The interleukin-7 (IL-7) receptor is expressed throughout T-cell differentiation and, although lacking a tyrosine kinase domain, mediates tyrosine phosphorylation in T cells. We have identified IL-7-induced activation of three cytoplasmic tyrosine kinases in T cells, Jak1, Jak3, and the src-like kinase p56lck. Many members of the cytokine receptor superfamily activate the Jak protein tyrosine kinase family, with resultant phosphorylation of the Stat transcriptional activator factors. We describe here a novel function of the Jak kinases, because Jak kinase activity is not only required for Stat activation but also for PI3 kinase response to IL-7 in human T cells. We show that IL-7 receptor-mediated Jak activation can occur independently of p56lck activity. IL-7-induced PI3 kinase activation, mediated by tyrosine phosphorylation of the PI3 kinase p85 subunit, is essential to the IL-7 proliferative signal and also occurs in the absence of src family kinase activity. Jak3 is found associated with the p85 subunit of PI3 kinase in an IL-7-responsive manner in T cells and appears to regulate IL-7-induced PI3 kinase activation by mediating tyrosine phosphorylation of the p85 subunit. Specific inhibition of IL-7-induced Jak kinase activity ablates p85 tyrosine phosphorylation, subsequent PI3 kinase activation, and, ultimately, proliferation. The ability to regulate PI3 kinase activity indicates a more generalized role for the Jak family than activation of gene transcription via the Stat family in cytokine receptor signal transduction.

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Materials and Methods

Isolation of human thymocytes and lymphocytes. Mononuclear cells were isolated from the thymus of juvenile open heart surgery patients using Ficoll-Hypaque gradients of single-cell suspensions. Adherent cells were removed by adherence to plastic culture dishes for 60 minutes at 37°C. The resultant thymocytes are 98% to 99.5% CD3+. Lymphocytes were isolated from tonsil tissue and peripheral blood by Ficoll-Hypaque gradient centrifugation, followed by rosetting with neuraminidase-treated sheep red blood cells to isolate T lymphocytes. After isolating rosettes by Ficoll-Hypaque gradient centrifugation, T cells were released with ACT treatment (0.75% NH₄Cl in 20 mmol/L Tris, pH 7.2) of the rosettes to lyse the red blood cells. The resultant T lymphocytes are typically 98% to 99.5% CD3+, with less than 1% B-cell contamination.

Reagents and antibodies. A polyclonal antibody to Jak3 was the gift of Dr J.J. O'Shea (National Institutes of Health, Bethesda, MD) and was raised to a Jak3 peptide as described. This antibody does not cross-react with Jak1, Jak2, or Tyk2. Antibodies to Jak1 and Jak2 were obtained from Upstate Biotechnology Inc (Urbana, New York, NY) and to Tyk2, p62yes, and p60src were obtained from Santa Cruz Biotech (Santa Cruz, CA). Lypates for immunoprecipitation with anti-Jak2 were precleared with anti-Jak3-protein A-sepharose conjugates, because the antibody to Jak2 cross-reacts strongly with human Jak3. Anti-tyrosine-phosphotyrosoine and anti-p85 were also purchased from Santa Cruz Biotech. Reagents and antibodies to Tyk2, p62yes, and p60src were obtained from Dako (Glostrup, Denmark). A polyclonal antibody to Jak3 was the gift of Dr L. Dilli (University of Toronto, Toronto, Ontario, Canada). Typhostin blocker AG-490 was kindly provided by Dr A. Levitzki (Hebrew University of Jerusalem, Jerusalem, Israel).

Immunoprecipitation and Western blotting. Cells were resuspended in RPMI 1640 medium at a density of 2 x 10⁷ peripheral blood T lymphocytes [PBL-T] and tonsil T lymphocytes or 5 x 10⁶ (thymocytes) and incubated at 37°C with 30 ng/mL recombinant IL-7 (Gibco-BRL, Gaithersburg, MD) for the indicated periods of time. Cells were then quickly pelleted and resuspended in ice-cold lysis buffer containing 10 mmol/L Tris, pH 7.6, 150 mmol/L NaCl, 5 mmol/L EDTA, 1 mmol/L NaVO₄, 1 mmol/L phenylmethylsulfonyl fluoride (PMSF), and 1% NP-40. For Jak1 immunoprecipitation, cells were lysed in RIPA buffer rather than in 1% NP-40 (1% Triton, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate [SDS]), 150 mmol/L NaCl, 20 mmol/L EDTA, 1 mmol/L NaVO₄, 1 mmol/L PMSF). After 15 minutes on ice, debris was removed by centrifugation at 12,000g for 10 minutes at 4°C. Antibodies and 20 µL of 50% protein A sepharose-CL4B were added to cleared lysates and incubated at 4°C with constant agitation for 4 to 16 hours. Immunoprecipitates were collected by a brief centrifugation and washed three or four times in lysis buffer (without PMSF) before the addition of SDS sample buffer. Samples were separated on 8% polyacrylamide gels and transferred to nitrocellulose membranes (Amersham, Arlington Heights, IL). Membranes were blocked overnight with either 5% bovine serum albumin (BSA) or 5% Biotto in TBS-T at 4°C. Blotting antibodies were added at the recommended dilutions in TBS-T containing 1% BSA or Biotto and incubated at 4°C for 2 hours. After washing, antibodies were detected using horseradish-conjugated donkey antirabbit or sheep antimouse antibodies and ECL reagents (GIBCO-BRL).

In vitro kinase assays. Immunoprecipitations were performed in 1% NP-40 lysis buffer as described. Precipitates were washed three times with EDTA-free NP-40 lysis buffer and then twice with kinase assay buffer (20 mmol/L HEPES, pH 7.5, 10 mmol/L MgCl₂, 10 mmol/L MnCl₂, and 0.1 mmol/L NaVO₄). After resuspension in a 35 µL volume of kinase buffer, 2 µmol/L ATP containing 10 µCi [γ^32P]-ATP (>5,000 Ci/mmol; Amersham) was added to initiate the reaction. Reactions were performed at 25°C and stopped by the addition of SDS-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer. Samples were analyzed by 8% gel electrophoresis and autoradiography.

P43 kinase reactions. Reactions and lipid extraction were essentially performed according to the method of Whitman et al. Reactants were washed three times with lysis buffer (1% NP-40, 0.1% Brij-35, 1% detergent) and to Tyk2, p62yes, and p60src were obtained from Dako (Glostrup, Denmark). A polyclonal antibody to Jak3 was the gift of Dr L. Dilli (University of Toronto, Toronto, Ontario, Canada). Typhostin blocker AG-490 was kindly provided by Dr A. Levitzki (Hebrew University of Jerusalem, Jerusalem, Israel).

MATERIALS AND METHODS

Jak1 and Jak3 are activated by IL-7 in T cells. Mature T lymphocytes express most members of the Jak kinase family, including Jak3, which has been reported to be strongly expressed only in terminally differentiated or activated cells. Activation of Jak1 and Jak3 by IL-7 has recently been described in mature T cells, including helper T cell clones and in vitro phytohemagglutinin (PHA)-activated peripheral T lymphocytes (PBL-T). In agreement with these reports, when IL-7–stimulated activation of the Jak kinases was examined in human tonsil T lymphocytes, a substantial increase in the level of Jak3 tyrosine phosphorylation was detected (Fig 1). Activation of Jak3 was detected in tonsil T cells within 1 minute of IL-7 stimulation, reaching a maximum level after 10 to 15 minutes. IL-7–induced activation of Jak3 occurred in all primary human T cells examined, including purified human T lymphocytes and peripheral and tonsillar human T lymphocytes (manuscript submitted).

Although Jak3 was heavily phosphorylated, we were unable to detect tyrosine phosphorylation of the Jak family members Jak2 and Tyk2. A low level of Jak1 tyrosine phosphorylation was also observed in response to IL-7 (Fig 2). Jak1 phosphorylation was not detected under exposure conditions at which Jak3 activation was already clearly observed. IL-7–induced Jak1 activation could only be detected after much longer exposure of antiphosphotyrosine Western blots of immunoprecipitated Jak1 protein. The significant

RESULTS

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Fig 1. Activation of Jak3 by IL-7 in tonsil T lymphocytes. Jak3 was immunoprecipitated from IL-7–stimulated tonsil T cells (30 ng/mL rIL-7 [GIBCO-BRL] for the indicated times), subjected to 8% SDS-PAGE, and transferred to nitrocellulose. Immunoblotting was performed with monoclonal antiphosphotyrosine (UBI) and detection by ECL (GIBCO-BRL). The lower panel displays Jak3 quantitation by immunoblotting, which was performed after stripping the antiphosphotyrosine with 62.5 mmol/L Tris, pH 6.8, 2% SDS, 100 mmol/L β-mercaptoethanol at 50°C.

Fig 2. Activation of Jak1. Jak1 was immunoprecipitated from IL-7–stimulated thymocytes as previously detailed. Activation was detected by immunoblotting with antiphosphotyrosine and quantitation was performed after stripping with anti-Jak1 (lower panel).

Fig 3. Activation of Ick kinase activity by IL-7. Cell lysates were prepared from thymocytes (±30 ng/mL rIL-7 for 10 minutes) and immunoprecipitated with anti-p56lck (UBI). In vitro kinase assays of immunoprecipitates were performed in 20 mmol/L HEPES, pH 7.5, 10 mmol/L MgCl₂, 10 mmol/L MnCl₂, and 10 μCi γ³²P-ATP for 10 minutes at 25°C. Reactions were stopped by the addition of SDS-PAGE sample buffer and reaction products were visualized by autoradiography at −70°C after gel electrophoresis.

The difference between the levels of IL-7–inducible tyrosine phosphorylation of Jak1 and Jak3 suggests that Jak3 will play a greater role than Jak1 in IL-7R signal transduction. Although IL-7–mediated Jak1 activation has been shown in PHA-treated PBL-T, Zeng et al., although detecting Jak3 activation, did not observe phosphorylation of Jak1 in helper T cells. Having established the potential of the Jak kinases to participate in IL-7 signal transduction, we examined the ability of the src-like protein tyrosine kinases expressed in T lymphocytes to respond to IL-7.

p56lck is activated by IL-7 in T cells. p56lck and p59fyn are the most highly expressed members of the src kinase family in both thymocytes and T lymphocytes. In the mature T lymphocytes of the periphery, these kinases play an important role in transduction of signals not only from the T-cell receptor complex, but also from some members of the cytokine receptor superfamily. The IL-2R is known to activate both of these kinases in transduction of its signal, in addition to activating members of the Jak family.

In vitro kinase assays of immunoprecipitated kinases showed for the first time that IL-7 induces activation of the p56lck kinase (Fig 3). The rapidity with which p56lck activation occurred in response to IL-7 stimulation was difficult to assess owing to a high level of basal kinase activity. However, a twofold to threefold increase in p56lck kinase activity was consistently detected after 5 to 10 minutes of stimulation.

Despite the high level of p59fyn expression and kinase activity present in T cells, we could not detect IL-7 stimulation of this or any other src family kinase (data not shown). This finding is in contrast to previous findings in IL-7–stimulated pre-B cells, in which the rapid activation of both p59fyn and p53/56lyn, but not p56lck, kinase activities has been reported. In contrast to its activity in the B lineage, the IL-7R does not appear to activate multiple members of the src family within T cells.

Jak3 is activated independently of src family kinase activity. IL-7–induced activation of p56lck raised the possibility that Jak activation may be dependent on phosphorylation by this kinase. Activation of Jak kinase activity is accompanied by an increase in Jak tyrosine phosphorylation. Thus far, it has not been possible to determine whether tyrosine phosphorylation is required for Jak activation by IL-7.
phosphorylation occurs as a consequence of kinase activation or as a causal event. Furthermore, this phosphorylation may occur by means of autophosphorylation or through the action of another kinase family. Because no temporal sequence could readily be assigned to the activation of Jak3 (or Jak1) and p56lck because of the high background of p56lck kinase activity encountered, IL-7–induced Jak kinase activation was examined in p56lck-deficient T-cell lines. T cells deficient in p59fyn kinase expression were also examined because of the potential for redundancy in src family kinase usage by the IL-7R. p59fyn appeared to be the src-like kinase most likely to be capable of substituting for p56lck because it is activated by IL-7 in B cells.\(^9,10\) Because Jak3 is expressed at a higher level than Jak1 and its activation is easily detectable, IL-7–induced Jak3 activation was examined in these cells.

T-cell lines lacking in fyn expression \(lck^{-}/fyn^{-}\) (C8), lck expression \(lck^{+}/fyn^{+}\) (KIT 225), and both lck and fyn \(lck^{-}/fyn^{-}\) (SIT) were stimulated with IL-7 and the activation of Jak3 was monitored. These cell lines have been extensively analyzed for p56lck and p59fyn expression by Northern blotting, RT-PCR, Western blotting, and in vitro kinase assays.\(^34\) Figure 4 shows that, even in the absence of both the p56lck and p59fyn kinases, IL-7 induces the activation of Jak3. No significant difference could be detected between cell lines expressing low levels of either src kinase and the \(lck^{-}/fyn^{-}\) line. The other src-like kinases known to be expressed by SIT cells (p60src and p62yes) were also examined by in vitro kinase assay but were not activated by IL-7 (data not shown), showing that src-like kinase redundancy does not appear to occur in these T cells.

The use of these T-cell lines has not only enabled us to determine that Jak3 activation can occur independently of p56lck, but also that Jak3 phosphorylation may be mediated by its own kinase domains. The activation of Jak3 in the absence of p56lck may indicate that the IL-7R uses two independent branches of tyrosine kinase activity, although the potential for p56lck activation in the absence of Jak3 has yet to be examined.

IL-7 activates PI3 kinase in the absence of src-like kinase activity—association of Jak3 and p85. IL-7 stimulation of both thymocytes and mature T lymphocytes induces the activation of PI3 kinase. This rapid and transient response is dependent on tyrosine phosphorylation of the p85-regulatory subunit of PI3 kinase. In many systems, this is accomplished by the src family of tyrosine kinases and we have established that IL-7 does induce activation of the src-like kinase p56lck in T cells. However, demonstration of Jak activation by IL-7 in the absence of detectable src-like kinase activity presented the opportunity to determine whether Jak3 (or Jak1) was capable of activating PI3 kinase. Available evidence suggested that certain cytokine receptors are able to activate PI3 kinase in the absence of receptor or src-like kinase activity. The IL-2R, although activating several src family kinases, can induce proliferation in their absence.\(^35-37\) To initiate proliferation, most cytokine receptors, including IL-7, have an absolute requirement for PI3 kinase activation, suggesting that the IL-2R uses an alternative kinase to the src family to regulate PI3 kinase activity. Similar to IL-2, the IL-7R activates both the Jak1 and Jak3 kinases (as well as src-like kinase activity), suggesting that both receptors may use Jak kinases to activate PI3 kinase.

The likelihood of this possibility was strengthened when p85 subunit immunoprecipitated from NP-40 lysates of T cells was found to be associated with Jak3 kinase. Although displaying a relatively high level of constitutive association, IL-7 induced a significant increase (Fig 5A), which peaked after 5 to 10 minutes of stimulation. This coincides with the peak of PI3 kinase activity observed in response to IL-7.\(^23\) If the association had functional significance, then it was possible that IL-7 would still be able to activate PI3 kinase in the absence of p56lck activity. Indeed, in vitro assays of PI3 kinase showed that IL-7–induced activation of PI3 kinase occurred not only in normal T cells, but also in those lacking expression of p56lck and p59fyn (Fig 5B). Therefore, both IL-7–induced activation of PI3 kinase and Jak appear to occur independently of src family activation in T cells. Although both the Jak1 and Jak3 tyrosine kinases are activated by IL-7, we could not detect an association between Jak1 and the p85 subunit of PI3 kinase in T cells. This failure to detect Jak1-p85 association may simply reflect limitations in the sensitivity of the technique or may delineate a potential difference in the roles of these kinase.

In light of this evidence, the IL-7R must use an alternative pathway to the recognized activation signals provided by intrinsic receptor kinase activity or src-like kinases to activate PI3 kinase. Considering the IL-7–induced increase in Jak3-p85 association, it appeared likely that Jak3 was responsible for tyrosine phosphorylation of the p85 subunit of PI3 kinase.

Jak kinase activity is required for PI3 kinase activation. We have previously shown that IL-7–mediated activation of PI3 kinase activity and phosphorylation of the p85 subunit can be blocked by the tyrosine kinase inhibitor AG-490.\(^38\) This tyrphostin inhibits the tyrosine phosphorylation induced by the IL-7R and ultimately prevents the mitogenic response
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Fig 5. Association of Jak3 with PI3 kinase. (A) After IL-7 stimulation (30 ng/ml) for the indicated times at 37°C, T-cell lysates were prepared in 1% NP-40 lysis buffer and immunoprecipitation was performed with anti-p85 (UBI). Precipitating complexes were washed and analyzed by immunoblotting with anti-Jak3. (B) Lysates were prepared from cells incubated (±30 ng/ml IL-7) for 10 minutes at 37°C and antiphosphotyrosine (UBI) used to precipitate PI3 kinase activity. After washing (see Materials and Methods), immunoprecipitates were resuspended in in vitro kinase buffer, 20 mmol/L Tris, pH 7.5, 100 mmol/L NaCl, 0.5 mmol/L EGTA containing 2 mg/mL PI (Sigma) and incubated at 37°C for 10 minutes. Reactions were initiated by the addition of 5 μCi γ32P-ATP (>5,000 Ci/mmoll) and 25 mmol/L MgCl2, and allowed to proceed for 10 minutes at room temperature. Lipids were extracted as described and the phosphorylated inositol products were separated by TLC. Radioactive products were visualized by autoradiography of the TLC plate at -70°C.

of thymocytes to IL-7 while having no effect on tyrosine kinase activities of the EGF and PDGF receptors or on antigen receptor-mediated responses or src family kinases. We therefore examined the possibility that AG-490 may prevent IL-7-induced p85 tyrosine phosphorylation, and consequent stimulation of PI3 kinase activity, by the specific inhibition of Jak3 kinase activity.

Single-cell suspension cultures of human thymocytes were incubated at 37°C for 16 hours with a dose of AG-490 (50 μmol/L) known to have a significant inhibitory effect on IL-7-induced tyrosine phosphorylation. Stimulation of these cells showed an inhibition of IL-7–induced Jak3 activation, as measured in a substantial decrease in the tyrosine phosphorylation of Jak3 immunoprecipitates (Fig 6A). Similar in nature to the coincident events of Jak activation, the decreased tyrosine phosphorylation paralleled a significant inhibition of Jak3 in vitro kinase activity (Fig 6B). AG-490 appears to inhibit IL-7R signal transduction by the specific inhibition of the Jak kinases rather than src-like kinases, because purified p56lck kinase activity is not inhibited by AG-490 (data not shown). As we have previously shown, AG-490 inhibited the IL-7–induced activation of the total PI3 kinase activity (Fig 6C) and, even more specifically, decreased the amount of PI3 kinase activity coprecipitating with the Jak3 protein (Fig 6D). These data are consistent with an AG-490 induced reduction in Jak kinase activity being responsible for the decrease in p85 phosphorylation and, therefore, of PI3 kinase activation.

Anti-Jak3 immunoprecipitates show a higher basal level of PI3 kinase activity than do antiphosphotyrosine immunoprecipitates, which is consistent with the high basal level of association detected between p85 and Jak3 (this is similar to the high basal activity seen when immunoprecipitating with antibodies to the p85 subunit itself). AG-490 also shows a more complete inhibition of the PI3 kinase activity associated with theJak protein, suggesting that this pool is selectively targeted, whereas only the Jak-associated activity is inhibited within the total PI3 kinase pool, resulting in a higher level of residual activity.

DISCUSSION

We have provided evidence indicating that the Jak kinase family mediates functions other than transcriptional activation through the Stat family. The data obtained in this study consistently support the hypothesis that the interaction of Jak3 with p85 mediates the activation of PI3 kinase induced by IL-7. We have shown that IL-7–induced activation of PI3 kinase occurs in the apparent absence of activated src-like kinases (Fig 5B), conditions under which Jak activation proceeds normally (Fig 4), and that Jak3 and the p85-regulatory subunit of PI3 kinase are associated within the cell in an IL-7–responsive manner (Fig 5A). Furthermore, inhibition of IL-7R signal transduction by specifically inhibiting IL-7–induced tyrosine phosphorylation38 is accomplished with a tyrophostin that inhibits Jak (Fig 6A and B) but not src-like kinase activity. Although p56lck is activated by IL-7 in T cells, it is the specific inhibition of Jak3 activity that ablates PI3 kinase activation. These data strongly argue against a role for the src kinase family in directly mediating PI3 kinase activation in this system. In the absence of src-like
kinase or receptor kinase activity, the IL-7R would appear to have no recognized method of mediating P13 kinase activation, because IL-7–induced ras activation cannot be detected in primary T cells (N.S., unpublished observations).

An SH2 domain recognition motif (YXXM) in the cytoplasmic domain of the IL-7Rα chain also participates in recruitment of the p85 subunit to the IL-7R signalling complex. Mutation of this tyrosine residue significantly inhibits association of p85 with transfected IL-7Rα in pre-B cells, while both the C and N terminal SH2 domains of p85 bind phosphorylated peptide sequences based on this motif with high affinity in vitro. Although the structural basis for the interaction between Jak3 and P13 kinase remains undetermined, the high level of basal association between p85 and Jak3 suggests that this interaction may not be completely dependent on activation signals such as phosphorylation. Although the relative contributions of Jak- and receptor-mediated contacts to activation are difficult to directly assess, prior phosphorylation of the receptor motif is absolutely required for p85 recruitment. P13 kinase SH2 domains do not bind peptides containing this motif unless they are tyrosine phosphorylated. Unless Jak-mediated binding is simply sufficient to permit P13 kinase activation in lck/fyn-deficient lines, it appears that the Jak kinase may be responsible for phosphorylation of the receptor chain in addition to phosphorylating p85.

By virtue of a common IL-2Rγ chain, the IL-7R belongs to a cytokine receptor subfamily including the IL-2R and IL-4R, which also activate the kinase pairing of Jak3 and Jak1. However, the IL-7R is unique in possessing an SH2 domain recognition motif for the P13 kinase p85 subunit. This motif does not occur in either of the IL-2Rβ or IL-4α chains or in the common IL-2Rγ chain, implying that the IL-7R may use a slightly different mechanism of P13 kinase activation. However, the lack of an SH2 recognition motif does suggest that these receptors may use Jak-mediated contacts to recruit P13 kinase. This hypothesis is further supported by deletion studies of the IL-2R chains, because deletion of the serine-rich region of the IL-2Rβ chain, which is required for binding and activation of Jak1 and Jak3,
ablates IL-2–induced activation of PI3 kinase and proliferation.46

The relative contributions of Jak1 and Jak3 to the IL-7 transduction pathway are difficult to determine. Although Jak1-p85 complexes could not be detected, Jak1 may also be capable of regulating events such as PI3 kinase activation, although the necessity of activating two kinases to perform the same task is debatable. However, the ability to inhibit Jak3 kinase activity and the IL-7–stimulated PI3 kinase activity specifically associated with Jak3 does indicate a significant role for Jak3 in PI3 kinase activation. The inability to show Jak1-p85 association suggests that this kinase is likely to play a lesser role in activating PI3 kinase or may, in fact, show a difference in function.

The ability of Jak3 to regulate PI3 kinase implies that transcriptional activation via Stat phosphorylation may not necessarily be the primary signalling event downstream of the Jak kinases (Fig 7). Although the precise downstream function of the phospho-inositol lipid second messengers generated remains poorly defined, the activation of PI3 kinase by phosphorylation is central and essential to signalling from many receptors. The inhibition of Jak3 activation, which in turn prevents PI3 kinase activation, completely abrogates normal IL-7–induced thymocyte proliferation,48 indicating that Jak activation is absolutely essential to transduction of the IL-7 signal. We therefore suggest that Jak kinases play a much more generalized and yet essential role in cytokine receptor signal transduction than currently believed.

Inhibition of IL-7–induced Jak kinase activity by AG-490 also resulted in a decreased level of Jak tyrosine phosphorylation. Because AG-490 does not demonstrably inhibit tyrosine kinases other than the Jak family, it appears that the Jak kinases autophosphorylate in vivo as well as in vitro. Tyrosine phosphorylation of Jak3 in the apparent absence of other activated kinases supports this and lends credence to the view that Jak kinases are activated by receptor chain dimerization. Dimerization in response to ligand may simply serve to colocalize Jak proteins with resultant autophosphorylation. This appears to be sufficient to activate the kinase domains to phosphorylate receptor chains and other substrates.

The specificity of Jak kinase-mediated activation of the Stat family of transcriptional activators has been shown to be determined by the receptor chains rather than by the Jak kinase activated.47,48 The receptor chain provides a binding site for Stat factors, which serves to colocalize the Jak and Stat proteins. Similarly, Jak3 phosphorylation of PI3 kinase may be primarily determined by p85 binding to the receptor. Whether activated Jak kinases are capable of performing a physiologic role when detached from the activating receptor remains to be determined. If in vivo substrate specificity is determined in such a manner, the Jak kinases may potentially phosphorylate a much wider range of proteins than PI3 kinase and the Stat family.

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JAK3 protein tyrosine kinase mediates interleukin-7-induced activation of phosphatidylinositol-3' kinase

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