Activation-induced Association of a 145-kDa Tyrosine-phosphorylated Protein with Shc and Syk in B Lymphocytes and Macrophages*

(Received for publication, June 7, 1995, and in revised form, September 21, 1995)

Mary T. Crowley†‡‡, Stacey L. Harmer‡‡‡, and Anthony L. DeFranco‡‡‡

From the G. W. Hooper Foundation and the Departments of Microbiology and Immunology and the Department of Biochemistry and Biophysics, University of California, San Francisco, California 94143-0552

Engagement of many cell surface receptors results in tyrosine phosphorylation of an overlapping set of protein substrates. Some proteins, such as the adaptor protein Shc, and a frequently observed Shc-associated protein, p145, are common substrates in a variety of receptor signaling pathways and are thus of special interest. Tyrosine-phosphorylated Shc and p145 coprecipitated with anti-Shc antibodies following B cell antigen receptor (BCR) cross-linking or interleukin-4 (IL-4) receptor activation in B cells, and after lipopolysaccharide (LPS) treatment or IgG Fc receptor (FcγR) cross-linking in macrophages. In the case of BCR stimulation, we have shown that this represented the formation of an inducible complex. Furthermore, in response to LPS activation or FcγR cross-linking of macrophages (but not IL-4 treatment) of B cells, we observed a similar tyrosine-phosphorylated p145 protein associated with the tyrosine kinase Syk. We did not detect any Shc associated with Syk, indicating that a trimeric complex of Shc, Syk, and p145 was not formed in significant amounts. By several criteria, the Syk-associated p145 was very likely the same protein as the previously identified Shc-associated p145. The Syk-associated p145 and the Shc-associated p145 exhibited identical mobility by SDS-polyacrylamide gel electrophoresis and identical patterns of induced tyrosine phosphorylation. The p145 protein that coprecipitated with either Shc or Syk bound to a GST-Shc fusion protein. In addition, a monoclonal antibody developed against Shc-associated p145 also immunoblotted the Syk-associated p145. The observations that p145 associated with both Shc and Syk proteins, in response to stimulation of a variety of receptors, suggest that it plays an important role in coordinating early signaling events.

Receptors for antigens, cytokines, and growth factors utilize tyrosine phosphorylation of proteins to initiate and propagate intracellular events that result in cellular responses. As many of these receptors lack intrinsic tyrosine kinase activity, this increase in cellular phosphorylation can result from the recruitment and activation of cytoplasmic tyrosine kinases including Syk, ZAP-70,Src family tyrosine kinases (1), and JAK family tyrosine kinases (2). Targets of these kinases include enzymes that generate second messengers, regulators of Ras and other Ras-like G proteins, transcription factors, and a variety of other proteins that are believed to play a role in receptor signaling (3).

Recently, attention has focused on the Shc protein, which is a ubiquitously expressed adaptor protein that is tyrosine-phosphorylated following stimulation of B or T lymphocyte antigen receptors (BCR) and TCR (4–6), growth factor receptors (7), and cytokine receptors (8–11). The SHC1 gene encodes the two major Shc isoforms, p52SHC and p46SHC. These two proteins are produced by utilization of two in-frame translation initiation sites. Each Shc isoform contains a C-terminal SH2 domain, a proline-rich central domain with multiple collagen-like type repeats, and at the N terminus, a recently identified phosphotyrosine interaction domain (12–15). All of these domains are likely to be important for mediating protein-protein interactions (12). Phosphorylation at Tyr-317 of the central domain of Shc directs binding of the Grb-2-SOS-1 complex to Shc via the SH2 domain of Grb-2 (16). By virtue of this association, Shc has been implicated in Ras activation. The localization of SOS-1 to the plasma membrane is necessary for the activation of Ras (17). Although Shc is not itself a membrane protein, it can bind to tyrosine-phosphorylated activated IL-2 receptor (18), erythropoietin receptor (10), or TCR (5). Presumably this binding, coupled with Grb-2 binding to phosphorylated Shc, would direct Grb-2-SOS complexes to the membrane following receptor activation. Other reports have implicated Shc in Ras activation in non-hematopoietic cells as well (19, 20).

In different cell types activated by a variety of stimuli, Shc also associates with a highly tyrosine-phosphorylated protein of approximately 145 kDa (4, 11, 21, 22). Depending upon the cell type analyzed, this protein has been referred to as p140, p145, or p150. It can appear as a single band or as several closely spaced bands. These Shc-associated proteins may well be the same protein or very similar isoforms of the same protein. In this report, we used BCR-stimulated B lymphocytes as a model system to demonstrate that the p145-Shc association is an inducible event and that complex formation and maintenance correlates with levels of tyrosine phosphorylation of these proteins. In examining responses via other signaling...
receptors, we found association of tyrosine-phosphorylated p145 with Shc in response to IL-4 treatment of B lymphocytes and FcεR cross-linking or LPS treatment of macrophages. To address which tyrosine kinases may phosphorylate Shc and p145, we looked for direct association of these substrates with specific kinases. We found that Syk kinase coprecipitated with the p145 protein following most but not all of the same stimuli that induced p145 to associate with Shc. These observations suggest that p145 may have an important role in coordinating signaling pathways from a number of receptors.

MATERIALS AND METHODS

Reagents—Goat anti-mouse IgM antibodies were obtained from Jackson Immunological Research Laboratories (West Grove, PA). Murine IL-4 was purchased from Genzyme (Cambridge, MA). Antibodies from ATCC and was used to cross-link 2.4G2. Anti-Syk antibody was purchased from Pharmingen (San Diego, CA). The mouse anti-rat immunoglobulin κ chain monoclonal antibody, MAR18.5, was obtained from ATCC and was used to cross-link 2.4G2. Anti-Syk antibody was produced by immunizing rabbits with a GST fusion protein containing amino acids 299–333 of murine Syk. Horseradish peroxidase (HRP)-conjugated sheep anti-mouse IgG antibody, Pro-Mix [35S]methionine and [35S]cysteine cell labeling mix, and [35S]methionine were purchased from RIBI adjuvant as per the manufacturer's protocol (DuPont).

RESULTS

BCR-associated Induction of Shc with p145—Recent reports have demonstrated that stimulation through a variety of receptors in different cell types results in tyrosine phosphorylation of the signaling adapter protein Shc and the association of Shc with a 145-kDa tyrosine-phosphorylated protein. For example, BCR cross-linking in B lymphocytes (4), macrophage colony-stimulating factor treatment of myeloid cells (21), and IL-3, Steel factor, and erythropoietin treatment of responsive hematopoietic stem cell lines (22) all induce the appearance of the signaling adapter protein Shc and the association of Shc with a 145-kDa tyrosine-phosphorylated protein. For anti-phosphotyrosine immunoblotting, 4610 hybridoma supernatant was used at a 1:5 dilution. Alternatively, anti-Shc monoclonal antibody was used at 1 μg/ml or anti-Syk antisemur was used at 1:1,000 dilution. Blots were incubated with HRP-conjugated secondary antibody (1 μg/ml in TBST) for 30–60 min. Bands were visualized using the Renaissance chemiluminescence detection system (DuPont).

Activation-induced Association of p145 with Shc and Syk

Department of Microbiology and Immunology, Duke University Medical Center, Durham, North Carolina 27710-0262.
was immunoprecipitated by the stimulating antibody. In addition to p145, proteins of 40, 45, 100, and 110 kDa coprecipitated with Shc at 15 and 30 min following stimulation. The identity of these bands was unknown, and none were recognized by anti-phosphotyrosine antibodies. However, anti-phosphotyrosine immunoblotting of the same samples showed the prominent doublet of Shc-associated tyrosine-phosphorylated proteins at 140 and 145 kDa (Fig. 1B). In addition, a weaker tyrosine-phosphorylated band of 150 kDa was sometimes seen. To confirm that the inducibly associated 140- and 145-kDa proteins were indeed the tyrosine-phosphorylated species observed, the 35S-labeled Shc immunoprecipitates were dissociated by treatment with 2% SDS and then reimmunoprecipitated with anti-phosphotyrosine antibody. The metabolically labeled protein doublet at 140–145 kDa was reprecipitated in this way (data not shown), indicating that these bands corresponded to the p145 tyrosine-phosphorylated proteins. We believe that these bands were all isoforms of the same protein as each of these proteins exhibited the same properties in all of the experiments reported here. The nature of the changes that caused differential migration are not known at this time. These bands retained their different mobilities after de-phosphorylation with calf intestinal phosphatase (data not shown), suggesting that some property other than phosphorylation was responsible for the differential mobility of these bands.

The Formation of the Shc-p145 Complex and Its Persistence in the Cell Were Correlated with Tyrosine Phosphorylation—We also examined the kinetics of the Shc-p145 complex formation to determine whether this was a transient response or a prolonged signaling event following BCR stimulation (Fig. 1). The phosphotyrosine content of Shc and p145 increased rapidly and then decreased sharply by 30 min after stimulation. This response peaked at 8–10 min (data not shown). There was only faintly detectable tyrosine-phosphorylated p145 associated with Shc by 90 min after stimulation. The association of p145 with Shc as detected with biosynthetically labeled protein declined over a similar time course (Fig. 1A), except that the sensitivity of detection by metabolic labeling was less so the small amount of complex remaining at 90 min could only be detected by anti-phosphotyrosine immunoblotting. The correlation observed between tyrosine phosphorylation and association was consistent with a requirement for tyrosine phosphorylation of Shc and/or p145 proteins for the complex to remain intact. As Shc has two domains capable of interacting with other tyrosine-phosphorylated proteins, it is likely that BCR stimulation induced the tyrosine phosphorylation of p145 and this in turn led to Shc binding. Indeed, it was recently reported that p145 isolated from fibroblasts must be tyrosine-phosphorylated for in vitro association with Shc (13).

Syk Kinase Association with p145—The induction of tyrosine phosphorylation of signaling proteins by BCR cross-linking requires the activities of intracellular tyrosine kinases. As the tyrosine kinases Syk and Lyn play important roles in signaling by the BCR (28), we employed coimmunoprecipitation experiments to examine whether either of these kinases associated with Shc and/or Shc-associated p145 upon stimulation of B cells. The Syk- or Lyn-immunoprecipitated proteins were resolved by SDS-PAGE and immunoblotted with anti-phosphotyrosine antibody. In agreement with previous reports (29, 30), we found that Syk was tyrosine-phosphorylated in response to BCR stimulation in B cells. Additionally, Syk immunoprecipitates (but not Lyn immunoprecipitates; data not shown) from BCR-stimulated B cells contained a p145 tyrosine-phosphorylated doublet (Fig. 2A). This Syk-associated doublet comigrated with the Shc-associated p145 doublet, suggesting they might be the same proteins. This suggested the possibility that Shc and Syk might interact to form a trimolecular complex with p145. However, there was no detectable Shc protein in the anti-Syk immunoprecipitates (Fig. 2B). Correspondingly, there was no Syk detected in the anti-Shc immunoprecipitates. Thus, the Syk-p145 complex did not include detectable amounts of Shc.

The p145 protein associated with Syk appeared to be the same as the p145 associated with Shc both by electrophoretic mobility and pattern of anti-phosphotyrosine blotting. To de-
termine in a more direct manner whether these tyrosine-phosphorylated p145 proteins were indeed the same protein, we examined whether Shc could associate with p145 protein that was isolated by virtue of its association with Syk. For this purpose, we made use of previous observations that recombinant proteins containing full-length Shc fused to GST (GST-Shc) can interact with Shc-associated p145 resolved by SDS-PAGE and transferred to nitrocellulose (13). Shc or Syk immunoprecipitates from stimulated and unstimulated WEHI-231 cells were resolved by SDS-PAGE (B) and the possible presence of a coprecipitating component was assessed by immunoblotting in parallel with polyclonal anti-Syk antibodies (upper section) or monoclonal anti-Shc antibodies (lower section).

Finally, the Shc-associated and Syk-associated p145 proteins were immunologically related. Hybridomas were generated from spleen cells of mice immunized with purified, Shc-associated p145. Monoclonal antibodies were selected on the basis of reactivity with Shc-associated p145 by immunoblotting. One monoclonal antibody, 4U2, reacted in an immunoblot with both the Shc-associated, and Syk-associated p140, p145, and p150.
Activation-induced Association of p145 with Shc and Syk

Figure 4. Monoclonal anti-Shc-associated p145 antibody immunoblots both Shc- and Syk-associated p145. Shc or Syk immunoprecipitates from anti-IgM-stimulated WEHI-231 cell lysates were separated by SDS-PAGE and transferred to nitrocellulose. Membranes were immunoblotted with anti-phosphotyrosine (P-Y) antibody (3 × 10⁷ cells/lane) or 4U2, an anti-p145 antibody (13 × 10⁷ cells/lane) and reactions were detected by use of an HRP-conjugated anti-mouse secondary antibody, which did not produce any signal by itself (not shown).

Figure 5. Immunoprecipitation of p145 with anti-Syk requires Syk expression. Chicken B cells DT40 (parental) and S1.10 (syk−) were stimulated with or without added pervanadate solution (400 μM) for 4 min. Since total cellular phosphorylation was consistently lower in the syk− cells as compared to the parental B cells, a greater amount of protein of the former was used for the immunoprecipitations to give similar phosphotyrosine signals. Cells were lysed and subjected to immunoprecipitation with anti-Shc antibody (0.6 × 10⁶ cells or 1.8 × 10⁶ cells/point) or anti-Syk antibody (1.5 × 10⁶ or 4.5 × 10⁶ cell/point). Immunoprecipitates were resolved by SDS-PAGE and then analyzed by anti-phosphotyrosine immunoblotting. The migration positions of the tyrosine-phosphorylated p145, Syk, and Shc proteins are indicated.

Phosphorylation and association in B lymphocytes, we treated WEHI-231 B cells or splenic B cells with IL-4 and then examined tyrosine phosphorylation of immunoprecipitated proteins by anti-phosphotyrosine immunoblotting. IL-4 treatment resulted in clear tyrosine phosphorylation of Shc and an increase in Shc-associated tyrosine-phosphorylated p145 (Fig. 6 and data not shown). This response was less dramatic than that seen in response to anti-IgM but was still readily detectable. In contrast, the tyrosine phosphorylation of Syk was not induced by IL-4 stimulation and tyrosine-phosphorylated p145 protein was not immunoprecipitated with Syk (data not shown). As Syk activation is correlated with increased tyrosine phosphorylation of Syk, it is likely that Syk was not activated in B cells responding to IL-4. Thus, the association of tyrosine-phosphorylated p145 with Syk was dependent on the nature of the stimulation and did not necessarily correlate with the tyrosine phosphorylation of p145. This observation suggests first, that Syk may require tyrosine phosphorylation and/or activation to associate with p145 and second, that tyrosine kinases other than Syk can phosphorylate p145 and Shc under certain physiological conditions.

Phosphorylated Shc:p145 and Syk:p145 Complexes in Macrophages—To see if the association of p145 with Shc and with Syk occurs in response to stimulation of other receptors, we examined tyrosine phosphorylation of p145, Shc, and Syk in macrophages stimulated through Fcγ receptors. Like the BCR and the TCR, the FcγR complexes have cytoplasmic domains with immunoreceptor tyrosine-based activation motifs (ITAM) sequences (38) and therefore activate signal transduction events by a very similar mechanism. Cross-linking the FcγRs in the murine macrophage cell line RAW 264.7 by addition of the anti-FcγR antibody 2.4G2 and of a secondary cross-linking antibody resulted in tyrosine phosphorylation of Shc and the association of Shc with tyrosine-phosphorylated p145 (Fig. 7). Cross-linking the FcγRs in these macrophages also induced tyrosine phosphorylation of Syk and the appearance of a low level of tyrosine-phosphorylated p145 associated with Syk, which was easily visible upon longer exposure of immunoblots (Fig. 7B). The low levels of Syk tyrosine phosphorylation and Shc:p145 complex formation that were observed in cells not receiving cross-linking treatment were equivalent to those seen in untreated cells.

Another potential stimulator of macrophages is bacterial LPS. LPS treatment of macrophages induces them to produce numerous cytokines and proinflammatory mediators (39, 40). LPS binding to the glycosylphosphatidylinositol-linked protein CD14 leads to increased tyrosine phosphorylation in macrophages, and this event is important for downstream responses (41–43). Although CD14 lacks any cytoplasmic tail sequences for direct association and activation of cytoplasmic protein kinases or signaling proteins, LPS rapidly activates the Src family tyrosine kinases Lyn, Hck, and Fgr in human monocytes suggesting an important role for these tyrosine kinases in LPS responses (44, 45). Interestingly, RAW 264.7 cells stimulated with LPS exhibited induced tyrosine phosphorylation of Shc and Shc-associated p145 (Fig. 7A). The induced tyrosine phosphorylation and association of Shc and p145 was greater than that which occurred in response to FcγR cross-linking. Moreover, these were rapid events in macrophages, being evident as early as 1 min and peaking at about 10 min after stimulation (data not shown). Unexpectedly, LPS signaling also induced
tyrosine phosphorylation of Syk and its association with p145 (Fig. 7A). As was true for p145-Shc association, the p145-Syk association (as detected by anti-phosphotyrosine immunoblotting) was greater in response to LPS than in response to FcγR cross-linking. This is most likely due to the relatively low level of FcγR expression on these cells. Cross-linking of higher levels of FcγR expressed on other macrophage lines elicited a signal equivalent to or greater than that induced by LPS. Syk and Shc did not appear to associate in a precipitable complex from FcγR- or LPS-stimulated macrophages, similar to the situation with BCR-stimulated B cells (data not shown). Although it has been reported that Syk becomes tyrosine-phosphorylated in response to FcγR cross-linking and is important in phagocytosis (46, 47), a role for Syk in LPS receptor signaling in macrophages had not been previously suggested. These observations were particularly interesting, as few downstream targets of tyrosine kinases in LPS-activated macrophages have been identified. Aside from the observations regarding Syk and Shc reported here, the only other reported early substrate for tyrosine kinases play critical roles in signaling by antigen receptors (52), and by FcεRI and FcγR (46, 53). Upon immunoprecipitation of Lyn and Syk from BCR-stimulated B cells, we found tyrosine-phosphorylated p145 associated with Syk but not Lyn. In contrast, we did not detect any association of Shc with Syk. Recently, an interaction between Syk and Shc was detected following overexpression of Syk in B cells (54). It could be that a very small amount of ShcSyk complex did form in vivo in our BCR-stimulated B cells but was below our limit of detection. Such a small amount of Shc would not be sufficient to account for the prominent association of p145 with Syk, however, so p145-Syk association must either be direct or mediated via an association of both Syk and p145 with a protein or proteins other than Shc. In any case, the association of p145 with Syk is provocative and may reflect a role for Syk in phosphorylating p145 and possibly Shc as well.

The Syk-associated 145-kDa doublet appeared to be the same as the Shc-associated p145 doublet by several criteria. First, the distinctive pattern of tyrosine phosphorylation of the Shc-associated p145 was identical to that produced by the Syk-associated p145. In murine cells, the upper band was a more highly phosphorylated and more abundant protein than the lower band. In chicken B cells, the banding pattern was different from that found in mammalian B cells, yet the Syk-associated p145 again resembled the Shc-associated p145. Second, the association of p145 with Syk or Shc occurred with similar kinetics (data not shown). Third, murine GST-Shc protein was able to bind Shc-associated p145 or Syk-associated
p145 immunoprecipitated from both murine and chicken cells and immobilized on nitrocellulose. And finally, a monoclonal antibody specific for Shc-associated p145 was reactive with Syk-associated p145 protein as well. Thus, the Syk-associated p145 and the Shc-associated p145 behaved identically in many respects, strongly suggesting their identity.

The tyrosine phosphorylation of Shc and the association of Shc with tyrosine-phosphorylated p145 were also seen in B cells stimulated through the IL-4 receptor, a member of the cytokine/hematopoietic receptor superfamily, and in macrophages stimulated either through FcγRs or by LPS. We observed that FcγR cross-linking or LPS stimulation of macrophages also induced tyrosine phosphorylation of Syk and its association with p145. IL-4 stimulation of B cells did not lead to Syk phosphorylation or its association with tyrosine-phosphorylated p145. Thus, association of p145 with Syk correlated with the tyrosine phosphorylation of Syk and not with the tyrosine phosphorylation of p145. While the mechanism by which p145 and Syk associate with each other remains to be determined, these results suggest that Syk may not associate via its SH2 domains with p145 or that the SH2 domains of Syk are inaccessible when the kinase is inactive. In support of the first suggestion, we have not detected an interaction between a GST-Syk(SH2)2 fusion protein and p145 immobilized on membranes.3

The Syk-p145 association observed in response to BCR-, FcγR-, and LPS-mediated activation suggests a role for Syk in phosphorylating either p145 or Shc. If that is the case then the IL-4 receptor presumably utilizes a different mechanism for phosphorylating p145 and Shc. Additionally, our results comparing BCR cross-linking and IL-4 receptor signaling in B cells indicated that different p145 associations were induced by kinases activated through different receptors within the same cell. This result raises the possibility that phosphorylated p145, although it is an early substrate of tyrosine kinases, may function in separate and distinct signaling pathways depending on the nature of the signaling receptor.

The functional significance of the p145-Shc and p145-Syk complexes is not yet known, although several possibilities can be considered. These complexes appeared to be primarily membrane-bound in BCR-stimulated B cells.4 Association with p145 might concentrate Shc in a particular location in the cell, such as the plasma membrane. Thus, p145 could function to bring Shc to the membrane where its subsequent tyrosine phosphorylation and association with Grb-2/SOS-1 complex could promote Ras activation. In agreement with this idea, Saxton et al. (4) have shown that Shc-p145 complexes are present in cytosolic and membrane fractions of cells and that BCR stimulation resulted in elevated levels of Shc complexes in the membrane-bound fraction. It is unclear whether p145 or some other component is responsible for this membrane localization. Moreover, studies in other cell types have given different results regarding the molecular nature of the interactions between Shc, p145, and Grb-2, the intracellular localization of these complexes, and whether these proteins are within a single complex (4, 11, 21, 22). It is unclear at this time whether these differences are due to the different experimental approaches utilized or, as reflected in our own results regarding Syk-p145 and Shc-p145 complexes, whether the exact nature of the complex may vary with the cell type and route of stimulation.

It is possible that p145 membrane localization is due to its association with tyrosine-phosphorylated receptor cytoplasmic domains, as has been reported for Syk. In this model, the coprecipitation of Syk and p145 would reflect a trimerolecular complex consisting of phosphorylated receptor tails interacting with both p145 and Syk. Interestingly, it has recently been reported that stimulation of the FcγRI on mast cells resulted in common coprecipitation of a tyrosine-phosphorylated protein of 145 kDa with the FcγRI β chain (53). In this system, a GST-Syk(SH2)2 fusion protein was used to precipitate not only the γ and β chains of the FcγRI, but also a tyrosine-phosphorylated doublet resembling p145 (53, 55). These observations may reflect associations of GST-Syk(SH2)2 and p145 with the FcγRI γ chain and β chain cytoplasmic domains. Interestingly, we have observed a small amount of p145 protein to coprecipitate with Igλ from lysates of BCR-stimulated B cells.3 Thus, one possible scenario is that BCR and FcγR stimulation leads to p145 association with tyrosine-phosphorylated receptor tails, followed by tyrosine phosphorylation of p145, dissociation of Syk, and subsequent association of Shc with p145. This could be a means whereby Shc would achieve recruitment to the membrane and subsequent phosphorylation on Tyr-317. Phosphorylated Shc could then be bound by the Grb-2(SH2) domain of a Grb-2/SOS-1 complex resulting in activation of Ras at the membrane. The exact nature of the interactions between Syk, ITAM-containing receptor chains and p145 and whether this association is used as a mechanism for Ras activation remain to be determined.

The observations reported here suggest that p145 may be an important signaling component. The membrane localization of p145 and its association with Shc in B cells stimulated through the BCR are consistent with p145 playing an important role in Ras activation. It is equally possible that p145 is a signaling effector that is regulated by Shc and therefore represents a non-Ras Shc-signaling pathway. Two of the receptors that induce Shc tyrosine phosphorylation and association with p145, IL-4R in B cells and FcγR in macrophages, have not been reported to result in Ras activation. LPS may activate Ras in monocytes and macrophages, although this has not been uniformly observed to date (56, 57). Thus the downstream consequences of Shc and p145 tyrosine phosphorylation in B cells or in macrophages remains unknown. The cloning of the gene for p145 and subsequent characterization of its primary sequence may shed light on these issues. In any case, the ability of p145 to interact with the cytoplasmic signaling components Shc and Syk suggests that it plays an important role in the initiation of signaling events in activated B cells and macrophages.

Acknowledgments—We thank Dr. Tomohiro Kurosaki for the generous gift of the chicken B cell lines DT40 and S1.10. We thank Drs. Khoi Lee and Lewis Williams for the plasmid pG-IKS. We thank Dr. P. G. Pellici for the Shc cDNA clone. We thank Steve Robbins, Steve Weinstein, Julie Hambleton, Debbie Law, and Jim Richards for many helpful discussions and for critical reading of the manuscript.

REFERENCES

1. Howe, L., and Weiss, A. (1995) Trends Biochem. Sci. 20, 59–64
2. Ziemiecki, A., Harpur, A. G., and Wilks, A. F. (1994) Trends Cell Biol. 4, 207–212
3. DeFranco, A. L. (1994) Curr. Opin. Immunol. 6, 364–371
4. Saxton, T. M., van Oostveen, I., Bowtell, D., Aebersold, R., and Gold, M. R. (1994) J. Immunol. 153, 623–636
5. Ravichandran, K. S., Lee, K. K., Songyang, Z., Cantley, L. C., Burn, P., and Burakoff, S. J. (1993) Science 262, 902–905
6. Sieh, M., Balzer, A., Schlessinger, J., and Weis, A. (1994) Mol. Cell Biol. 14, 4435–4442
7. Ruff, A., Bonnet, J., Gourse, R., and Baltimore, D. (1993) J. Biol. Chem. 268, 610–618
8. Matsuguchi, T., Salgia, R., Hailik, M., Dederer, B., Ernst, T. J., and Griffin, J. D. (1994) J. Biol. Chem. 269, 5016–5021
9. Cutler, R. L., Liu, L., Damen, J. E., and Krystal, G. (1993) J. Biol. Chem. 268, 21463–21465
10. Damens, J. E., Liu, L., Cutler, R. L., and Krystal, G. (1993) Blood 82, 2296–2303
11. Welham, M. J., Duronio, V., Leslie, K. B., Bowtell, D., and Schrader, J. W. (1994) J. Biol. Chem. 269, 21165–21176
12. Pelicci, G., Lanfrancone, L., Grignani, G., Glade, J., Cavallo, F., and Forni, G., unpublished results.

3 S. L. Harmer and A. L. DeFranco, unpublished observations.
4 M. T. Crowley and A. L. DeFranco, unpublished results.
Activation-induced Association of p145 with Shc and Syk

Nicoletti, I., Grignani, F., Pawson, T., and Pelicci, P. G. (1992) Cell 70, 93–104

13. Kavanaugh, W. M., and Williams, L. T. (1994) Science 266, 1862–1865
14. Pawson, T. (1995) Nature 373, 573–579
15. Bork, P., and Margolis, B. (1995) Cell 80, 693–694
16. Salcini, A. E., McGlade, J., Pelicci, G., Nicoletti, I., Pawson, T., and Pelicci, P. G. (1994) Oncogene 9, 2827–2836
17. Aronheim, A., Engelberg, D., Li, N., al-Alawi, N., Schlessinger, J., and Karin, M. (1994) Cell 78, 949–961
18. Ravichandran, K. S., and Burakoff, S. J. (1994) J. Biol. Chem. 269, 1599–1602
19. Basu, T., Warne, P., and Downward, J. (1994) Oncogene 9, 3483–3491
20. Skoblin, E. Y., Lee, C. H., Batzer, A., Vicentini, L. M., Zhou, M., Daly, R., Myers, M. J., Backer, J. M., Ullrich, A., and White, M. F. (1993) EMBO J. 12, 1929–1936
21. Lioubin, M. N., Myles, G. M., Carlberg, K., Böttcher, D., and Rohrschneider, L. R. (1994) Mol. Cell Biol. 14, 6562–6569
22. Liu, L., Degen, J. E., Cutler, R. L., and Krystal, G. (1994) Mol. Cell Biol. 14, 6926–6935
23. Gold, M. R., Law, D. A., and DeFranco, A. L. (1990) Nature 345, 810–813
24. Evans, G. A., Garcia, G. G., Erwin, R., Howard, O. M., and Farrar, W. L. (1994) J. Biol. Chem. 269, 23407–23412
25. Ashwell, J. D., DeFranco, A. L., Paul, W. E., and Schwartz, R. H. (1984) J. Exp. Med. 159, 801–803
26. Gold, M. R., Crowley, M. T., Martin, G. A., McCormick, F., and DeFranco, A. L. (1993) J. Immunol. 150, 377–386
27. Blanar, M. A., and Rutter, W. J. (1992) Science 256, 1014–1018
28. DeFranco, A. (1995) Curr. Opin. Cell Biol. 7, 163–175
29. Hutchenrecher, J. E., Harrison, M. L., and Geahlen, R. L. (1992) J. Biol. Chem. 267, 8613–8619
30. Burg, D. L., Furlong, M. T., Harrison, M. L., and Geahlen, R. L. (1994) J. Biol. Chem. 269, 573–579
31. Takata, M., Sabe, H., Hata, A., Inazu, T., Homma, Y., Nukada, T., Yamamura, H., and Kuroaki, T. (1994) EMBO J. 13, 1341–1349
32. Ho, J., Schindler, U., Hierholz, W. J., Ho, T. C., Brassier, M., and McKnight, S. L. (1994) Science 265, 1701–1706
33. Kirken, R. A., Rui, H., Malabarba, M. G., and Farrar, W. L. (1994) J. Biol. Chem. 269, 19136–19141
34. Miyazaki, T., Kawahara, A., Fujii, H., Nakagawa, Y., Minami, Y., Liu, Z. J., Oishi, I., Silverthorne, O., Wittuhun, B. A., and Ihle, J. N. (1994) Science 266, 1045–1047
35. Zeng, Y. X., Takahashi, H., Shibata, M., and Hirokawa, K. (1994) FEBS Lett. 353, 289–293
36. Yin, T., Tsang, M. L., and Yang, Y. C. (1994) J. Biol. Chem. 269, 26614–26617
37. Izuhara, K., Feldman, R. A., Greer, F., and Harada, N. (1994) J. Biol. Chem. 269, 18623–18629
38. Ravetch, J. V. (1994) Cell 78, 553–560
39. Adams, D. O., and Hamilton, T. A. (1984) Annu. Rev. Immunol. 2, 283–318
40. Morrison, D. C., and Ryan, J. L. (1987) Annu. Rev. Med. 38, 417–432
41. Weinstein, S. L., Sanghera, J. S., Lemke, K., DeFranco, A. L., and Pelech, S. L. (1992) J. Biol. Chem. 267, 14955–14962
42. Weinstein, S. L., June, C. H., and DeFranco, A. L. (1993) J. Immunol. 151, 3829–3838
43. Tobias, P. S., and Ulevitch, R. J. (1993) Immunobiology 187, 227–232
44. Stefanova, I., Corcoran, M. L., Horak, E. M., Wahl, L. M., Bolen, J. B., and Horak, I. D. (1993) J. Biol. Chem. 268, 20725–20728
45. Beaty, C. D., Franklin, T. L., Uehara, Y., and Wilson, C. B. (1994) Eur. J. Immunol. 24, 1278–1284
46. Indik, Z. K., Park, J., Pan, X. Q., and Schreiber, A. D. (1995) Blood 85, 1175–1181
47. Park, J., and Schreiber, A. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 7381–7385
48. Geng, Y., Gulbins, E., Altman, A., and Lotz, M. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 8602–8606
49. Lankester, A. C., van Schijndel, G. M., Rood, P. M., Verhoeven, A. J., van der Wolde, J. R., and van der Horst, I. D. (1993) J. Biol. Chem. 268, 20725–20728
50. Yamanishi, Y., Fukui, Y., Bolen, J. B., Kurosaki, T., and Yamamoto, T. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 1118–1122
51. Prasad, K. V., Janssen, O., Kapeller, R., Repe, H., Duke-Cohan, J. S., Cantley, L. C., and Ruddle, C. E. (1993) Mol. Cell Biol. 13, 7708–7717
52. Wess, A., and Littman, D. R. (1994) Cell 76, 263–274
53. Shu, L., Green, J., O’Mara, G., Karas, J. L., and Damon, J. P., Ram, M. K., Taylor, M. K., Zoller, M. J., Zvyetov, L. D., and Bolen, J. B. (1995) Mol. Cell Biol. 15, 272–281
54. Napi, K., Takata, M., Yamamura, H., and Kurosaki, T. (1995) J. Biol. Chem. 270, 6824–6829
55. Kihara, H., and Siragian, R. P. (1994) J. Biol. Chem. 269, 22427–22432
56. Buscher, D., Hipkiss, R. A., Krautwald, S., Reimann, T., and Baccarin, M. (1995) Mol. Cell Biol. 15, 466–475
57. Geng, Y., Gulbins, E., Altman, A., and Lotz, M. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 8602–8606