. J. Biol. Chem. **268:**6765–6770.
28. **Minami, Y., Y. Nakagawa, A. Kawahara, T. Miyazaki, K. Sada, H. Yamamura, and T. Taniguchi.** 1995. Protein tyrosine kinase Syk is associated with and activated by the IL-2 receptor: possible link with the c-myc induction pathway. Immunity **2:**89–100.
29. **Miyazaki, T., A. Kawahara, H. Fujii, Y. Nakagawa, Y. Minami, Z. Liu, I. Oishi, O. Silvennoinen, B. Witthuhn, and J. Ihle.** 1994. Functional activation of Jak1 and Jak3 by selective association with IL-2 receptor subunits. Science **266:**1045–1047.
30. **Miyazaki, T., Z.-J. Liu, A. Kawahara, Y. Minami, K. Yamada, Y. Tsujimoto, E. L. Barsoumian, R. M. Perlmutter, and T. Taniguchi.** 1995. Three distinct IL-2 signaling pathways mediated by bcl-2, c-myc, and lck cooperate in hematopoietic cell proliferation. Cell **81:**223–231.
31. **Murata, Y., S. Takaki, M. Migita, Y. Kikuchi, A. Tominaga, and K. Takatsu.** 1992. Molecular cloning and expression of the human interleukin 5 receptor. J. Exp. Med. **175:**341–351.
32. **Nakamura, Y., S. Russell, S. Mess, M. Friedmann, M. Erdos, C. Francois, Y. Jacques, S. Adelstein, and W. Leonard.** 1994. Heterodimerization of the IL-2 receptor beta- and gamma-chain cytoplasmic domains is required for signalling. Nature (London) **369:**330–333.
33. **Nelson, B. H., J. D. Lord, and P. D. Greenberg.** 1994. Cytoplasmic domains of the interleukin-2 receptor beta and gamma chains mediate the signal for T-cell proliferation. Nature (London) **369:**333–336.
34. **Noguchi, M., Y. Nakamura, S. M. Russell, S. F. Ziegler, M. Tsang, X. Cao, and W. J. Leonard.** 1993. Interleukin-2 receptor gamma chain: a functional component of the interleukin-7 receptor. Science **262:**1877–1880.
35. **Noguchi, M., H. Yi, H. M. Rosenblatt, A. H. Filipovich, S. Adelstein, W. S. Modi, O. W. McBride, and W. J. Leonard.** 1993. Interleukin-2 receptor gamma chain mutation results in X-linked severe combined immunodeficiency in humans. Cell **73:**147–157.
36. **Park, L. S., U. Martin, R. Sorensen, S. Luhr, P. J. Morrissey, D. Cosman, and A. Larsen.** 1992. Cloning of the low-affinity murine granulocyte-macrophage colony-stimulating factor receptor and reconstitution of a high-affinity receptor complex. Proc. Natl. Acad. Sci. USA **89:**4295–4299.
37. **Peschon, J. J., P. J. Morrissey, K. H. Grabstein, F. J. Ramsdell, E. Maraskovsky, B. C. Gliniak, L. S. Park, S. F. Ziegler, D. E. Williams, C. B. Ware, et al.** 1994. Early lymphocyte expansion is severely impaired in interleukin 7 receptor-deficient mice. J. Exp. Med. **180:**1955–1960.
38. **Polotskaya, A., Y. Zhao, M. B. Lilly, and A. S. Kraft.** 1994. Mapping the intracytoplasmic regions of the alpha granulocyte-macrophage colony-stimulating factor receptor necessary for cell growth regulation. J. Biol. Chem. **269:**14607–14613.
39. **Puck, J. M., S. M. Deschenes, J. C. Porter, A. S. Dutra, C. J. Brown, H. F. Willard, and P. S. Henthorn.** 1993. The interleukin-2 receptor gamma chain maps to Xq13.1 and is mutated in X-linked severe combined immunodeficiency, SCIDX1. Hum. Mol. Genet. **2:**1099–1104.
40. **Quelle, F. W., N. Sato, B. A. Witthuhn, R. C. Inhorn, M. Eder, A. Miyajima, J. D. Griffin, and J. N. Ihle.** 1994. JAK2 associates with the β_c chain of the receptor for granulocyte-macrophage colony-stimulating factor, and its activation requires the membrane-proximal region. Mol. Cell. Biol. **14:**4335–4341.
41. **Russell, S., J. Johnston, M. Noguchi, M. Kawamura, C. Bacon, M. Friedmann, M. Berg, D. McVicar, B. Witthuhn, and O. Silvennoinen.** 1994. Interaction of IL-2R beta and gamma c chains with Jak1 and Jak3: implications for XSCID and XCID. Science **266:**1042–1045.
42. **Russell, S. M., A. D. Keegan, N. Harada, Y. Nakamura, M. Noguchi, P. Leland, M. C. Friedmann, A. Miyajima, R. K. Puri, W. E. Paul, and W. J. Leonard.** 1993. Interleukin-2 receptor gamma chain: a functional component of the interleukin-4 receptor. Science **262:**1880–1883.
43. **Satoh, T., Y. Minami, T. Kono, K. Yamada, A. Kawahara, T. Taniguchi, and Y. Kaziro.** 1992. Interleukin 2-induced activation of ras requires two domains of interleukin 2 receptor beta subunit, the essential region for growth stimulation and lck-binding domain. J. Biol. Chem. **267:**25423–25427.
44. **Schorle, H., T. Holtschke, T. Hunig, A. Schimpl, and I. Horak.** 1991. Development and function of T cells in mice rendered interleukin-2 deficient by gene targeting. Nature (London) **352:**621–624.
45. **Shibuya, H., M. Yoneyama, J. Ninomiya-Tsuji, K. Matsumoto, and T. Taniguchi.** 1992. IL-2 and EGF receptors stimulate the hematopoietic cell cycle via different signaling pathways: demonstration of a novel role for c-myc. Cell **70:**57–67.
46. **Sugamura, K., T. Takeshita, H. Asao, S. Kumaki, K. Ohbo, K. Ohtani, and M. Nakamura.** 1990. IL-2-induced signal transduction: involvement of tyrosine kinase and IL-2 receptor gamma chain. Lymphokine Res. **9:**539–542.
47. **Takaki, S., H. Kanazawa, M. Shiiba, and K. Takatsu.** 1994. A critical cytoplasmic domain of the interleukin-5 (IL-5) receptor alpha chain and its function in IL-5-mediated growth signal transduction. Mol. Cell. Biol. **14:**7404–7413.
48. **Takaki, S., A. Tominaga, Y. Hitoshi, S. Mita, E. Sonoda, N. Yamaguchi, and K. Takatsu.** 1990. Molecular cloning and expression of the murine interleukin-5 receptor. EMBO J. **9:**4367–4374.
49. **Takeshita, T., H. Asao, K. Ohtani, N. Ishii, S. Kumaki, N. Tanaka, H. Munakata, M. Nakamura, and K. Sugamura.** 1992. Cloning of the gamma chain of the human IL-2 receptor. Science **257:**379–382.
50. **Takeshita, T., H. Asao, J. Suzuki, and K. Sugamura.** 1990. An associated molecule, p64, with high-affinity interleukin 2 receptor. Int. Immunol. **2:**477–480.
51. **Takeshita, T., K. Ohtani, H. Asao, S. Kumaki, M. Nakamura, and K. Sugamura.** 1992. An associated molecule, p64 with IL-2 receptor beta chain. Its possible involvement in the formation of the functional intermediate-affinity IL-2 receptor complex. J. Immunol. **148:**2154–2158.
52. **Tanaka, N., H. Asao, K. Ohbo, N. Ishii, T. Takeshita, M. Nakamura, H. Sasaki, and K. Sugamura.** 1994. Physical association of Jak1 and Jak2 tyrosine kinases with the interleukin 2 receptor beta and gamma chains. Proc. Natl. Acad. Sci. USA **91:**7271–7275.
53. **von Freeden-Jeffry, U., P. Vieira, L. A. Lucian, T. McNeil, S. E. Burdach, and R. Murray.** 1995. Lymphopenia in interleukin (IL)-7 gene-deleted mice identifies IL-7 as a nonredundant cytokine. J. Exp. Med. **181:**1519–1526.
54. **Voss, S. D., P. M. Sondel, and R. J. Robb.** 1992. Characterization of the interleukin 2 receptors (IL-2R) expressed on human natural killer cells activated in vivo by IL-2: association of the p64 IL-2R gamma chain with the IL-2R beta chain in functional intermediate-affinity IL-2R. J. Exp. Med. **176:**531–541.
55. **Yaseen, N. R., J. Park, T. Kerppola, T. Curran, and S. Sharma.** 1994. A central role for Fos in human B- and T-cell NFAT (nuclear factor of activated T cells): an acidic region is required for in vitro assembly. Mol. Cell. Biol. **14:**6886–6895.