Identification, cDNA Cloning, and Targeted Deletion of p70, a Novel, Ubiquitously Expressed SH3 Domain-Containing Protein

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In a screen for proteins that interact with Jak2, we identified a previously uncharacterized 70-kDa protein and cloned the corresponding cDNA. The predicted sequence indicates that p70 contains an SH3 domain and a C-terminal domain with similarities to the catalytic motif of phosphoglycerate mutase. p70 transcripts were found in all tissues examined. Similarly, when an antibody raised against a C-terminal peptide to analyze p70 protein expression was used, all murine tissues examined were found to express p70. To investigate the in vivo role of p70, we generated a p70-deficient mouse strain. Mice lacking p70 are viable, develop normally, and do not display any obvious abnormalities. No differences were detected in various hematological parameters, including bone marrow colony-forming ability, in response to cytokines that utilize Jak2. In addition, no impairment in B- and T-cell development and proliferative ability was detected.

A variety of cytokine receptors recruit and mediate the activation of Jak2, including the receptor for erythropoietin (Epo) (7, 13). The essential role that Jak2 plays in the function of these receptor complexes is illustrated by the loss of their function in Jak2-deficient cells (15, 16). The activation of Jak2 results in the tyrosine phosphorylation of multiple sites on the receptor chains and the subsequent recruitment of a variety of proteins to the receptor complex (5, 20, 24). The proteins recruited to the receptor complex are hypothesized to be critical for the subsequent cellular responses (see, for example, reference 4). However, in the case of the receptor for Epo, mice have been derived in which the distal half of the receptor cytoplasmic domain has been deleted and in which the single remaining tyrosine has been mutated to a phenylalanine (27). Remarkably, these mice are relatively normal, and, specifically, the Epo receptor retains its ability to support erythroid lineage expansion and differentiation. In the absence of evidence that receptor tyrosines are required for recruitment of essential signaling proteins to the receptor complex, we have focused on the possibility that sites of tyrosine phosphorylation on Jak2 may play essential roles in recruiting signaling proteins (10, 21, 27).

The activation of Jak2 initially requires transphosphorylation of a critical tyrosine in the activation loop, Y1007, comparable to most tyrosine kinases (6). Following activation of kinase activity, there are a number of additional tyrosine residues that are phosphorylated. One of the major sites of autophosphorylation within murine Jak2 is Y966 (T. Matsuda and J. N. Ihle, unpublished data). In assessing the potential role for this site, we initially sought to determine which of the known signaling proteins would bind to the site by affinity isolation. Strikingly, a number of proteins known to be involved in signal transduction bound a tyrosine-phosphorylated peptide derived from the amino acid sequence surrounding...
Among these proteins were phospholipase (PLC)\textsubscript{1}, PLC\textsubscript{2}, the phosphatidylinositol (PI) 3-kinase adapter subunits p85\textsubscript{1} and p85\textsubscript{2}, the PI 3-kinase catalytic subunit p110, SHC, and Stat5a and -b. The binding was unique to Jak2-Y966 among the phosphorylation sites examined and specifically required the phosphorylation of Y 966. Because of the unique properties of Y 966, we used a large-scale affinity purification approach to identify novel proteins that would be recruited into complexes at this site and identified a novel protein of 70 kDa. Here we report the properties of p70, demonstrate that mice lacking p70 are not detectably altered relative to wild-type mice, and show that p70 is not required for the responses of cytokines that utilize Jak2.

**MATERIALS AND METHODS**

**Peptide pulldown assay.** DA3 cells (11) growing in media containing 10% fetal calf serum (FCS) and 5 ng of interleukin 3 (IL-3)/ml were washed twice with phosphate-buffered saline (PBS) and were incubated overnight in media containing 0.5% FCS. Unstimulated cells or cells treated for 10 min with 50 ng of IL-3/ml (10^6/assay) were lysed in buffer containing 50 mM Tris, pH 8.0, 50 mM NaCl, 5 mM EDTA, 1 mM EGTA, 1% Triton X-100, 0.5 g of leupeptin/ml, 0.1 g of aprotinin/ml, 100 g of phenylmethylsulfonyl fluoride (PMSF)/ml, and 1 mM Na\textsubscript{3}VO\textsubscript{4}. Following removal of the nuclei by centrifugation at 10,000 g, cytoplasmic lysates were incubated with 20 l of Sepharose beads that had been conjugated by glutaraldehyde either with the peptide TSQICKGMEYLGK or the peptide TSQICKGMEpYLGTKR. The beads were incubated with the cytoplasmic lysates for 20 min at 4 °C, washed five times with buffer containing 20 mM Tris, pH 7.8, 150 mM NaCl, 1 mM EDTA, 0.1% Triton X-100, 100 g of PMSF, and 1 mM Na\textsubscript{3}VO\textsubscript{4}. Bound proteins were eluted with Laemmli sample buffer.
buffer, resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and visualized by Western blot analysis as described below.

Isolation of p70 protein. Fifty liters of DA3 cells was grown to a confluence of 2 × 10^6 cells/ml in RPMI medium (Gibco-Life Technologies) containing 10% FCS and 5 ng of IL-3/ml. Following two washes in PBS to remove growth factor, cells were cultured overnight in RPMI medium containing 1% FCS, pelleted, and lysed in ice-cold lysis buffer (see above). Nuclei were removed by centrifugation at 10,000 × g. Approximately 100 ml (15 mg/ml) of lysate was applied to a Q-Sepharose column (2.5 by 10 cm) previously equilibrated in ice-cold buffer containing 50 mM Tris, pH 8.0, 50 mM NaCl, 5 mM EDTA, 100 μM of PMSF/ml, and 1 mM Na3VO4. The column was washed extensively in lysis buffer, and bound proteins were step eluted with lysis buffer containing 150 mM NaCl. The eluate was passed sequentially over a Sepharose column to which had been conjugated the peptide TSQICKGMEYLGTKR (1 by 2 cm), and a Sepharose column to which had been conjugated the tyrosine-phosphorylated peptide TSQICKGMEMGLGTKR (1 by 1 cm). The latter column was washed extensively with load buffer and distilled water, and bound proteins were eluted with 2% acetic acid. Following removal of the solvent by lyophilization, the eluted material was suspended in Laemmli sample buffer, resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and visualized by Western blot analysis as described below.

Transfections, immunoprecipitations and Western blots. COS7 cell transfections were performed with Polyfect (Qiagen) according to the manufacturer’s instructions. Cell lysates were made by lysing cells in ice-cold lysis buffer (see above), clarified by centrifugation, rotated at 4°C with specific antibody for 2 h, and incubated 1 h with 20 μl of protein A Sepharose. Beads were washed five times with cold wash buffer (20 mM Tris, pH 7.8, 150 mM NaCl, 1 mM EDTA, 0.1% Triton X-100, 100 μM PMSF, and 1 mM Na3VO4), and bound protein was eluted with Laemmli sample buffer and separated by SDS-PAGE. Protein was transferred to nitrocellulose using a semidy transfer apparatus (Bio-Rad), blocked with 5% bovine serum albumin in TBST (10 mM Tris, pH 8.0, 150 mM NaCl, 0.1% Tween 20), incubated at 23°C, washed with TBST, incubated for 1 h with horseradish peroxidase-conjugated secondary antibody (Amer sham), and developed according to the manufacturer’s instructions.

Generation of p70-deficient mice. p70 genomic clones were isolated from a W9.5 embryonic stem cell BAC library (Incyte Genomics, St. Louis, Mo.) by screening with [α-32P]dCTP-labeled EST no. AA198028 as a probe. A restriction enzyme map of the p70 locus was determined using BAC clones and murine genomic DNA. A 10-kb fragment of p70 genomic sequence containing two exons was replaced with a cDNA encoding the neomycin resistance gene driven by the thymidine kinase promoter oriented in the direction opposite to that of p70 transcription. A cDNA encoding the diphtheria toxin gene driven by the thymidine kinase promoter (25) to be used in negative selection was then placed 3′ of the p70 homology region to complete the targeting construct. p70-deficient mice were generated essentially as previously described (22). Briefly, 129/SvJ E14 ES cells were electroporated with 20 μg of SalI-linearized targeting vector and selected with 350 μg of G418 (Gibco-BRL)/ml. Four hundred fifty G418-resistant clones were isolated, expanded, and analyzed by Southern analysis for correct integration of the mutation, by probing with a BamHI genomic fragment 3′ to the region of homology within the targeting construct. Five clones heterozygous for the induced mutation were in-
...jected into C57Bl/6 blastocysts, which were then implanted into pseudo-pregnant females. Male chimeras from three clones successfully passed the induced mutation through the germ line. Heterozygote agouti mice were interbred to produce wild-type and p70-deficient litters on a mixed 129/Sv/JBL/6 background. Genotype was determined by PCR using the following primers: wild-type allele, CCGTGATGAATCCGCTCAGTA and CACATGTCGCTGAA CGCTTCTT (300 bp); and knockout allele, CCGGTAGTAAGAATCCGCTC AGTA and GCAATGCTGTATACGCTTGT (150 bp). Animals were housed under specific-pathogen-free conditions under institutional guidelines.

DNA and RNA analysis. Genomic DNA was isolated by lysing tissue in buffer (100 mM Tris, pH 8.0, 5 mM EDTA, 0.2% SDS, 200 mM NaCl, and 250 μg of proteinase K/ml) incubating 12 h at 55°C, extracting with phenol-chloroform, and precipitating with ethanol. After restriction enzyme digestion, DNA was incubated by agarose gel electrophoresis and transferred to positively charged nylon (Amersham) for hybridization with labeled probes (Rediprime; Amersham) according to standard techniques. RNA was prepared by extracting tissue with Trizol according to the manufacturer’s instructions (Gibco BRL), separated by formaldehyde gel electrophoresis, and analyzed as described above. A Northern blot of murine poly(A)⁺ RNA (Clontech) was probed with a radio-labeled fragment of p70 cDNA corresponding to nucleotides encoding amino acid residues 469 to 501.

Histology. For organ histological analysis, tissues were fixed overnight in 10% buffered formalin (Fisher Scientific). Fixed tissues were embedded in paraffin, sectioned (10 μm), and stained with hematoxylin and eosin.

FACS analysis and cell purification. Whole spleens, thymus, or lymph nodes were dissected from littersmates and crushed through a 70-μm-pore-size nylon mesh in PBS containing 2% FCS. Bone marrow was obtained by flushing femurs and tibias with the same buffer. Red blood cells were lysed by addition of buffer (pH 7.2) containing 150 mM NH₄Cl, 1 mM KHCO₃, and 0.1 mM EDTA; debris and tibias with the same buffer. Red blood cells were lysed by addition of buffer. For proliferation assays, B and T cells were purified and lysates were subjected to immunoprecipitation (IP)/Western blot analysis with the indicated antibody.

RESULTS

Identification and cloning of p70. Jak2 is activated in the context of a number of cytokine receptors (12). As a consequence, it is tyrosine phosphorylated at 10 predominant sites (Matsuda and Ihle, unpublished data). Among these sites, murine Jak2-Y966 was identified as a potentially important site based on mutational analysis and on the ability of a phosphopeptide containing this site to bind a number of proteins known to be involved in signal transduction. These proteins include such molecules as Stat5a and -b, PLC-γ1, PLC-γ2, the PI 3-kinase adapter subunits p85α and -β, the PI 3-kinase catalytic subunit p110β, and SHC (Fig. 1). To identify novel proteins that might be recruited to this site, a large-scale affinity column approach was used as described in Materials and Methods and the affinity-purified proteins were sequenced. Protein microsequencing of a 70-kDa protein identified five peptides, three of which were found to be encoded in the sequence of a cDNA for an uncharacterized EST (GenBank accession no. AA190028). To clone the complete p70 cDNA, one of the ESTs was used to isolate four overlapping cDNA fragments from a murine library. The predicted protein from the compiled cDNA sequences would contain 603 amino acids with a calculated molecular mass of 67.5 kDa and included all five peptides identified by microsequencing (Fig. 2A).

The protein sequence of p70 reveals two previously defined protein domains. Residues 211 to 271 are predicted to contain an SH3 domain, a domain that interacts with protein sequences containing prolines and hydrophobic residues (17, 26). A second region in the carboxyl-terminal portion is 46% similar to the catalytic domain motif found in members of the bis-phosphoglycerate mutase family. Members of this family are structurally related enzymes that catalyze the transfer of phosphogroups from small molecules such as phosphoglycerate and fructose-2,6-phosphate (1, 18). Modeling of the catalytic domain of family members reveals a hydrophobic core surrounding a constellation of active site residues (2, 9), including two conserved histidines, two conserved arginines, and a glutamic acid (14). Five residues of murine p70, Arg-344, His-345, Arg-427, Glu-455, and His-530, are present in an orientation consistent with the existence of an evolutionary relationship between p70 and members of the bis-phosphoglycerate mutase family (Fig. 2B).

Screening of databases resulted in the identification of both human and Drosophila homologues of murine p70, and, importantly, both genes contain the SH3 and potential catalytic domain of the bis-phosphoglycerate mutase family.

Biological characterization of p70. To initially characterize the biological functions of p70, an antiserum against a C-terminal peptide sequence was generated. As illustrated (Fig. 2C), this antibody recognizes a protein of 70 kDa and this recognition was blocked by the immunizing peptide but not by an unrelated peptide. Moreover, this antiserum detected a 70-kDa protein that was specifically isolated by the Jak2 Y966 phosphopeptide affinity column and was not obtained from a peptide affinity column that contained the nonphosphorylated peptide (Fig. 2D). To further assess the ability of p70 to interact with Jak2, COS7 cells were cotransfected with expression vectors for Jak2 and a C-terminal Flag-tagged version of p70. As illustrated in Fig. 3, p70 was readily detected in the immu-
Northern analysis with a probe downstream of the deleted fi
rmed by Southern analysis (Fig. 5B).

Mice heterozygous for the induced mutation were interbred to
mice that transmitted the mutated allele through the germ line. 

dent heterozygous ES cell lines were used to generate chimeric 

vector was introduced into E14 ES cells, and
replace them with a neomycin resistance gene. The targeting 

would remove two coding exons (amino acids 9 to 88) and 

indicated in Fig. 5A, a targeting vector was constructed that 

encoding amino acid residues 9 to 26, designated as exon 2. As 

most amino terminal residues of p70 but did contain an exon 

W9.5 ES cell BAC genomic library, and four partially overlap-

gene targeting (3). Genomic p70 clones were isolated from a 

function of the
p70

were indicated in a variety of cell lines as well as various mouse tissues. 

As illustrated in Fig. 4A, there are two transcripts of 4 and 6 kb

that contain p70 sequences. Both of these transcripts are larger 

than the compiled cDNA sequence, suggesting that additional

5’ and/or 3’ untranslated regions exist. By Northern analysis, 

p70 transcripts are present in a variety of tissues. Consistent 

with this, a broad pattern of p70 protein expression was de-

ected in a variety of cell lines as well as various mouse tissues. 

Derivation of p70-deficient mice. To explore the in vivo 

function of the p70 gene, p70-deficient mice were generated by 
gene targeting (3). Genomic p70 clones were isolated from a 

W9.5 ES cell BAC genomic library, and four partially overlapping 
clones were obtained. These clones did not contain the

most amino terminal residues of p70 but did contain an exon 

encoding amino acid residues 9 to 26, designated as exon 2. As 

indicated in Fig. 5A, a targeting vector was constructed that 

would remove two coding exons (amino acids 9 to 88) and 

replace them with a neomycin resistance gene. The targeting 

vector was introduced into E14 ES cells, and five correctly 
targeted, independent clones were obtained. Three indepen-
dent heterozygous ES cell lines were used to generate chimeric 
mice that transmitted the mutated allele through the germ line. 

Mice heterozygous for the induced mutation were interbred to 
produce homozygous mutant mice. Transmission of the mu-
tated allele was confirmed by Southern analysis (Fig. 5B). 

Northern analysis with a probe downstream of the deleted 
exons was utilized to confirm the absence of p70 mRNA in 
mice homozygous for the mutation (Fig. 5C). Finally, anti-p70
antibody was utilized to confirm the absence of p70 protein or 
an amino-truncated protein in homozygous mice (Fig. 5D).

Analysis of p70-deficient mice. Intercrossing of p70 hetero-
zygote mice produced litters with the expected Mendelian ratio of +/+, +/-, and -/- mice (1:2:1). At a gross level, p70-
deficient mice were indistinguishable from their wild-type and 
p70 heterozygote littermates, developed normally, appeared 
healthy, did not succumb to any unusual pathology, and sur-
vived with a comparable longevity. Similarly, male and female 
p70 and p70 mice did not display any defects in their reproductive 
abilities. Histological examination of various organs revealed 
no differences between wild-type and p70-deficient mice. In 
addition, the architectures of the thymus, spleen, and lymph 
nodes were indistinguishable from those in wild-type mice. 

Blood serum chemistry (levels of albumin, alanine aminotrans-
ferase, amylase, bilirubin, creatinine, cholesterol, glucose, 
globulin, and cations) was not significantly different from that 
of wild-type mice. Additionally, wild-type and knockout mice 
that were starved overnight and administered glucose dis-
played similar responses to intraperitoneal glucose tolerance 
tests.

Because of the relatively high expression of p70 in hemato-
poietic tissues, various hematological parameters were exam-
inred. There were no significant differences between wild-type 
and mutant mice in the number of red cells, hemoglobin levels, 
or hematocrits. Similarly, there were no significant effects on 
the numbers and morphology of peripheral blood leukocytes, 
including neutrophils, eosinophils, basophils, monocytes, and 
lymphocytes. The recovery of bone marrow cells was normal, 
and in colony assays of bone marrow hematopoietic progeni-
tors, there were no significant alterations in the frequency and 
morphology of colonies in response to a variety of cytokines

FIG. 4. p70 expression analysis. (A) Northern blots containing poly(A)
RNA from multiple murine tissues were probed with a p70 cDNA 
probe (upper panel) or a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe (lower panel). Positions of p70 mRNA and GAPDH
mRNA are indicated, as are positions of standard markers, in kilobases. (B) Lysates prepared from the indicated cell lines were subjected to 
immunoprecipitation/Western blot analysis utilizing anti-p70 peptide antibody. (C) Lysates prepared from the indicated murine tissues were 
subjected to immunoprecipitation/Western blot analysis utilizing anti-p70 peptide antibody.
Bone marrow CD117−p70−deficient mice (data not shown) (8). Similarly, levels of B220 and CD8 numbers of pro-B (IgM−) were detectably affected by the absence of p70. Several known adapter proteins. In particular, although binding is specific for the phosphorylated peptide, binding occurs in the absence of any known phosphotyrosine binding domains. It is therefore likely that an adapter protein is involved in the binding. For example, a catalytic subunit of PI 3-kinase, p110α, is isolated from the Y966 phosphopeptide affinity column through its association with the PI 3-kinase adapter subunits p85α and β-S (S.-T. Jou and Ihle, unpublished data). Studies are in progress to assess the potential for p70 to interact with several known adapter proteins. As illustrated, interaction with Jak2 can also be shown to

![Diagram](http://mcb.asm.org/)

**FIG. 5.** p70 targeting. (A) Structure of the p70 targeting construct. The two filled boxes represent putative exons 2 and 3. NheI (N), BamHI (B), and SphI (S) sites are indicated, as is the location of the probe utilized in Southern analysis to distinguish wild-type and targeted alleles (black bar). Neo, neomycin resistance cassette; DTA, diphtheria toxin cassette. (B) Southern blot of BamHI-digested genomic DNA from +/+ , +/−, and −/− mice probed with the external probe illustrated in Fig. 4A. Bands generated by the wild-type (+/+), heterozygote (+/−), and targeted (−/−) alleles are indicated. WT, wild type; KO, knockout. (C) Northern analysis of RNA isolated from wild-type (+/+) or p70-deficient (−/−) thymi. The blot was probed with a p70 cDNA probe that was 3′ to the targeted exons as indicated in Materials and Methods. The bottom panel indicates the 28S and 18S RNAs as loading controls. (D) Immunoprecipitation (IP)/Western blot analysis of levels of p70 protein in splenic lysates prepared from +/+ (WT), +/− (Het), and −/− (KO) littersmates.

(Table 1). Importantly, the response to cytokines that are known to require Jak2, including Epo and IL-3, was not detectably affected by the absence of p70.

With regard to the lymphoid lineages, we observed normal numbers of pro-B (IgM− B220low, CD43−, and CD25−), pre-B (IgM−, B220+, CD43−, and CD25−), and mature B (IgM+, B220+, CD43−, and CD25−) cells in the bone marrow of p70-deficient mice (data not shown) (8). Similarly, levels of bone marrow CD117+, Ter119+, Gr1+, and Mac1+ cells were normal in the knockout mice. Flow cytometry analysis of developing thymocytes revealed no difference in numbers and proportions of double-negative (CD4− CD8−), double-positive (CD4+ CD8+), and single-positive (CD4+ CD8− or CD4− CD8+) (Fig. 6A) cells, indicating that T-cell development is unaffected by p70 deficiency. Peripheral T-cell numbers, as measured by anti-Thy1.2 or anti-CD3 reactivity, were likewise unaffected by the absence of p70, as were the levels and ratio of CD4 or CD8 single-positive T cells (Fig. 6B). To assess whether the deletion of p70 affected mature B-cell development, we analyzed by flow cytometry B-cell populations in the spleen and lymph nodes by using antibodies to B220, CD19, IgM, and IgD. There was no significant difference in numbers and maturation of peripheral B cells between +/+ and −/− mice (Fig. 6C). We then analyzed the proliferative ability of peripheral lymphocytes when stimulated by different agents. B and T cells were purified by FACS and were cultured in the presence of various mitogenic agents. Figure 7 demonstrates the absence of a significant difference between the ability of wild-type and p70-deficient B and T cells to respond to different stimuli.

**DISCUSSION**

The studies presented here are part of a general effort to identify proteins that are recruited to cytokine receptor complexes through the direct, or indirect, interaction with tyrosine-phosphorylated Jak2. As illustrated, a novel 70-kDa protein was identified that specifically binds to a peptide containing one of the major sites of autophosphorylation of murine Jak2, Tyr-966, and that coimmunoprecipitates with Jak2 in transfected cells. The basis for the interaction with Jak2 has not been precisely defined. In particular, although binding is specific for the phosphorylated peptide, binding occurs in the absence of any known phosphotyrosine binding domains. It is therefore likely that an adapter protein is involved in the binding. For example, a catalytic subunit of PI 3-kinase, p110α, is also isolated from the Y966 phosphopeptide affinity column through its association with the PI 3-kinase adapter subunits p85α and β-S (S.-T. Jou and Ihle, unpublished data). Studies are in progress to assess the potential for p70 to interact with several known adapter proteins.
occur in cells in which the two proteins are overexpressed. However, numerous attempts to demonstrate coimmunoprecipitation of endogenous proteins under conditions of activation of Jak2 have failed. It is possible that the fraction of Jak2 activated in these situations, which would be limited by the receptor numbers, is too low to allow detection of the proteins. Alternatively, it is possible that overexpression gives rise to associations that are not physiologically evident. Similarly, isolation on affinity columns may be occurring at protein concentrations that are not present in a physiologically relevant receptor complex. In this regard it is important to note that, although Stat5a and Stat5b bind specifically to the tyrosine-phosphorylated mJak2-Y966 peptide, the recruitment and activation of Stat5a and -b in the context of the Epo receptor require specific receptor tyrosines (19). Since it was not possible to strictly rule in, or out, a role for p70 in Jak2 activity based on these biochemical approaches, we also utilized a genetic approach.

Beyond a potential role in signaling from cytokine receptors utilizing Jak2, the properties of p70 provide few additional clues regarding its physiological role. The gene is conserved, as evidenced by the existence of homologues in other species. However, in these species, p70 is similarly an uncharacterized gene product of an EST and consequently nothing is known regarding its function. The gene is widely expressed in mouse tissues, which does not allow a focus for function on a particular lineage of cells or tissue. The protein contains an SH3 domain that would implicate it in signal transduction. The protein also contains a domain with homology to the catalytic domain of members of the bis-phosphoglycerate mutase family of enzymes. However, we have been unable to demonstrate a catalytic function for this region of p70. Members of the phosphoglycerate mutase family of enzymes are not known to have protein interaction domains, such as an SH3 domain. The function of the SH3 domain of p70 might be either to target it to a specific molecular complex or to enable its interaction with regulatory proteins.

One important approach to defining gene function is to determine the consequences of eliminating the gene in the general context of mouse development. As illustrated here, we were able to develop a strain of mice in which the p70 gene

**TABLE 1.** In vitro colony formation of bone marrow hematopoietic progenitors from wild-type and p70-deficient littermates in response to various cytokines

<table>
<thead>
<tr>
<th>Colony type</th>
<th>Cytokine</th>
<th>+/+ (No. of colonies formed)</th>
<th>-/- (No. of colonies formed)</th>
</tr>
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<tbody>
<tr>
<td>CFU-E</td>
<td>Epo</td>
<td>121 ± 9</td>
<td>100 ± 13</td>
</tr>
<tr>
<td>BFU-E</td>
<td>Epo, IL-3</td>
<td>27 ± 5</td>
<td>25 ± 5</td>
</tr>
<tr>
<td>CFU-G</td>
<td>G-CSF</td>
<td>39 ± 3</td>
<td>37 ± 16</td>
</tr>
<tr>
<td>CFU-GM</td>
<td>GM-CSF</td>
<td>27 ± 7</td>
<td>33 ± 8</td>
</tr>
<tr>
<td>CFU-Mix</td>
<td>IL-3</td>
<td>43 ± 12</td>
<td>41 ± 10</td>
</tr>
<tr>
<td>CFC-Mix</td>
<td>IL-3, IL-6, SCF, Epo</td>
<td>12 ± 2</td>
<td>16 ± 3</td>
</tr>
<tr>
<td>CFU-Eos</td>
<td>IL-5</td>
<td>37 ± 9</td>
<td>29 ± 5</td>
</tr>
<tr>
<td>CFU-Meg</td>
<td>Tpo</td>
<td>11 ± 2</td>
<td>13 ± 5</td>
</tr>
<tr>
<td>CFU-Mix</td>
<td>SCF</td>
<td>32 ± 5</td>
<td>37 ± 4</td>
</tr>
<tr>
<td>Pre-B</td>
<td>IL-7</td>
<td>13 ± 4</td>
<td>10 ± 2</td>
</tr>
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* Bone marrow cells from four animals of each genotype were prepared for colony assay.
FIG. 6. Flow cytometry analysis of T and B cells. Calculated percentages are displayed within each quadrant. (A) Thymocytes from wild-type and p70-deficient mice were stained with Cyconjugated anti-CD4 and FITC-conjugated anti-CD8. (B) Splenocytes from wild-type and p70-deficient mice were stained with Cyconjugated anti-B220 and FITC-conjugated anti-CD3 antibodies (top pair). Alternatively, splenocytes were stained with PE-conjugated anti-Thy1.2, Cyconjugated anti-CD4, and FITC-conjugated anti-CD8. The CD4/CD8 profile of splenocytes gated on Thy 1.2-positive cells is displayed (bottom panel). (C) Splenocytes from +/- or -/- mice were stained with FITC-conjugated anti-IgD, PE-conjugated anti-IgM, and Cyconjugated anti-B220. The IgM/IgD profile of splenocytes gated on B220-positive cells is displayed. Shown are representative FACS analyses of at least six mice of each genotype.
contained an internal deletion that resulted in a protein null phenotype. Extensive characterization of these mice has failed to identify any phenotypic characteristics that are different from those of wild-type mice. Most specifically, none of the receptor systems that utilize Jak2 that we examined were affected by the absence of p70, demonstrating the lack of a nonredundant, essential role in signaling. The absence of any phenotypic alterations in such cases can often be attributed to functional redundancy between family members.

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REFERENCES


FIG. 7. T- and B-cell proliferation assays. FACS-purified splenic T cells (left) or FACS-purified splenic B cells (right) were cultured in the presence of the indicated stimuli for 48 h. Cell proliferation was measured by adding 1 μCi of [3H]thymidine to the cultures. Standard deviations for triplicate samples are indicated by the error bars. These data are representative of three T-cell and B-cell proliferation assays. LPS, lipopolysaccharide; WT, wild type; KO, knockout.


