quenced, about 35% carried mutations in the SRE. Of these mutants, we obtained 13 of the 18 possible single substitutions, as well as five mutants with multiple substitutions. Mobility shift probes of similar specific activities were prepared by primer extension of phagemid DNA in the presence of α-^{32}P-dCTP. DNA was annealed to universal sequencing primer, and the primer was extended with Klenow polymerase for 60 min at room temperature. The Klenow was heat-inactivated and the reaction was treated with Hind III and Eco RI, which excises a 79-bp internally labeled fragment that carried the SRE derivative. The labeled fragments were gel purified, eluted from the gel slice by diffusion, and used directly without further purification. DNA-binding assays were performed as described, with heparin-agarose–purified protein fractions (*16*). SRF was eluted from the heparin-agarose column at ~450 mM KCl, p62^{DBF} at 350 mM KCl, and p62^{TCF} flowed through the column.

19. R. Graham and M. Gilman, unpublished data.
20. To assay the activity of the mutant SRE's in vivo, we transferred them to a c-*fos*–CAT fusion plasmid that carried mouse c-*fos* sequences from −151 to +109 (*4, 8*). Phage DNA inserts were amplified by the polymerase chain reaction (PCR) in 50-μl reactions that contained phage DNA (~1 ng), forward and reverse sequencing primers (50 pmol of each), deoxynucleoside triphosphates (250 μM), and Taq polymerase (Perkin-Elmer Cetus) (1.25 units). The amplification cycle was 30 s at 94°C, 30 s at 55°C, 60 s at 72°C, and was repeated for 25 cycles. The PCR products were digested with Hind III and Sal I, and the fragments that carried the SRE were isolated from a polyacrylamide gel and ligated into the c-*fos*–CAT plasmid that had been cleaved with the same enzymes. This construction placed the SRE oligonucleotide sequence immediately upstream of position −151, separated by 7 bp. All clones were sequenced to determine that the correct SRE oligonucleotide had been subcloned. Transfection and serum stimulation of BALB/c 3T3 cells and ribonuclease (RNase) protection assays were performed as described (*5*) [L. A. Berkowitz, K. T. Riabowol, M. Z. Gilman, *Mol. Cell. Biol.* **9**, 4272 (1989)] with one modification. A total of 10 μg of cytoplasmic RNA was subject to RNase protection analysis. Half of this material (representing 5 μg of RNA from each culture) was analyzed by gel electrophoresis to compare signals from endogenous c-*fos* to ensure that all cultures were equally treated. The remaining samples were refractionated in amounts adjusted to equalize the signals from the α-globin internal control; this controls for variations in transfection efficiency and RNA recovery and facilitates comparison of signals from the transfected c-*fos*–containing plasmids. These are the gels shown in the figures. Therefore, signals from the transfected c-*fos* plasmids may be directly compared, but signals from the endogenous c-*fos* gene may not, because they represent signals from different amounts of cellular RNA.
21. U. Kikkawa, A. Kishimoto, Y. Nishizuka, *Annu. Rev. Biochem.* **58**, 31 (1989).
22. M. K. L. Collins and E. Rozengurt, *J. Cell Physiol.* **118**, 133 (1984).
23. P. Chavrier *et al.*, *Mol. Cell. Biol.* **9**, 787 (1989); B. Christy and D. Nathans, *ibid.*, p. 4889; S. Orita *et al.*, *Gene* **75**, 13 (1989).
24. T. E. Hayes, P. Sengupta, B. H. Cochran, *Genes Dev.* **2**, 1713 (1988); C. Norman, M. Runswick, R. M. Pollock, R. Treisman, *Cell* **55**, 989 (1988); G. Ammerer, *Genes Dev.* **4**, 299 (1989); E. E. Jarvis, K. L. Clark, G. F. Sprague, Jr., *ibid.* **3**, 936 (1989); S. Passmore, R. Elble, B.-K. Tye, *ibid.*, p. 921.
25. S. Tan and T. J. Richmond, *Cell* **62**, 367 (1990).
26. I. Herskowitz, *Nature* **342**, 749 (1989).
27. E. E. Jarvis *et al.*, *Mol. Cell. Biol.* **8**, 309 (1988).
28. B. Errede and G. Ammerer, *Genes Dev.* **3**, 1349 (1989).
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Association of B Cell Antigen Receptor with Protein Tyrosine Kinase Lyn

YUJI YAMANASHI, TERUTAKA KAKIUCHI, JUNICHIRO MIZUGUCHI, TADASHI YAMAMOTO,* KUMAO TOYOSHIMA†

Antigen is thought to cross-link membrane-bound immunoglobulins (Igs) of B cells, causing proliferation and differentiation or the inhibition of growth. Protein tyrosine kinases are probably involved in signal transduction for cell proliferation and differentiation. The Src-like protein tyrosine kinase Lyn is expressed preferentially in B cells. The Lyn protein and its kinase activity could be coimmunoprecipitated with IgM from detergent lysates. Cross-linking of membrane-bound IgM induced a rapid increase in tyrosine phosphorylation of at least ten distinct proteins of B cells. Thus, Lyn is physically associated with membrane-bound IgM, and is suggested to participate in antigen-mediated signal transduction.

B CELLS HAVE MEMBRANE-BOUND immunoglobulins (mIgs) on their surface, which are receptors for specific antigens. Cross-linking of the mIgs of resting B cells by antigens or antibodies to Ig generally activates B cells to enter the G_1 phase of the cell cycle, in which they become susceptible to proliferative signals provided by helper T cells (*1, 2*). Cross-linking can also cause B cell tolerance, by inhibiting growth of immature and mature B cells (*3*). These responses are preceded by phosphatidylinositol (PI) turnover, activation of protein kinase C, and Ca^{2+} mobilization (*2, 4, 5*), which are apparently dependent on the function of GTP-binding proteins (G proteins) (*6*). Because B cell antigen receptors have few cytoplasmic amino acids (*7*), mIgs might be associated with other signal transducing molecules. mIgM and mIgD associate with at least three sets of polypeptide chains at the plasma membrane (*8*). However, the molecular mechanisms by which the mIgs transmit signals intracellularly have not yet been elucidated, partly because no catalytic molecules associated with mIgs are identified.

Protein tyrosine kinases probably participate in signal transduction systems that control cell proliferation and differentiation (*9, 10*). Protein tyrosine kinases can be divided into receptor-type kinases and nonreceptor-type kinases (or Src-like kinases). The Src-like kinases are generally associated with the internal portion of the plasma membrane and have no extracellular or transmembrane sequences (*10*). Thus, Src-like kinases may act as signal transducers in association with surface receptors that lack an intracellular catalytic domain. The T lymphocyte–specific Src-like kinase p56^{lck} is physically and functionally associated with the T cell surface antigens CD4 and CD8 (*11*). Possible involvement of protein tyrosine kinases in B cell activation is suggested by the observation that antibody to CD45, a membrane-bound phosphotyrosine phosphatase, inhibits anti-IgM–induced proliferation of B cells (*12, 13*). The *lyn* gene product is an Lck-like protein tyrosine kinase that is preferentially expressed in B cells, but not T cells (*14, 15*). Our present study with antibodies to Lyn suggests that the Lyn protein is physically associated with mIgM in B cells and functions in mIgM-mediated signaling.

WEHI-231 cells are murine B cell line that carry mIgM (*5, 16*). These B cells behave as normal B cells in terms of initial biochemical events such as PI turnover and Ca^{2+} mobilization after mIgM cross-linking (*5*). This suggests that these cells possess a proper signal transduction system for early responses to mIgM cross-linking. To determine if the Lyn protein is physically associated with mIgM, we examined immunoprecipitates of IgM from detergent (digitonin) lysates of WEHI-231 cells by immunoblotting with antibodies to Lyn (anti-Lyn), which were affinity-purified from antiserum (*15*) (Fig. 1A). Both forms of Lyn, p56^{lyn} and p53^{lyn} (*17*), were coimmunoprecipitated with IgM, but not with the class I nor class II major histocompatibility complex (MHC) molecules. No Lyn protein was detected in anti-IgM immunoprecipitates from digitonin lysates of A-20 cells, which do not express mIgM. Faint and broad bands detected in each precipitate were from the antibody solutions used for the immunoprecipitation (*18*). These data support the conclusion that the Lyn protein is physically associated with mIgM in B cells.

Y. Yamanashi, T. Yamamoto, K. Toyoshima, Department of Oncology, Institute of Medical Science, University of Tokyo, Minato-ku, Tokyo 108, Japan.
T. Kakiuchi, Department of Allergology, Institute of Medical Science, University of Tokyo, Minato-ku, Tokyo 108, Japan.
J. Mizuguchi, Department of Applied Immunology, National Institute of Health, Shinagawa-ku, Tokyo 141, Japan.

*To whom correspondence should be addressed.

†Present address: Department of Oncogene Research, Research Institute for Microbial Diseases, Osaka University Suita, Osaka 565, Japan.

Densitometric analysis of the results (Fig. 1A, lanes 1 and 2) revealed that the recoveries of $p56^{lyn}$ and $p53^{lyn}$ in anti-IgM immunoprecipitates were low (1 to 2%)(*19*). We could not detect the Lyn protein in anti-IgM immunoprecipitates from Nonidet P-40 (NP40) lysates of WEHI-231 cells (*18*), whereas many other protein associations have been detected in NP40 lysates of cells. As NP40 is a stronger detergent than digitonin (*20*), the affinity of Lyn for mIgM is probably low. However, it is unlikely that only 1 to 2% of $p56^{lyn}$ is associated with mIgM in vivo, because at least 50% of $p56^{lyn}$ was comodulated with mIgM after cross-linking of mIgM (*18*). Thus, it is difficult to determine what fraction of the Lyn protein is mIgM-associated by coimmunoprecipitation analysis.

WEHI-231 cells produce two types of μ chains; only the membrane (μm), not the soluble (μs), IgM μ chains were significantly coimmunoprecipitated with the Lyn protein by anti-Lyn (Fig. 1B). The μm chain was not detected in MHC class I immunoprecipitates or in anti-Lyn solutions after immunoprecipitation. Control rabbit antibodies did not precipitate the μm polypeptide (*18*). The two forms of μs might represent heterogeneous glycosylation of the μs polypeptide (*21*), and we confirmed that the upper band comigrated with the heavy chain of secreted IgM in SDS–polyacrylamide gel electrophoresis (PAGE) analysis (*18*). Thus, the Lyn protein is associated with mIgM.

We then evaluated the kinase activity in anti-IgM immunoprecipitates from digitonin lysates of WEHI-231 cells by an in vitro kinase assay (Fig. 2). Two species of phosphorylated proteins (pp56 and pp53) comigrated with the autophosphorylated Lyn protein in anti-IgM immunoprecipitates. However, no corresponding phosphoprotein was detected in anti–class I nor anti–class II MHC immunoprecipitates. To verify that pp56 and pp53 are the phosphorylated Lyn proteins, we did reimmunoprecipitation experiments. After the in vitro phosphorylation of the anti-IgM immunoprecipitates, the immune complex was incubated in solubilizing buffer (*22*) to dissociate the proteins of the mIgM complex. The reextracted proteins were then reimmunoprecipitated with anti-Lyn. The phosphorylated $p56^{lyn}$ and $p53^{lyn}$ were detected specifically in anti-IgM, but not in anti–class I nor anti–class II MHC immunoprecipitates (Fig. 2B). Moreover, the intensities of phosphorylation of the pp56 and pp53 (Fig. 2A, lane 3) and those of reimmunoprecipitated $p56^{lyn}$ and $p53^{lyn}$ (Fig. 2B, lane 2) were about 1% of those of $p56^{lyn}$ and $p53^{lyn}$ (Fig. 2A, lane 2, and Fig. 2B, lane 1) immunoprecipitated with anti-Lyn(*19*). These data further indicate that $p56^{lyn}$ and $p53^{lyn}$ are associated with mIgM.

We reimmunoprecipitated the tyrosine phosphorylated proteins with an antibody to phosphotyrosine (*23*) after in vitro phosphorylation of anti-IgM immunoprecipitates (Fig. 2C). In addition to autophosphorylated $p56^{lyn}$ and $p53^{lyn}$, at least four

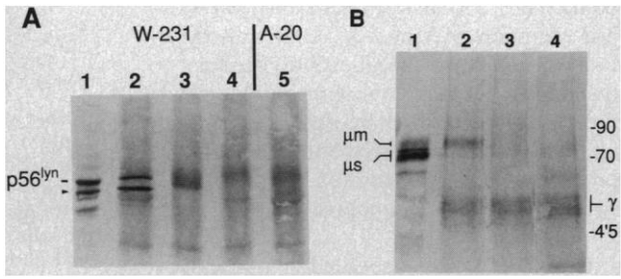

Fig. 1. Coimmunoprecipitation of $p56^{lyn}$ and $p53^{lyn}$ with mIgM. Growing cells (1×10^7) were collected by centrifugation and suspended at 0°C for 30 min in 1 ml of lysis buffer [1% digitonin, 10 mM triethanolamine, 150 mM NaCl, 10 mM iodoacetoamide, 1 mM EDTA, aprotinin (10 μg/ml), pH 7.8] (*20*). The lysate was centrifuged and the supernatant incubated with excess protein G–Sepharose. Samples of the cleared lysate were incubated with various monoclonal antibodies (MAbs) or anti-Lyn and the immune complexes were precipitated with protein G–Sepharose. The immunoprecipitates were washed with lysis buffer, suspended in sample buffer, denatured by boiling, and then tested for Lyn protein or μ chain by immunoblotting (*15*). (**A**) Lyn protein in anti-IgM immunoprecipitates. Immunoprecipitates from the lysates of WEHI-231 cells (lanes 2 to 4) and A-20 cells (lane 5) (1×10^7 cells per lane) were examined for the presence of Lyn protein. MAbs were LO.MM.9 (anti-IgM) (lanes 2 and 5), MK-D6C (anti–class II MHC) (lane 3), and M1/42.3.98 (anti–class I MHC) (lane 4). As a positive control, whole cell lysate of 4×10^5 WEHI-231 cells was examined (lane 1). The position of $p53^{lyn}$ is indicated by the arrowhead. (**B**) Anti-Lyn immunoprecipitates μm. Anti-IgM, anti-Lyn, and anti–MHC class I immunoprecipitates were prepared with LO.MM.9, anti-Lyn, and M1/42.3.98 from 1×10^5, 1×10^7, and 1×10^7 WEHI-231 cells, respectively, and were examined for the presence of μ chain with affinity-purified goat antibodies to IgM. Lane 1, IgM immunoprecipitates; lane 2, Lyn immunoprecipitates; lane 3, anti-Lyn immunoprecipitates of the lysis buffer; lane 4, MHC class I immunoprecipitates. The positions of μm, μs, and γ chains are indicated, and the positions of standard protein markers are indicated in kilodaltons. The γ and μs chains faintly detected in lanes 2 to 4 by immunoblotting originated from the antibodies used for the immunoprecipitations.

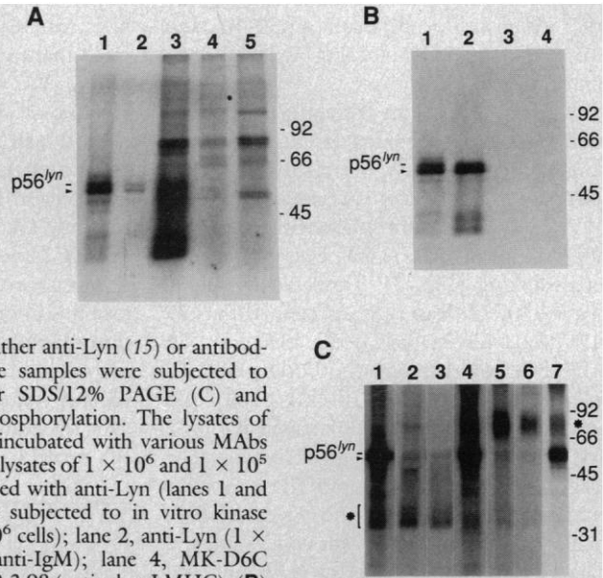

Fig. 2. Immune complex kinase assay. The immune complexes with various MAbs were prepared as described in Fig. 1, and subjected to the kinase reaction (*27*). The reaction was stopped by addition of sample buffer (reducing) or 2-mercaptoethanol–free sample buffer (nonreducing). For reimmunoprecipitation experiments, the products of the kinase reaction were reextracted from immunoprecipitates with solubilizing buffer (*22*), and the extracts were reimmunoprecipitated with either anti-Lyn (*15*) or antibodies to phosphotyrosine (*23*). The samples were subjected to SDS/8.5% PAGE (A and B) or SDS/12% PAGE (C) and autoradiography. (**A**) In vitro phosphorylation. The lysates of WEHI-231 cells (1×10^7) were incubated with various MAbs indicated below (lanes 3 to 5). The lysates of 1×10^6 and 1×10^5 WEHI-231 cells were also incubated with anti-Lyn (lanes 1 and 2). The immune complexes were subjected to in vitro kinase reaction. Lane 1, anti-Lyn (1×10^6 cells); lane 2, anti-Lyn (1×10^5 cells); lane 3, LO.MM.9 (anti-IgM); lane 4, MK-D6C (anti–class II MHC); lane 5, M1/42.3.98 (anti–class I MHC). (**B**) Reimmunoprecipitation of the autophosphorylated Lynproteins. After kinase reaction, the immune complexes (from 1×10^7 cells) indicated below were reimmunoprecipitated with anti-Lyn (lane 2 to 4). Lane 1, autophosphorylated anti-Lyn immunoprecipitates (from 1×10^5 WEHI-231 cells); lane 2, IgM; lane 3, class II MHC; lane 4, class I MHC. (**C**) Tyrosine phosphorylation in vitro of IgM-associated polypeptides. After the kinase reaction, the Lyn and IgM immune complexes (from 1×10^7 cells) were lysed in reducing (lanes 1 and 2) or nonreducing (lanes 4 and 5) sample buffer. The phosphorylated proteins in IgM immune complexes were reimmunoprecipitated with antibodies to phosphotyrosine, and the precipitates were lysed in reducing (lane 3) or nonreducing (lane 6) sample buffer. Lane 1, the Lyn immune complex; lane 2, the IgM immune complex; lane 3, reimmunoprecipitates; lane 4, the Lyn immune complex; lane 5, the IgM immune complex; lane 6, reimmunoprecipitates; lane 7, shorter time exposure of lane 4. The position of $p53^{lyn}$ is indicated by the arrowhead and those of pp30-40 and their dimerized forms are indicated by an asterisk. The positions of standard protein markers are indicated in kilodaltons.

phosphorylated proteins of 30 to 40 kD (pp34, pp35, pp37, and pp39) were specifically reimmunoprecipitated. These phosphoproteins resisted 1 M potassium hydroxide treatment (*18*), which preferentially hydrolyzes phosphoserine and phosphothreonine. Thus, a small fraction of the pp30-40 was reimmunoprecipitated along with the Lyn protein from the phosphorylated extracts of anti-IgM immunoprecipitates and the Lyn kinase may phosphorylate the pp30-40 species in vitro. Similar sizes of proteins were also identified in phosphorylated anti-Lyn immunoprecipitates (Fig. 2, A and C).

Membrane IgM is associated with at least three polypeptide chains, IgM-α/mb-1 (32 to 34 kD), Ig-β (37 to 39 kD) and Ig-γ (35 kD), which are phosphorylated at tyrosine residues in vivo (*8, 24*). The IgM-α protein forms a disulfide-linked heterodimer with either Ig-β or Ig-γ. We therefore examined the possibility that the pp30-40 described above was also disulfied-linked to each other (Fig. 2C). Mobility of the pp30-40 in anti-Lyn, anti-IgM, and anti-IgM/anti-phosphotyrosine-immunoprecipitates shifted to the 80-kD region under nonreducing conditions, whereas no shift was apparent in migration of autophosphorylated Lyn proteins. These observations indicate that the pp30-40 exist as disulfide-linked dimers. Further experiments are needed to clarify the relationship between pp30-40 and mIgM-associated molecules, IgM-α, Ig-β, and Ig-γ.

Association of Lyn with mIgM suggests that mIgM cross-linking up-regulates Lyn kinase activity, which, in turn, would phosphorylate its substrates on tyrosine residues. We examined the phosphotyrosine-containing proteins in WEHI-231 cells after mIgM cross-linking (Fig. 3). Tyrosine phosphorlyation of at least ten species, about 39 kD(p39), 42 kD(p42), 53 kD(p53), 56 kD(p56), 66 kD(p66), 70 kD(p70), 80 kD(p80), 90 kD(p90), 130 kD(p130), and 160 kD(p160) proteins, increased within 2 to 15 min after mIgM cross-linking. The degree of phosphorylation of these proteins was variable. Tyrosine phosphorylation of p39, p42, p53, and p56 was very rapid, and peaked within 2 min of cross-linking, returning to basal levels within 60 min. In contrast, tyrosine phosphorylation of p66, p70, p80, p90, p130, and p160 increased with slower kinetics. The p53 and p56 phosphoproteins comigrated with $p53^{lyn}$ and $p56^{lyn}$, and may represent autophosphorylated Lyn proteins. These rapid increases in tyrosine prosphorylation suggest that a protein tyrosine kinase participates early in the mIgM-mediated signal transduction pathway. This is consistent with the observation

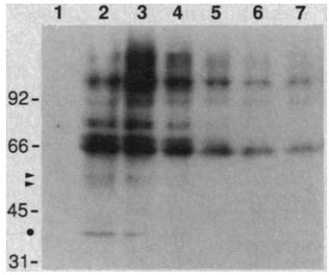

Fig. 3. Rapid induction of tyrosine phosphorylation by mIgM cross-linking. WEHI-231 cells (1×10^6 per milliliter) were incubated in the presence of affinity-purified goat antibodies [$F(ab')_2$] to IgM (10 μg/ml) for the times indicated below. Then the cells (4×10^5) were lysed in sample buffer and phosphotyrosine containing proteins were examined by immunoblotting with antibodies to phosphotyrosine as described (*23*). Incubation time: Lane 1, untreated control; lane 2, 2 min; lane 3, 15 min; lane 4, 30 min; lane 5, 60 min; lane 6, 3 hours; lane 7, 6 hours. The positions of p56 and p53 (see text) are indicated by the arrowheads, and that of p39 is indicated by the closed circle. The positions of standard protein markers are indicated in kilodaltons.

that an antibody to CD45, a membrane-bound phosphotyrosine phosphatase, inhibits its B cell activation induced by mIgM cross-linking (*12*). Two genes, *lyn* and *blk*, encoding Src-like protein kinases, are expressed preferentially in B cells (*15, 25*). However, the *blk* mRNA was reportedly undetectable in one of three mature B cell lines, whereas the *lyn* mRNA was detected in all mature B cell lines examined (ten cell lines). We could detect no significant amount of autophosphorylated Scr-like kinase, which is to be about 60 kD in size, other than Lyn after in vitro phosphorylation of anti-IgM immunoprecipitates (Fig. 2, A and C). Thus, we suggest that the Lyn protein associated with mIgM becomes autophosphorylated and phosphorylates other proteins in mIgM-mediated signal transduction. However, a clarification of possible involvement of Blk kinase in mIgM cross-linking–induced tyrosine phosphorylation awaits a study with antibodies specific to the Blk protein.

REFERENCES AND NOTES

1. A. L. DeFranco, E. S. Raveche, R. Asofsky, W. E. Paul, *J. Exp. Med.* **155**, 1523 (1982).
2. G. G. B. Klaus *et al.*, *Immunol. Rev.* **99**, 19 (1987).
3. G. J. V. Nossal, *Annu. Rev. Immunol.* **1**, 33 (1983); A. L. DeFranco, *Nature* **342**, 340 (1989); C. C. Goodnow *et al.*, *ibid.*, p. 385.
4. M. K. Bijsterbosch, C. J. Meade, G. A. Turner, G. G. B. Klaus, *Cell* **41**, 999 (1985); S. A. Grupp and J. A. K. Harmony, *J. Immunol.* **134**, 4087 (1985).
5. J. Mizuguchi, W. Tsang, S. L. Morrison, M. A. Beave, W. E. Paul, *J. Immunol.* **137**, 2162 (1986).
6. M. R. Gold, J. P. Jakway, A. L. DeFranco, *ibid.* **139**, 3604 (1987); M. M. Harnett, G. G. B. Klaus, *ibid.* **140**, 3135 (1988); J. G. Monroe, S. Haldar, *Biophysics* **1013**, 273 (1989).
7. J. Rogers *et al.*, *Cell* **20**, 303 (1980); H-L. Cheng, F. R. Blattner, L. Fitzmaurice, J. F. Mushinski, P. W. Tucker, *Nature*, **296**, 410 (1982); B. M. Tyler *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 2008 (1982); Y. Yamawaki-Kataoka, S. Nakai, T. Miyata, T. Honjo, *ibid.*, p. 2623.
8. K. S. Campbell and J. C. Cambier, *EMBO J.* **9**, 441 (1990); J. Wienands, J. Hombach, A. Radbruch, C. Riesterer, M. Reth, *ibid.*, p. 449; J. Hombach *et al.*, *Nature* **343**, 760 (1990).
9. T. Hunter and J. A. Cooper, *Annu. Rev. Biochem.* **54**, 897 (1985); A. Ullrich and J. Schlessinger, *Cell* **61**, 203 (1990).
10. J. A. Cooper, in *Peptides and Protein Phosphorylation*, B. Kemp, Ed. (CRC Press, Boca Raton, FL, 1990), pp. 85–113; K. Semba and K. Toyoshima, in *Genes and Cancer*, K. Sikora and D. Carney, Eds. (Wiley, Chichester, 1990), pp. 73–83.
11. C. E. Rudd, J. M. Trevillyan, F. E. Dasgupta, L. L. Wong, S. F. Schlossman, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 5190 (1988); A. Veillette, M. A. Boolman, E. M. Horak, J. B. Bolen, *Cell* **55**, 301 (1988); A. Veillette, M. A. Bookman, E. M. Horak, L. E. Samelson, J. B. Bolen, *Nature* **338**, 257 (1989); E. K. Barber, J. D. Dasgupta, S. F. Schlossman, J. M. Trevillyan, C. E. Rudd, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 3277 (1989).
12. N. K. Tonks *et al.*, *Biochemistry* **27**, 8697 (1988); M. F. Gruber, J. M. Bjorndahl, S. Nakamura, S. M. Fu, *J. Immunol.* **142**, 4144 (1989).
13. During preparation of this manuscript, induction of tyrosine phosphorylation after antigen receptor-mediated stimulation of B lymphocytes have been reported independently (*26*).
14. Y. Yamanashi *et al.*, *Mol. Cell. Biol.* **7**, 237 (1987).
15. Y. Yamanashi *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 6538 (1989).
16. K. J. Kim, C. Kanellppoulod-Langevin, R. M. Merwin, D. H. Sachs, R. Asofsky, *J. Immunol.* **122**, 549 (1979); A. W. Boyd and J. W. Schrader, *ibid.* **126**, 2466 (1981).
17. Anti-Lyn detects two species of proteins, $p56^{lyn}$ and the p53 (*15*). V8 peptide maps of these proteins labeled metabolically with [^{35}S]methionine were virtually identical (*18*). Thus, we tentatively concluded the p53 is the *lyn* gene product and called it $p53^{lyn}$.
18. Y. Yamanashi, T. Kakiuchi, J. Mizuguchi, T. Yamamoto, K. Toyoshima, unpublished observations.
19. The same amount of either Lyn or the kinase activity, as compared to that immunoprecipitated in Figs. 1 and 2, was immunoprecipitated (or coimmunoprecipitated) with twice as much anti-Lyn or MAb to IgM as that used in the experiments for those figures (*18*). This indicates that either Lyn or IgM in the lysates of various numbers of cells was saturated by the antibody used, and that the recovery of Lyn in these experiments is linearly related to lysed cell number.
20. H. C. Oettgen *et al.*, *Nature* **320**, 272 (1986).
21. R. G. Vasilov and H. L. Ploegh, *J. Immunol.* **12**, 804 (1982).
22. Tris-HCl (10 mM), pH 7.4, 1% NP40, 0.1% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 1 mM EDTA, aprotinin (10 μg/ml).
23. M. Hamaguchi, C. Grandori, C. Hanafusa, *Mol. Cell. Biol.* **8**, 3035 (1988).
24. N. Sakaguchi, S. Kashiwamura, M. Kimoto, P. Thalmann, F. Melchers, *EMBO J.* **7**, 3457 (1988).
25. S. M. Dymecki, J. E. Niederhuber, S. V. Desiderio, *Science* **247**, 332 (1990).
26. M. Campbell and B. M. Sefton, *EMBO J.* **9**, 2125 (1990); M. R. Gold, D. A. Law, A. L. DeFranco, *Nature* **345**, 810 (1990).
27. The immunoprecipitates were suspended in 20 μl of kinase buffer containing 10 μCi of [γ-^{32}P]ATP (5000 Ci/mmol) and incubated for 15 min.

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