Reconstitution of the B Cell Antigen Receptor Signaling Components in COS Cells*

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To elucidate interactions occurring between B cell protein tyrosine kinases and the signaling components of the B cell antigen receptor, we have co-transfected into COS cells individual tyrosine kinases together with chimeric cell surface receptors containing the cytoplasmic domains of Igα or Igβ. Of the tyrosine kinases transfected (Lyn, Blk, Hck, Syk, Fyn), only Blk was able to phosphorylate and subsequently associate with co-transfected Igα and Igβ chimeras in vivo. Association between Blk and the Igα and Igβ cytoplasmic domains was shown by mutational analyses to be the result of an SH2-phosphotyrosine interaction. We identified the tyrosine residues of the Igα and Igβ cytoplasmic domains phosphorylated by Blk. The enzymatic activity and membrane association of Blk were required for the observed phosphorylation of the Igα and Igβ chimeras. Sequences within the amino-terminal unique domain of Blk are responsible for recognition and subsequent phosphorylation of the Igα chimera since transfer of the unique region of Blk to Fyn results in the chimeric kinase’s ability to phosphorylate the cytoplasmic domain of Igα. These findings indicate that the unique domain of Src family kinases may direct recognition of certain substrates leading to their phosphorylation.

Signal transduction through the B cell antigen receptor (BCR) involves the interaction of many distinct types of signaling molecules. Cross-linking of surface immunoglobulin results in tyrosine phosphorylation of numerous proteins, including the cytoplasmic domains of the immunoglobulin-associated signaling chains Igα and Igβ, which exist as disulfide-linked heterodimers on the surface of B lymphocytes (1–3). Igα and Igβ are necessary for signal transduction through the BCR since mutation of the surface immunoglobulin molecule such that it does not associate with the Igα/β heterodimer results in B cell nonresponsiveness to antigen receptor engagement (4). Igα and Igβ each contain one immune receptor tyrosine-based activation motif (ITAM) in their cytoplasmic domains (5). Mutation of the ITAM tyrosine residues in Igα results in abrogation of B cell activation (6). The cytoplasmic domains alone of Igα and Igβ are able to mediate signal transduction in both B and T lymphocytes (7, 8). Although the signaling chains of B and T cells differ in overall sequence, the common ITAM is sufficient to mediate signaling in diverse cell types.

Tyrosine kinases expressed in B cells and implicated in induction of signaling through the BCR are Blk, Lyn, Fyn, Hck, Syk, and Btk. These kinases have been shown to be activated following BCR engagement and are activated in a sequential order (9–12). The Src and Syk classes of tyrosine kinases have been found to be associated with the Ig receptor complex (11, 13–17), but elucidation of the interactions between Src kinases and the cytoplasmic domains of the receptor complex is lacking. We have therefore reconstituted the signaling components of the BCR in COS cells to define the domains of the cytoplasmic regions of Igα and Igβ and the domains of the tyrosine kinases involved in physical and functional interactions possibly important in B cell antigen receptor-mediated signal transduction.

MATERIALS AND METHODS
COS Cell Expression Constructs
Construction of platelet-derived growth factor receptor (PDGFR)-Igα, Igβ, TCR-α, and ForRIβ Chimeras—DNA fragments encoding the extracellular domain of the PDGFR (amino acids 1–499) (18) or the transmembrane and cytoplasmic domain of Igα (amino acid 138–220), Igβ (amino acid 159–228), TCR-α chain (amino acid 31–164), and ForRIβ (amino acid 200–243) (19) were amplified from their respective cDNAs by polymerase chain reaction (PCR), fused at a BamHI site introduced by the amplification primers and cloned into pBluescript SKII+ (Stratagene). Sequencing and in vitro transcription-translation reactions showed that the fusion was in-frame and that the resulting molecule was translated into a protein of the expected size. The plasmids pSVPDGFRα, pSVPDGFRβ, pSVPDGFRαβ, and pSVPDGFRαβForRIβ were generated by subcloning fusion genes into the COS expression vector pSV7C (20). Point mutations of tyrosine residues to phenylalanine were introduced into the ITAMs of Igα and Igβ by PCR using an oligonucleotide encoding the required mutation (21).

Cloning and Mutagenesis of B Cell Protein Tyrosine Kinases—The B cell protein tyrosine kinases Blk, Lyn, Fyn, Hck, and Syk were cloned by reverse transcriptase-PCR as described elsewhere (22, 23). Point mutation of the SH2 domain of Blk was performed by overlap extension PCR (24) to change the conserved arginine residue at position 145 to lysine. Using the same technique the conserved tryptophan residues at positions 88 and 89 of the Blk SH3 domain were mutated to leucine. The double mutant of Blk was made by combining the SH2 and SH3 domain mutants via a common restriction enzyme site followed by ligation. Mutation of the myristylation site and ATP binding site of Blk was performed by PCR as described (22). To construct the Blk-Fyn chimeric kinase an Xhol site was inserted by PCR at the end of the Blk unique domain (nucleotide 156 of open reading frame) and at the beginning of the Fyn SH3 domain (nucleotide 246 of open reading frame). PCR-amplified products were subcloned and digested with Xhol and restriction enzymes outside the coding region to fuse the kinases together resulting in the BlkβFynα chimeric kinase. All cDNA constructs were subcloned into the pSV7C COS expression vector (20), sequenced to confirm mutations, and transcribed and translated in vitro to confirm that a protein of the predicted appropriate size was produced. Where appropriate, in vitro kinase assays were performed on the translated products to confirm enzymatic activity.

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1 The abbreviations used are: BCR, B cell antigen receptor; ITAM, immune receptor tyrosine-based activation motif; PDGFR, platelet-derived growth factor receptor; PCR, polymerase chain reaction; GST, glutathione S-transferase; TCR, T cell receptor.
cells as determined by two-dimensional gel electrophoresis (not shown).

Co-expression of Igα or Igβ Chimeras with Tyrosine Kinases—To determine whether the chimeric receptors would interact with tyrosine kinases present in B cells, we co-transfected the Igα chimera or Igβ chimera with Lyn, Blk, Hck, Syk, or Fyn (Fig. 3). The chimeras were expressed at similar levels in each of the transfectants (Fig. 3B). The tyrosine kinases were enzymatically active when expressed in COS cells as shown by their ability to autophosphorylate in an in vitro kinase assay (Fig. 3C) and by their ability to phosphorylate an exogenous substrate (GST-Igβ) (Fig. 3D). The co-transfected kinases had similar levels of enzymatic activity. When transfected alone, the Igα and Igβ chimeras lacked tyrosine phosphorylation. However, co-expression with the Blk tyrosine kinase resulted in phosphorylation of the Igα and Igβ chimeras on tyrosine (Fig. 3A). No other tyrosine kinase tested, including Btk and Lck (not shown), was able to phosphorylate the Igα or Igβ chimera. To ensure that the tyrosine phosphorylation observed was occurring within the intact transfected cells rather than occurring post lysis of the cells, Igα and Igβ chimeras were co-transfected with Blk (Igα-Blk, Igβ-Blk) or chimeras were transfected separately from Blk and cell lysates from the two transfectants were mixed and incubated (Igα/Blk, Igβ/Blk) (Fig. 4). Tyrosine phosphorylation of the Igα or Igβ chimeras was detected only in cell lysates where the chimera and Blk were co-transfected, indicating that tyrosine phosphorylation of the cytoplasmic domains occurred in vivo (Fig. 4).

Co-expression of FcαRIβ and ζ Chimeras with Tyrosine Kinases—We tested the ability of tyrosine kinases to phosphorylate the cytoplasmic domains of the signaling proteins FcαRIβ and ζ from the T cell receptor complex. The PDGFR extracellular domain was fused to the transmembrane and cytoplasmic domains of FcαRIβ and ζ. When these chimeric proteins were co-expressed with tyrosine kinases in COS cells, Lyn, Blk, Hck, and Fyn phosphorylated the cytoplasmic domains, while Syk did not (Fig. 5). This indicated that a unique relationship exists between the B cell specific proteins Igα, Igβ, and Blk in COS cells, since only Blk phosphorylated the cytoplasmic domains of Igα and Igβ.

Mapping Tyrosine Phosphorylation Sites of Igα and Igβ Cytoplasmic Domains—The cytoplasmic domain of Igα has three potential sites for tyrosine phosphorylation, while the Igβ cytoplasmic domain has two such sites (Fig. 1). We mutated each of these sites individually and in combination to determine the tyrosine residues phosphorylated by Blk. No decrease in the intensity of tyrosine phosphorylation of the Igα chimera was detected with mutation of the most membrane proximal tyrosine residue (Igα1Y→), while phosphorylation of the 2Y and 3Y mutants was half of the wild type level (Fig. 6). Removal of the second and third tyrosine residues from the Igα chimera abolished its phosphorylation by Blk (Igα23Y→), indicating that only these two residues are sites for phosphorylation in vivo (Fig. 6). Mutation of each of the tyrosine residues in the Igβ cytoplasmic domain separately resulted in decreased phosphorylation of the chimera and mutation of both residues together yielded no phosphorylation of the chimera by Blk (Fig. 6). This indicates that both tyrosine residues in the Igβ cytoplasmic domain are substrates for Blk in vivo.

Physical Association of the Igα and Igβ Chimeras with Blk—Since tyrosine kinases have been found to associate with the immunoglobulin receptor complex in B lymphocytes (11, 13–17) we analyzed the potential of the kinases to associate with the Igα and Igβ chimeras. We co-transfected the Igα chimera with Lyn, Blk, Hck, or Syk, lysed the cells, and immunoprecipitated the kinase. An in vitro kinase assay was then performed fol-
followed by disruption of the immune complex and reimmunoprecipitation with antiserum to Igα. Therefore, any kinase associated with the Igα chimera would phosphorylate it in vitro with 32P, allowing detection of the chimera upon autoradiographic analysis after SDS-polyacrylamide gel electrophoresis. Only Blk was physically associated with the Igα chimera (Fig. 7A). A comparison was made between Blk’s ability to phosphorylate and associate with Igα and Igβ chimeras. As shown in Fig. 7B, Blk phosphorylates the chimeras equally and associates with them to the same extent.

Syk Binds the Igα Chimera when Co-expressed with Blk—
Syk contains two tandem SH2 domains in its amino terminus (27) and has been demonstrated to bind to the Igα/Igβ heterodimer in activated B lymphocytes via an SH2-phosphotyrosine dependent mechanism (9, 23). Since the cytoplasmic domain of Igα becomes phosphorylated on tyrosine residues when co-transfected with Blk, we sought to determine whether Syk could bind to tyrosine-phosphorylated Igα. We co-transfected the Igα chimera, Blk, and Syk into COS cells and assessed the kinases’ ability to associate with Igα. Upon co-transfection with Blk, Syk became associated with the Igα cytoplasmic domain (Fig. 8), whereas expression of Syk alone with the Igα chimera did not result in association (see Fig. 7A). Co-transfection of Lyn with Blk and the Igα chimera did not result in Lyn’s association with the Igα chimera although Blk was associated with the chimera (Fig. 8).

Mutation of Blk’s SH3 and SH2 Domains—
To determine the contribution of Blk’s SH3 and SH2 domains to phosphorylation of and association with the Igα and Igβ chimeras, we made point mutations in the SH3 and SH2 domains of Blk which destroy their interaction with proline-rich regions and phosphotyrosine, respectively. These mutants retained enzymatic activity when expressed in COS cells as measured by their ability to autophosphorylate (Fig. 9C). The Blk mutants were co-expressed with the Igα or Igβ chimeras (Fig. 9D) and were assessed for association with the chimeras (Fig. 9A). Mutation of Blk’s SH2 domain alone or in combination with mutation of the SH3 domain abrogated Blk’s association with the cytoplasmic domains of Igα and Igβ (Fig. 9A). Mutation of Blk’s SH3 domain had no effect on Blk’s association with Igα or Igβ.
Phosphorylation of the Igα and Igβ chimeras was unaffected by mutation of the SH3 and SH2 domains of Blk (Fig. 9B).

Association of Blk with Igα and Igβ Chimeras—Results from an SH2-Phosphotyrosine Interaction—Since the SH2 domain of Blk is required for association with the chimeras, we assessed the effect of mutation of the Igα and Igβ tyrosine residues on association with wild type Blk. Mutation of the second and third cytoplasmic tyrosine residues of the Igα chimera resulted in decreased association with Blk (Igα Y2Y-, Igα Y3Y-) (Fig. 10). However, mutation of the most membrane proximal tyrosine, which we found to not be a substrate for phosphorylation by Blk, did not affect association of Blk with the Igα chimera (Igα Y1Y−) (Fig. 10). Mutation of the second and third tyrosine residues together (Igα Y2Y− Y3Y−) abrogated Blk’s association with the Igα chimera. Mutation of the two tyrosine residues in the Igβ cytoplasmic domain separately resulted in decreased association with Blk (Fig. 10). No association of Blk was detected when both tyrosine residues of the Igβ cytoplasmic domain were mutated together (Fig. 10).

Enzymatic Activity and Membrane Association of Blk—The protein interactions mediated by the SH3 and SH2 domains of Blk are not required for recognition and phosphorylation of the Igα and Igβ cytoplasmic domains (Fig. 9). We therefore determined if Blk’s enzymatic activity was necessary for phosphorylation of the substrates by mutating the ATP binding site of the kinase which results in a catalytically inactive enzyme.

Also, the Src kinase family is membrane associated due to myristylation of an amino-terminal glycine residue (28, 29). We therefore mutated this glycine residue, which results in cytoplasmic expression of the kinase (30). In order to assess the importance of plasma membrane localization of Blk on Igα and Igβ phosphorylation, the ATP binding site mutant lacks enzymatic activity as expected (Fig. 11). Enzymatic activity and membrane association of Blk are necessary for its phosphorylation of the Igα and Igβ chimeras (Fig. 11).

The Amino-terminal Unique Domain of Blk Confers Recognition and Phosphorylation of the Igα Chimera—Myristylation of Blk does not account for recognition of the Igα and Igβ chimeras by Blk alone, since all Src-family members are myristylated. We therefore replaced the amino-terminal unique domain of Fyn with that of Blk in order to localize the domain of Blk responsible for substrate recognition and phosphorylation. The chimeric kinase was enzymatically active and phosphorylated the cytoplasmic domain of the Igα chimera (Fig. 12). Wild type Fyn did not phosphorylate the Igα chimera. Blk’s amino-terminal unique region is responsible for Igα substrate recognition and tyrosine phosphorylation (Fig. 12).

DISCUSSION

Reconstitution of the B cell antigen receptor signaling components in nonlymphoid cells has allowed us to define interactions between the Igα and Igβ cytoplasmic domains and tyrosine kinases. A unique relationship between the B cell-specific proteins Blk, Igα, and Igβ has been discovered using this system. Although in B cells many Src family tyrosine kinases have been found to be associated with the antigen receptor complex under mild cell lysis conditions (11, 13–15), only Blk is able to phosphorylate and associate with the Igα and Igβ cytoplasmic domains in COS cells. The specificity of phosphorylation is observed only in vivo since each of the co-transfected tyrosine kinases is able to phosphorylate GST-Igβ in vitro (Fig. 3), indicating the inherent differences between in vivo and in vitro assays. The association detected between Blk and the Igα and Igβ chimeras is the result of an SH2- phosphotyrosine interaction, and occurs as a consequence of phosphorylation of the receptor components (Figs. 9 and 10). The low level of association detected between Blk and individual ITAM tyrosine mutants of Igα and Igβ may indicate that these phosphorylated tyrosine residues function in a cooperative manner to enhance kinase binding as has been suggested by in vitro studies (Fig. 10) (31). Phosphorylation of the cytoplasmic domains of Igα and Igβ by Blk occurs without a stable association of the proteins since the SH2 domain mutant of Blk retains its ability to phosphorylate Igα and Igβ cytoplasmic domains yet does not form a stable complex with the proteins (Fig. 9). Interestingly, only the tyrosine residues within the ITAM of Igα’s cytoplasmic domain are phosphorylated by Blk. The sequence surrounding the most membrane proximal tyrosine does not conform to the consensus motif found to be phosphorylated by Src family kinases in vitro (32), whereas the sequence surrounding the ITAM tyrosine residues fits the consensus motif. Phosphorylation of the ITAM tyrosine residues by Blk supports the importance of the ITAMs in receptor mediated signal transduction. These results differ somewhat from those of Flaswinkel and Reth (6) where they identified the first tyrosine residue of the Igα ITAM as the dominant tyrosine kinase phosphorylation site, without which no phosphorylation of Igα was detectable. This difference may be a function of the experimental systems used; however, the results from both systems indicate the importance of both ITAM tyrosine residues in B cell signal transduction.

The roles of the Igα and Igβ cytoplasmic domains in B cell antigen receptor signal transduction have been investigated.
Some findings support differing roles for the two proteins (15, 33, 34), whereas other results suggest that the cytoplasmic domains of these proteins are functionally equivalent in their ability to transduce signals (7, 35, 36). In the experimental system described here, the recognition and phosphorylation of the cytoplasmic domains of Igα and Igβ by Blk is equivalent, as is the association of the kinase with the proteins. The interac-

![FIG. 6. Mapping tyrosine phosphorylation sites of Igα and Igβ cytoplasmic domains.](image)

The Igα chimera was transfected alone (Igα w/o Blk) or co-transfected with Blk (Igα). Igα chimeras with tyrosine residues mutated to phenylalanine as indicated were co-transfected with Blk into COS cells. Wild type Igα chimera or Igβ chimeras with tyrosine residues mutated to phenylalanine as indicated were co-transfected with Blk. Cell lysates were immunoprecipitated with antibodies to Igα or Igβ and immunoblotted with antibodies to phosphorytrosine (top) or PDGFR (middle) to compare the amount of chimeric receptor expression. Equivalent enzymatic activity of co-transfected Blk was determined by in vitro autophosphorylation (bottom).

![FIG. 7. Blk associates with Igα and Igβ chimeras.](image)

A, COS cells were transfected with the Igα chimera alone, or were co-transfected with the chimera and the indicated tyrosine kinase. Association of the kinases with the Igα chimera was assessed by immunoprecipitating cell lysates with antisera to the indicated tyrosine kinase, followed by an immune complex kinase assay, and subsequent disruption of the immune complex followed by immunoprecipitation with antisera to Igα. Transfectants expressed equivalent levels of chimeric and equivalent kinase activity (not shown). B, the Igα or Igβ chimeras were co-transfected with Blk. Cell lysates were immunoprecipitated with the indicated antisera and immunoblotted with antisera to the PDGFR. Equivalent levels of Blk activity were present in each immunoprecipitate (not shown).

![FIG. 8. Syk binds to the Igα chimera when co-expressed with Blk.](image)

COS cells were co-transfected with the indicated cDNA constructs. Association of the kinases with the Igα chimera was assessed by immunoprecipitating cell lysates with antisera to the indicated tyrosine kinase, followed by an immune complex kinase assay, and subsequent disruption of the immune complex followed by immunoprecipitation with antisera to Igα (top). Enzymatic activity of the kinases was determined by in vitro autophosphorylation (bottom).

![FIG. 9. Effect of mutation of the SH3 and SH2 domains of Blk.](image)

Wild type Blk or Blk with point mutations in the SH3 or SH2 domain or both (Blk SH23) were co-transfected with the Igα or Igβ chimera. A, association of the kinases with the chimeras was assessed by immunoprecipitating cell lysates with antisera to Blk, followed by an immune complex kinase assay, and subsequent disruption of the immune complex followed by immunoprecipitation with antisera to Igα or Igβ. B, tyrosine phosphorylation of the chimeric receptors was assessed by immunoprecipitating cell lysates with antibody to phosphorytrosine and immunoblottting with PDGFR antisera. C, enzymatic activity of the kinases was determined in immune-complex kinase autophosphorylation assays. D, equivalence of chimeric receptor expression was determined by immunoprecipitating cell lysates with antisera to Igα or Igβ and immunoblotting with antisera to the PDGFR.

![FIG. 10. Association of Blk with Igα and Igβ cytoplasmic domains results from an SH2-phosphotyrosine interaction.](image)

The Igα chimera was transfected alone (Igα w/o Blk) or co-transfected with Blk (Igα). Igα chimeras with tyrosine residues mutated to phenylalanine as indicated were co-transfected with Blk into COS cells. Wild type Igβ chimera or Igβ chimeras with tyrosine residues mutated to phenylalanine as indicated were co-transfected with Blk. To assess the mutant chimeras' association with Blk, cell lysates were immunoprecipitated with antibody to phosphorytrosine and immunoblototted with PDGFR antisera. Transfectants expressed equivalent levels of chimeric receptor and equivalent Blk kinase activity (not shown).
containing the most divergent sequences between family members. Since the SH3 and SH2 domains were not involved in recognition and phosphorylation of the Igα and Igβ chimeras, and myristylation of Bk could not account for the specificity of Bk’s phosphorylation of the cytoplasmic domains because all Src kinases are myristylated, we examined the importance of the unique domain. The ability of Bk’s unique domain to transfer phosphorylation of the Igα chimera to Fyn indicated that sequences necessary for recognition and phosphorylation of this substrate are contained within Bk’s unique domain. There are 51 amino acids in Bk’s unique domain. A newly defined region of loose homology within the unique domain has been defined as the SH4 domain (42). This domain contains sequences important for subcellular localization of the Src kinases (43, 44). Since this domain contains variations within the Src family, the region of importance in Bk for recognition and phosphorylation of Igα may be within or outside this region. Association of Src family members with TCR (in vivo (45) and Igα in vitro (46) has been reported to involve the unique domain. The experiments described here indicate that the unique domain of Src kinases may direct recognition of certain substrates leading to their phosphorylation.

REFERENCES

1. Gold, M. R., Matsuuchi, L., Kelly, R. B., and DeFranco, A. L. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 3436.
2. Honbach, J., Tsutabata, T., Lederer, L., Steffert, H., and Reth, M. (1990) Nature 343, 760.
3. Campbell, K. S., and Camber, C. J. (1990) EMBO J. 9, 441–448.
4. Sanchez, M., Misulovin, Z., Burkhardt, A. L., Mahajan, S., Costa, T., Franke, R., Bolen, J. B., and Nussenzweig, M. C. (1993) J. Exp. Med. 178, 1049–1055.
5. Reth, M. (1989) Nature 338, 383–386.
6. Flavio, H., and Reth, M. (1994) EMBO J. 13, 83–89.
7. Taddie, J. A., Hurley, T. R., Hardwick, B. S., and Setton, B. M. (1994) J. Biol. Chem. 269, 13329–13335.
8. Burkhardt, A. L., Costa, T., Misulovin, Z., Steely, B., Bolen, J. B., and Nussenzweig, M. C. (1994) Mol. Cell. Biol. 14, 1095–1103.
9. Souaf, S. J., Mahajan, S., Rowley, R. B., Kut, S., Fargnoli, J., Burkhardt, A. L., Tsukada, S., Witte, D. N., and Bolen, J. B. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 9624–9628.
10. deWet, T. M., Brouns, G. S., Hinhelwood, S., Kinnet, C., Schuurman, R. K. B., Hendriks, R. W., and Borst, J. (1994) J. Biol. Chem. 269, 23857–23860.
11. Burkhardt, A. L., Brunswick, M., Bolen, J. B., and Mond, J. J. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 7410–7414.
12. Hutchcroft, J. E., Harrison, M. L., and Geahlen, R. L. (1991) J. Biol. Chem. 266, 14846–14949.
13. Yamanashi, Y., Kikuchi, T., Mizuguchi, J., Yamamoto, T., and Toyoshima, K. (1991) Science 251, 192–194.
14. Campbell, M. A., and Setton, B. M. (1992) Mol. Cell. Biol. 12, 2315–2322.
15. Clark, M. R., Campbell, K. S., Dazlauskas, A., Johnson, S. A., Hertz, M., Potter, T. A., Pleman, C., and Camber, J. C. (1992) Science 258, 123–126.
16. Hutchcroft, J. E., Harrison, M. L., and Geahlen, R. L. (1992) J. Biol. Chem. 267, 8633–8639.
17. Yamada, T., Taniguchi, T., Yang, C., Yasue, S., Saito, H., and Yamanura, H. (1993) Eur. J. Biochem. 213, 455–459.
18. Yarden, Y., Escobedo, J. A., Kuang, W. J., Yang-Feng, T. L., Daniel, T. O., Tremble, P. M., Chen, Y. E., Ando, M. E., Harkins, R. N., Franke, U., Fried, V. A., Ullrich, A., and Williams, L. T. (1986) Nature 323, 226–232.
19. Kinnet, J. P. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 6483–6487.
20. Escobedo, J. A., Keating, M. T., Ives, H. E., and Williams, L. T. (1988) J. Biol. Chem. 263, 1482–1487.
21. Young-Sharp, D., Thomson, N., and Kumar, R. (1990) J. Exp. Med. 171, 155–162.
22. Mahajan, S., Fargnoli, J., Burkhardt, A. L., Souaf, S. J., Kut, S. A., and Bolen, J. B. (1995) Mol. Cell. Biol. 15, 5304–5311.
23. Rowley, R. B., Burkhardt, A. L., Chao, H. G., Matsuoka, G. R., and Bolen, J. B. (1995) J. Biol. Chem. 270, 11590–11594.
24. Ho, S. N., Hunt, H. D., Horton, R. M., Pullen, J. K., and Pease, L. R. (1989) Gene (Amst.) 77, 51–59.
25. Veillet, A., Horak, I. D., Horak, E. M., Bookman, M. A., and Bolen, J. B. (1988) Mol. Cell. Biol. 8, 4353–4361.
26. Burkhardt, A. L., and Bolen, J. B. (1993) in Current Protocols in Immunology (Coligan, J. E., Kruisbeek, A. M., Margulies, D. H., Shevach, E. M., and Strober, W. eds) Vol. 11.4, pp. 1–18, Wiley, New York.
27. Taniguchi, T., Kiyoshi, T., Kondo, J., Takahashi, K., Nakamura, H., Suzuki, J., Nagai, H., Matsumoto, S., and Yamanura, H. (1991) J. Biol. Chem. 266, 15790–15796.
28. Buss, J. E., and Setton, B. M. (1985) J. Biol. Chem. 253, 7–12.
29. Cross, F. R., Garber, E. A., Pelman, D., and Hanafusa, H. (1985) Mol. Cell. Biol. 5, 1834–1842.
30. Buss, J. E., Kamps, M. P., Gould, K., and Sefton, B. M. (1986) J. Biol. Chem. 261, 468–474.
31. Clark, M. R., Johnson, S. A., and Camber, J. C. (1994) EMBO J. 13, 1911–1919.
B Cell Antigen Receptor and Tyrosine Kinase Interactions

32. Songyang, Z., Carraway, K. L., Eck, M. J., Harrison, S. C., Feldman, R. A., Mohammadi, M., Schlessinger, J., Hubbard, S. R., Smith, D. P., Eng, C., Lorenzo, M. J., Ponder, B. A. J., Mayer, B. J., and Cantley, L. C. (1995) Nature 373, 536–539
33. Kim, K. M., Alber, G., Weiser, P., and Reth, M. (1993) Eur. J. Immunol. 132, 125–146
34. Choquet, D., Ku, G., Cassard, S., Malissen, B., Korn, H., Fridman, W. H., and Bonnerot, C. (1994) J. Biol. Chem. 269, 6491–6497
35. Law, D. A., Chan, V. W. F., Datta, S. K., and DeFranco, A. L. (1993) Curr. Biol. 3, 645–657
36. Williams, G. T., Peaker, C. J. G., Patel, K. J., and Neuberger, M. S. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 474–478
37. Iwashima, M., Irving, B. A., Oers, N. S. C. v., Chan, A. C., and Weiss, A. (1994) Science 263, 1136–1139
38. Isakov, N., Wange, R. L., Burgess, W. H., Watts, J. D., Aebersold, R., and Samelson, L. E. (1995) J. Exp. Med. 181, 375–380
39. Deichaite, I., Casson, L. P., Ling, H. P., and Resh, M. D. (1988) Mol. Cell. Biol. 8, 4295–4301
40. Pellman, D., Garber, E. A., Cross, F. R., and Hanafusa, H. (1985) Nature 314, 374–377
41. Pellman, D., Garber, E. A., Cross, F. R., and Hanafusa, H. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 1623–1627
42. Resh, M. D. (1994) Cell 76, 411–413
43. Silverman, L., and Resh, M. D. (1992) J. Cell Biol. 119, 415–425
44. Alland, L., Pesockis, S. M., Atherton, R. E., Berthiaume, L., and Resh, M. D. (1994) J. Biol. Chem. 269, 16701–16705
45. Timson-Gauen, L. K., Kong, A.-N. T., Samelson, L. E., and Shaw, A. S. (1992) Mol. Cell. Biol. 12, 5438–5446
46. Pileman, C., Abrams, C., Timson-Gauen, L., Bedzyk, W., Jongstra, J., Shaw, A. S., and Cambier, J. C. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 4268–4272