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Immunology

The ζ chain is associated with a tyrosine kinase and upon T-cell antigen receptor stimulation associates with ZAP-70, a 70-kDa tyrosine phosphoprotein

(signal transduction/tyrosine phosphorylation/superantigen)

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ABSTRACT **Stimulation of the T-cell antigen receptor (TCR) leads to tyrosine phosphorylation of a number of cellular proteins, including phospholipase C (PLC) γ1 and the TCR ζ chain. We describe here a 70-kDa tyrosine phosphoprotein (ZAP-70) that associates with ζ within 15 sec following TCR stimulation. The phosphorylation of ZAP-70 and its association with ζ is independent of the other TCR chains since stimulation of a functional CD8/ζ chimeric receptor in a TCR-negative T cell leads to coprecipitation of ZAP-70 with the chimeric protein. In a Jurkat cell expressing the TCR and the CD8/ζ chimeric protein, tyrosine phosphorylation and association of ZAP-70 occurs exclusively with the stimulated receptor complex. In addition, a tyrosine kinase that does not appear to be fyn associates with the cytoplasmic domain of ζ and phosphorylates ζ and ZAP-70 *in vitro*.**

Stimulation of the T-cell antigen receptor (TCR) induces signal transduction events culminating in cellular proliferation and differentiation (1). The TCR consists of a ligand-specific α/β heterodimer noncovalently associated with the invariant CD3 and ζ subunits to form an eight-chain complex ($\alpha\beta\gamma\delta\varepsilon_2\zeta_2$) (2, 3). Stimulation of the TCR results in activation of a protein tyrosine kinase (PTK) with resultant phosphorylation of at least two known substrates: phospholipase C (PLC) γ1 and the TCR ζ chains (4, 5). Tyrosine phosphorylation of PLC γ1 results in increased PLC catalytic activity and activation of the inositol phosphate pathway (6). In addition to PLC γ1 and ζ, numerous other induced but as yet unidentified cellular tyrosine phosphoproteins have been observed following TCR stimulation (5, 7). Their significance and their potential interactions with the TCR complex, however, remain unclear.

Although the TCR is composed of at least eight chains, the cytoplasmic domain of the ζ chain alone is capable of transducing TCR signaling events (8). Expression of a chimeric protein (CD8/ζ) consisting of the extracellular and transmembrane domains of CD8 α and the cytoplasmic domain of ζ in a TCR-negative cell reconstitutes early {i.e., increases in cytoplasmic free calcium ($[Ca^{2+}]_i$), inositol phosphate turnover, and ζ phosphorylation} and late [i.e., interleukin 2 (IL-2) production and CD69 expression] events indistinguishable from those generated by the TCR. To identify molecules that interact with ζ and thus may play a critical role in TCR signal transduction, we determined whether any tyrosine phosphoproteins were associated with ζ. We describe here a 70-kDa tyrosine phosphoprotein (ZAP-70) that coprecipitates with ζ following TCR stimulation. Moreover, a tyrosine kinase activity is also associated with ζ and can phosphorylate ζ and ZAP-70 *in vitro*. ZAP-70 and the ζ-associated tyrosine kinase may play critical roles in TCR signal transduction.

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MATERIALS AND METHODS

Cells and Antibodies. The human leukemia T-cell lines Jurkat, its derived TCR β-chain negative cell (J.RT3-T3.5), and the CD8/ζ chimera transfected clones (JCD8/ζ2 and Jβ-CD8/ζ14) have been described (8). Murine monoclonal antibodies (mAbs) used and their specificities include: C305, Jurkat T_i β-chain; Leu4, CD3ε; 235, CD3; 4G10, phosphotyrosine; W6/32, human major histocompatibility complex class I heavy chain; OKT8, CD8 α; and 9.3, CD28. Anti-fyn antibody CFN-2 (9), anti-fyn peptide antisera (10, 11), rabbit anti-lck peptide antiserum (10), rabbit anti-ζ-chain peptide antiserum (12), and rabbit anti-raf antiserum SP-63 (13) were kindly provided by the investigators referenced.

Immunoprecipitation, Electrophoresis, and Western Blotting. Jurkat cells were stimulated with anti-TCR antibodies (1:500 dilution of C305 ascites fluid) at 37°C for 2 min unless otherwise specified. Cells were lysed and prepared for immunoprecipitations as described (8). In experiments with staphylococcal enterotoxin stimulation, Raji cells were coated with staphylococcal enterotoxin D (SED) (Toxin Technology, Madison, WI) at 100 ng/ml, washed with phosphate-buffered saline, and added to an equal number of Jurkat cells. The cells were rapidly sedimented to initiate cell contact and SED stimulation. Stimulation was terminated with lysis buffer. Immunoprecipitations, sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS/PAGE), and Western blotting were performed as described (8).

Biosynthetic Labeling. Jurkat cells were preincubated for 30 min in methionine-deficient medium and labeled for 4 hr with [^{35}S]methionine at 0.5–1 mCi/ml (>1000 Ci/mmol, ICN; 1 Ci = 37 GBq). Cells were stimulated and lysates were analyzed as described above. Quantitation of bands was performed using IMAGE QUANT software after analysis on a phosphorimager (Molecular Dynamics, Sunnyvale, CA).

***In Vitro* Kinase Assays and Phosphoamino Acid Analysis.** Immune complexes were washed with 10 mM Tris, pH 7.4/150 mM NaCl/1% Nonidet P-40 (NP-40), twice with 0.5 M LiCl/50 mM Tris, pH 7.4, and twice with water and resuspended in 30 μl of 20 mM Tris, pH 7.4/10 mM MnCl/10 μCi of [γ-^{32}P]ATP (>6000 Ci/mmol; NEN) for 10 min at room temperature. The labeled complex was then washed twice with water and analyzed by SDS/PAGE and autoradiography. Phosphoamino acids were analyzed by two-dimensional electrophoresis as described (14).

Abbreviations: $[Ca^{2+}]_i$, cytoplasmic free calcium; IL-2, interleukin 2; mAb, monoclonal antibody; PLC, phospholipase C; PTK, protein tyrosine kinase; NP-40, Nonidet P-40; SED, staphylococcal enterotoxin D; TCR, T-cell antigen receptor; ZAP-70, ζ-chain-associated 70-kDa protein.

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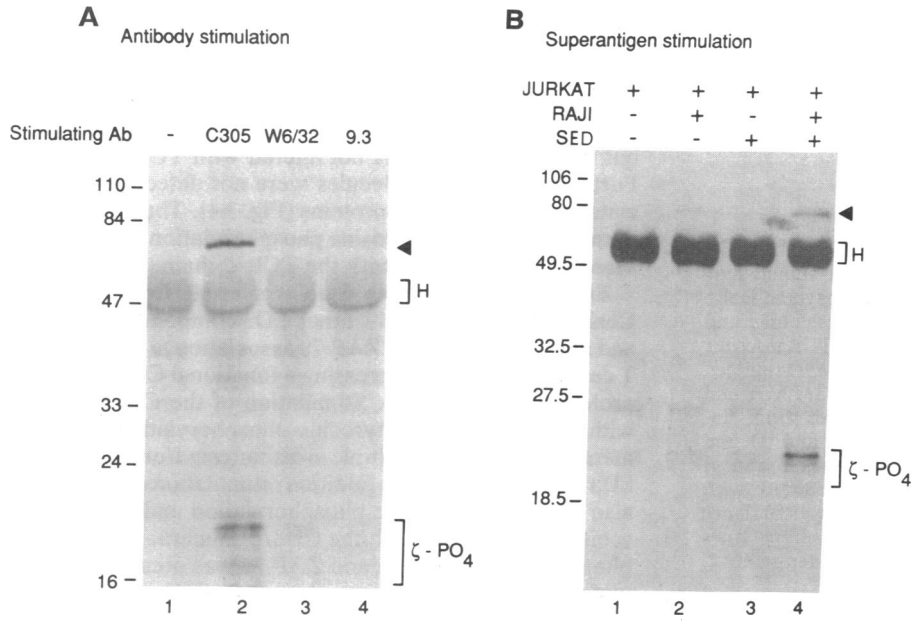

FIG. 1. TCR stimulation leads to association of a 70-kDa tyrosine phosphoprotein with ζ. (*A*) Jurkat cells were stimulated with medium (lane 1) or mAb C305 (lane 2), W6/32 (lane 3), or 9.3 (lane 4) for 2 min at 37°C. Cells were lysed and immunoprecipitated with anti-ζ antiserum (387). Immune complexes were then analyzed by SDS/PAGE and Western blotting with mAb 4G10. (*B*) Jurkat cells were stimulated with SED for 2 min and ζ-associated tyrosine phosphoproteins were analyzed as described. Lane 1, Jurkat cells alone; lane 2, Jurkat and Raji cells; lane 3, Jurkat cells stimulated with SED; and lane 4, Jurkat cells stimulated in the presence of Raji and SED. Closed arrowheads designate the phosphotyrosine-containing ZAP-70 (all figures), and H and L designate the heavy and light chains of immunoglobulin, respectively, used for immunoprecipitation. The bracket represents the complex of phosphorylated ζ chains. Size markers are shown in kDa (all figures).

RESULTS

Stimulation of the TCR Leads to Association of a 70-kDa Tyrosine Phosphoprotein with ζ. Jurkat cells were stimulated for 2 min using anti-TCR mAb C305 and lysed in 1% NP-40, and ζ immunoprecipitates were analyzed by Western blotting with anti-phosphotyrosine mAb (4G10). Following TCR stimulation, ζ underwent tyrosine phosphorylation, resulting in multiple tyrosine phosphorylated forms with molecular masses ranging from 16 kDa to 21 kDa (Fig. 1*A*, lane 2). This complex pattern most likely reflects the varying degrees of phosphorylation of the seven tyrosine residues in the cytoplasmic domain of ζ (15). In addition to phosphorylated ζ, a 70-kDa tyrosine phosphoprotein was also immunoprecipitated by the anti-ζ antiserum following TCR stimulation. Stimulation with two anti-CD3 antibodies (Leu4 and 235) produced similar results (data not shown). This 70-kDa phosphoprotein (designated ZAP-70 for ζ-associated protein) is not antigenically related to ζ since ZAP-70 was not recognized by anti-ζ antibodies in Western blotting experiments (Fig. 2*B*). In comparison to the stimulated state, there was a very low level of tyrosine phosphorylation of ζ and ZAP-70 in lysates from unstimulated cells (Fig. 1*A*, lane 1). Tyrosine phosphorylation of ZAP-70 is specific to TCR stimulation since incubation with mAbs directed against other membrane components (i.e., W6/32 or 9.3 mAb) did not give rise to any ζ-associated tyrosine phosphoproteins nor did they induce ζ phosphorylation (Fig. 1*A*, lanes 3 and 4). In addition to TCR stimulation in Jurkat, ZAP-70 was also detected as a ζ-associated phosphoprotein upon TCR stimulation in HUT-78 and HPB-ALL cells (data not shown). ZAP-70 was also coprecipitated with two other anti-ζ peptide antisera (388 and 391, ref. 12) generated against different cytoplasmic peptides of ζ, indicating that coprecipitation of ZAP-70 with ζ by 387 was not the result of cross-reacting epitopes (data not shown). Thus, ZAP-70 represents a 70-kDa tyrosine phosphoprotein that coprecipitates with ζ upon TCR stimulation.

The staphylococcal enterotoxins belong to a family of superantigens based on their ability to induce a potent proliferative response in T cells expressing the appropriate TCR V_β gene products (16). To examine superantigen stimulation of the TCR, Jurkat cells were stimulated with SED in the presence of Raji cells, which serve as the antigen-presenting cells (17). In SED-stimulated cells, as with antibody stimulation of the TCR, ZAP-70 was also detected as a tyrosine phosphoprotein that coprecipitates with ζ (Fig. 1*B*, lane 4). No tyrosine phosphorylation of ζ or ZAP-70 was detected with unstimulated Jurkat cells (lane 1), with Jurkat cells stimulated with Raji cells alone (lane 2), or with Jurkat cells stimulated with SED in the absence of an antigen-presenting cell (lane 3). Thus, ZAP-70 can be induced as a

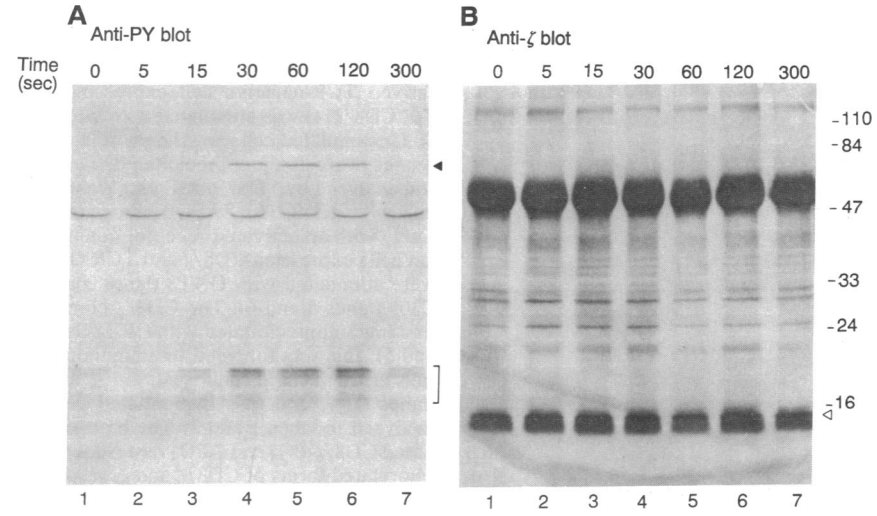

FIG. 2. Kinetics of ZAP-70 and ζ phosphorylation. Jurkat cells were stimulated with C305 for 0, 5 sec, 15 sec, 30 sec, 1 min, 2 min, and 5 min and ζ immunoprecipitates were analyzed by Western blotting with an anti-phosphotyrosine (PY) mAb, 4G10 (*A*), or anti-ζ antiserum (*B*). The open arrowhead designates unphosphorylated ζ.

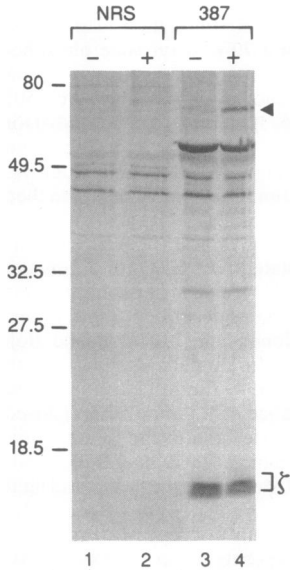

FIG. 3. Association of ZAP-70 with ζ requires TCR stimulation. Jurkat cells were biosynthetically labeled with [^{35}S]methionine and stimulated with anti-TCR mAb for 2 min, and immunoprecipitates were analyzed by SDS/PAGE and fluorography. Lysates from unstimulated (lanes 1 and 3) and stimulated cells (lanes 2 and 4) were immunoprecipitated with normal rabbit serum (NRS) (lanes 1 and 2) or anti-ζ antiserum (lanes 3 and 4). The bracket designates ζ.

ζ-associated tyrosine phosphoprotein with superantigen and antibody stimulation of the TCR.

Kinetics of ZAP-70 Phosphorylation and Association with ζ. To investigate the kinetics of ZAP-70 association with ζ, Jurkat cells were stimulated with anti-TCR mAb for various time periods and ζ-associated proteins were analyzed for tyrosine phosphoproteins. The kinetics of ZAP-70 coprecipitation with ζ paralleled that of ζ phosphorylation (Fig. 2*A*). Phosphorylated ZAP-70 was detected as early as 15 sec, peaked at 1 min, and was markedly decreased at 5 min. Similar amounts of ζ were present in each immunoprecipitate (Fig. 2*B*). ZAP-70 was undetectable or detected only at low levels as a tyrosine phosphoprotein in cell lysates of unstimulated cells (data not shown; ref. 4). The decreased amount of ZAP-70 found at 5 min may represent a combination of receptor internalization, degradation, dephosphorylation by regulatory elements, and/or dissociation from the ζ complex.

Association of ZAP-70 with ζ Increases with TCR Stimulation. To determine if the unphosphorylated form of ZAP-70 is associated with ζ in unstimulated cells, Jurkat cells were biosynthetically labeled with [^{35}S]methionine, and ζ immunoprecipitates from unstimulated and stimulated cells were analyzed. Without stimulation, a small amount of ZAP-70 coprecipitated with ζ (Fig. 3, lane 3). However, after TCR stimulation there was a >3-fold increase in the association of ZAP-70 with ζ (lane 4). Solubilization with more mild detergents such as digitonin also demonstrated minimal association of ZAP-70 with ζ in lysates prepared from unstimulated cells and a similar increase in the association of ZAP-70 with ζ following TCR stimulation (data not shown). Molecules with masses of 53 kDa and 30 kDa also coprecipitated with ζ, but their association was not altered with TCR stimulation. Furthermore, these molecules were not detected as ζ-associated tyrosine phosphoproteins (Fig. 1*A*). Thus, stimulation of the TCR results in tyrosine phosphorylation and increased association of ZAP-70 with the TCR ζ chain.

ZAP-70 Associates with the Cytoplasmic Domain of the ζ Chain. To determine if the other CD3 components (i.e., γ, δ, and ε) were required for ZAP-70 association, a TCR-negative T cell (Jβ$^-$CD8/ζ14) expressing a functional CD8/ζ chimeric protein was utilized (8). Stimulation of the CD8/ζ chimera with OKT8 resulted in tyrosine phosphorylation of the chimera migrating with multiple sizes ranging from 36 kDa to 41 kDa (Fig. 4*A*, lane 2). In addition, stimulation of the chimera also resulted in tyrosine phosphorylation and increased association of ZAP-70 with the CD8/ζ chimera. A low level of phosphorylated chimera and ZAP-70 was present in unstimulated cells (lane 1). Since the other CD3 chains are not expressed as cell surface molecules in the Jβ$^-$CD8/ζ14 cell and do not associate with the CD8/ζ chimera (8), ZAP-70 must associate with the TCR either directly with the cytoplasmic domain of ζ or indirectly through another molecule (e.g., the ζ-associated kinase; see below) that in turn interacts with ζ.

Phosphorylation of ZAP-70 Occurs only in Association with an Activated Receptor Complex. To examine further the specificity of ZAP-70 association and phosphorylation, a TCR-positive Jurkat-derived clone (JCD8/ζ2) that expresses the TCR and the transfected CD8/ζ chimera was used (8). JCD8/ζ2 can be activated either through the TCR or the CD8/ζ chimera by selectively stimulating with either anti-TCR or anti-CD8 mAb, respectively, and the receptor complexes can be analyzed by sequentially immunoprecipitating the CD8/ζ chimera followed by the TCR ζ chain. Stimulation of the TCR resulted in a marked increase in phosphorylation and association of ZAP-70 with the endogenous phosphorylated ζ chain (Fig. 4*B*, lane 6) but not with the CD8/ζ chimera (lane 5). Conversely, stimulation of the CD8/ζ chimera resulted in increased association of phosphorylated ZAP-70 with the phosphorylated CD8/ζ chimera (lane 3) but not with the endogenous ζ chain (lane 4). Low levels of the CD8/ζ chimera and ζ were present in the phosphorylated form in the unstimulated state (lanes 1 and 2, respectively), but this represented a small percentage of the phosphorylated com-

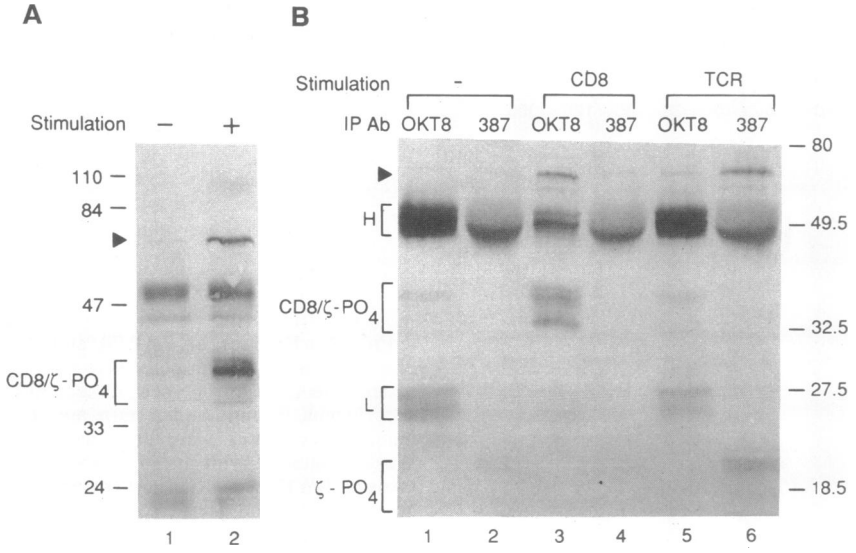

FIG. 4. (*A*) Association of ZAP-70 with the cytoplasmic domain of the ζ chain. A Jurkat-derived TCR-negative cell expressing CD8/ζ (Jβ$^-$CD8/ζ14) was stimulated with OKT8 (lane 2). Unstimulated cells are shown in lane 1. Cell lysates were immunoprecipitated with OKT8 and analyzed by SDS/PAGE and Western blotting with 4G10. (*B*) ZAP-70 associates exclusively with an activated receptor complex. Jurkat cells expressing CD8/ζ and TCR (JCD8/ζ2) were stimulated with OKT8 (lanes 3 and 4) or C305 (lanes 5 and 6). The CD8/ζ chimera was first immunoprecipitated with OKT8 (lanes 1, 3, and 5); this was followed by immunoprecipitation of the TCR ζ chain with anti-ζ antiserum (lanes 2, 4, and 6). Unstimulated cells were analyzed in lanes 1 and 2. The brackets designated CD8/ζ-PO_4 and ζ-PO_4 represent the phosphorylated forms of CD8/ζ and ζ, respectively. IP Ab, immunoprecipitating antibody.

plex seen after stimulation. Thus, association of tyrosine phosphorylated ZAP-70 with the cytoplasmic domain of ζ occurs exclusively with stimulated receptor complexes.

A Tyrosine Kinase That Is Not fyn Associates with the Cytoplasmic Domain of ζ. Since ZAP-70 undergoes tyrosine phosphorylation following TCR stimulation, ζ-associated molecules were analyzed for kinase activity. ζ immunoprecipitates were prepared in the presence of NP-40 and analyzed by *in vitro* kinase assays. Phosphorylation of ζ was seen in immunoprecipitates from unstimulated and stimulated cells (Fig. 5*A*, lanes 1 and 2). The presence of ζ-associated kinase activity *in vitro* in ζ immunoprecipitates prepared from unstimulated cells may reflect removal of inhibitory elements. An increase in kinase activity was observed in ζ immunoprecipitates prepared from stimulated cells. This increase may reflect an increase in the activity of the ζ-associated kinase following TCR stimulation and/or recruitment of other PTKs to the activated TCR complex. Phosphoamino acid analysis demonstrated that the ζ-associated kinase phosphorylates tyrosine residues exclusively (data not shown). Immunoprecipitates with W6/32, 9.3, or normal rabbit serum did not demonstrate any significant kinase activity (Fig. 5*B*, lanes 3–5). The tyrosine kinase activity also coprecipitated with the CD8/ζ chimera in the TCR-negative cell (Fig. 5*B*, lanes 1 and 2). Thus, the CD8/ζ chimera can serve as an *in vitro* substrate for the ζ-associated kinase derived from stimulated and unstimulated cells. Since the CD8/ζ chimera does not associate with any of the other TCR chains (8), the ζ-associated kinase must interact with the cytoplasmic domain of ζ.

fyn, a 59-kDa src family PTK, has been coprecipitated with the TCR in mild nonionic detergents (i.e., digitonin) (18). However, lysis in detergents with NP-40 or Triton X-100 results in dissociation of fyn from the TCR complex (18). Analysis of fyn immunoprecipitates prepared in NP-40 by an *in vitro* kinase assay demonstrated phosphorylation of molecules of 59 kDa and 72 kDa (Fig. 5*A*, lanes 3 and 4). No phosphorylated $p59^{fyn}$ was detected in ζ or CD8/ζ immunoprecipitates (Fig. 5 *A* and *B*, lanes 1 and 2) and no phosphorylated ζ was detected in fyn immunoprecipitates prepared in NP-40 (Fig. 5*A*, lanes 3 and 4). Furthermore, three sequential rounds of immunodepletion with anti-fyn mAbs did not alter the ζ-associated kinase activity (data not shown). Thus, the kinase activity associated with ζ does not appear to represent fyn.

ZAP-70 Is a Substrate for the ζ-Associated Tyrosine Kinase. In addition to tyrosine phosphorylation of ζ, tyrosine phosphorylation of ZAP-70 was also seen in the *in vitro* kinase assay of ζ immunoprecipitates, but only when prepared from stimulated cells (Fig. 5*A*, lane 2). Thus, ζ and ZAP-70 can serve as *in vitro* substrates for the ζ-associated kinase. Despite the presence of an active kinase in ζ immunoprecipitates from unstimulated cells, minimal amounts of ZAP-70 were seen. A 72-kDa molecule coprecipitates with $p59^{fyn}$ (9, 19). Analysis of *in vitro* kinase assays of ζ and fyn immunoprecipitates as described above demonstrated an ≈2-kDa difference between the 72-kDa protein associated with fyn and ZAP-70 (Fig. 5*A*, lanes 2–4). A similar difference was also seen between these two molecules from biosynthetically labeled Jurkat cells (data not shown). Definitive identification of ZAP-70 and its relationship to the 72-kDa fyn coprecipitating molecule will require protein sequencing and development of antisera directed against these molecules.

DISCUSSION

We describe a 70-kDa tyrosine phosphoprotein that coprecipitates with ζ following stimulation by anti-TCR mAbs or by SED. With both modes of TCR stimulation, ZAP-70 is the predominant tyrosine phosphoprotein associated with ζ. Tyrosine phosphorylation of ZAP-70 and its association with ζ appear tightly linked. Phosphorylation of ZAP-70, possibly by a ζ-associated tyrosine kinase (see below), may increase the affinity of ZAP-70 for ζ or the kinase. Alternatively, the association of ZAP-70 with ζ may precede and be required for its phosphorylation. Interestingly, in a cell expressing the TCR and a CD8/ζ chimeric receptor, the association and tyrosine phosphorylation of ZAP-70 occur exclusively with the activated receptor complex. Thus, an activated complex, possibly as a result of tyrosine phosphorylation, is essential for association of phosphorylated ZAP-70 with ζ.

One potential function for ZAP-70 is to couple the TCR through ζ to more distal TCR signaling molecules. Although the interaction of ZAP-70 to ζ appears to be tightly linked, the association may be indirect and be mediated by other molecules, such as the ζ-associated kinase. Thus, with TCR stimulation, the ζ-associated tyrosine kinase may phosphorylate ZAP-70, which, in turn, recruits and couples other cellular molecules to the activated TCR complex. Stimulation of the platelet-derived growth factor receptor results in

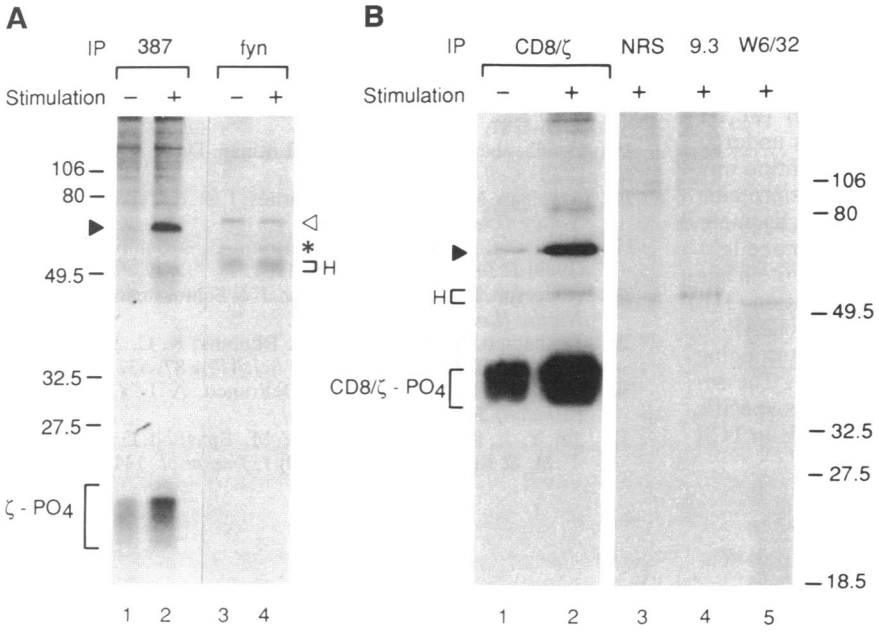

FIG. 5. ζ associates with a tyrosine kinase. (*A*) ζ (lanes 1 and 2) and fyn (lanes 3 and 4) immunoprecipitates of unstimulated (lanes 1 and 3) and stimulated (lanes 2 and 4) Jurkat cells were subjected to *in vitro* kinase assays and analyzed by SDS/PAGE and autoradiography. The bracket designates the *in vitro* phosphorylated forms of ζ, the open arrow designates a 72-kDa fyn coprecipitating protein, and the asterisk designates $p59^{fyn}$. Lanes 1 and 2 represent a 2-hr exposure, whereas lanes 3 and 4 represent a 12-hr exposure. (*B*) The kinase activity of the CD8/ζ chimera from unstimulated (lane 1) and stimulated cells (lane 2) was analyzed as described in *A*. Control immunoprecipitates with normal rabbit serum, mAb 9.3, and mAb W6/32 are represented in lanes 3–5. Lanes 1 and 2 represent a 15-min exposure, whereas lanes 3–5 represent a 12-hr exposure. The bracket designates the *in vitro* phosphorylated forms of the CD8/ζ chimera.

receptor dimerization (20, 21), trans-phosphorylation of the receptor, and tyrosine phosphorylation of a number of receptor-associated molecules, including phosphatidylinositol kinase, PLC γ1, $p60^{src}$, and GTPase-activating protein (reviewed in ref. 22). These receptor-associated proteins all contain SH2 domains that mediate the formation of high-affinity complexes with phosphotyrosine residues present in the activated receptor (23). Thus, SH2/tyrosine phosphoprotein interactions may form the basis for the association of activated receptors with their effector molecules. In a similar fashion, tyrosine phosphorylation of ZAP-70 may permit interaction of ZAP-70 with the SH2 domain of the ζ-associated kinase. In addition, ZAP-70 itself may have an SH2 domain that interacts with the phosphorylated ζ chain or other cellular tyrosine phosphoproteins to promote T-cell effector functions. More definitive structural information about ZAP-70 and the ζ-associated PTK is required to address these questions.

We also present evidence here of a ζ-associated tyrosine kinase. Using an *in vitro* kinase assay, a ζ-associated kinase phosphorylates ζ isolated from unstimulated cells and phosphorylates ZAP-70 and ζ following TCR stimulation. Since *in vitro* kinase activity is present in the unstimulated state with only minimal associated ZAP-70, it is unlikely that ZAP-70 itself represents the PTK. Two PTKs, lck and fyn, have been implicated in TCR signal transduction. lck, a member of the src protein tyrosine kinase family, associates with CD4 and CD8, which function as coreceptors by interacting with the major histocompatibility complex to promote T-cell responses (19, 24). In addition, the association of CD4 with lck is likely to play an important role in TCR signal transduction. In certain antigen-specific systems, expression of CD4 and its interaction with lck is required for TCR-mediated IL-2 production (25). fyn has also been implicated in TCR signaling and under low stringency conditions, $p59^{fyn}$ can be coprecipitated with the TCR complex (18). Furthermore, overexpression of fyn in thymocytes of transgenic animals as well as activated lck in hybridomas results in a heightened TCR response (11, 26). Thus, fyn and lck may represent potentially important PTKs in TCR signal transduction. The ζ-associated tyrosine kinase found in Jurkat cells is not likely to be fyn. The ζ chain does not have any known intrinsic enzymatic function and its amino acid sequence does not appear to code for any known kinase (15). Additional candidates for the ζ-associated kinase include other ζ-associated molecules, such as p53 seen in Fig. 3.

Molecules of 68–70 kDa have been previously reported in association with ζ. In peripheral blood lymphocytes, a 68- to 70-kDa protein has been coprecipitated with an anti-ζ mAb TIA-2 (27). In natural killer cells, a protein of 60–70 kDa has been described in association with ζ and CD16 ($Fc_{\gamma}III$ receptor) (28). However, whether these molecules undergo tyrosine phosphorylation after TCR or CD16 stimulation was not examined. Tyrosine phosphorylation of 70-kDa proteins has also been detected in mast cells after ligand engagement of the IgE receptor (29) and upon coupling of membrane IgM in B cells (30). Finally, a 68-kDa tyrosine phosphoprotein has been described in Jurkat lysates after TCR stimulation (31). These molecules may be identical or homologous to ZAP-70 and may represent a common mechanism for transducing membrane signals into more distal cellular events. The sequencing of ZAP-70 and development of antibodies specific for ZAP-70 will be required to further assess its role in TCR signal transduction.

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