Tyrosine Phosphorylation of the Fc Receptor γ-Chain in Collagen-stimulated Platelets*

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Stimulation of platelets by the extracellular matrix protein collagen leads to activation of a tyrosine kinase-dependent mechanism resulting in secretion and aggregation. Tyrosine phosphorylation of the tyrosine kinase Syk and phospholipase Cγ2 are early events in collagen-induced activation. We recently proposed that collagen signaling in platelets involves a receptor or a receptor-associated protein containing an immunoreceptor tyrosine-based activation motif (ITAM) enabling interaction with Syk. In this report we show that collagen stimulation of platelets causes rapid tyrosine phosphorylation of the ITAM containing Fc receptor γ-chain and that this is precipitated by the tandem Src homology 2 (SH2) domains of Syk expressed as a fusion protein. In addition we demonstrate an association between the Fc receptor γ-chain and endogenous Syk in collagen-stimulated platelets. The Fc receptor γ-chain undergoes tyrosine phosphorylation in platelets stimulated by a collagen-related peptide which does not bind the integrin α2β1 and by the lectin wheat germ agglutinin. In contrast, cross-linking of the platelet low affinity receptor for immune complexes, FcγRIIA, or stimulation by thrombin does not induce phosphorylation of the Fc receptor γ-chain. The present results provide a molecular basis for collagen activation of platelets which is independent of the integrin α2β1 and involves phosphorylation of the Fc receptor γ-chain, its association with Syk and subsequent phosphorylation of phospholipase Cγ2. Collagen is the first example of a nonimmunoreceptor stimulus to signal through a pathway closely related to signaling by immune receptors.

The adhesive and stimulatory properties of the extracellular matrix protein collagen on platelets are vital for the maintenance of hemostasis. Upon vascular damage, platelets adhere to subendothelial collagen which stimulates a tyrosine kinase dependent pathway leading to platelet degranulation, aggregation and development of a hemostatic plug. The mechanism of collagen stimulation of platelets is poorly understood, and the distinction between adhesion and stimulation ill defined. Several platelet glycoproteins have been implicated as potential collagen receptors, including the integrin α2β1, glycoprotein IV (GP1Iib, CD36) (2), glycoprotein VI (3), and uncharacterized 65-kDa (4) and 85–90-kDa glycoproteins (5). Patients whose platelets express abnormally low numbers of these proteins, or who possess autoantibodies to them, have limited bleeding defects (3, 5–9).

Collagen stimulation of platelets activates tyrosine kinase-dependent mechanisms which involve tyrosine phosphorylation of Syk and phospholipase Cγ2 (PLCγ2) (10–12). Syk is a nonreceptor tyrosine kinase which is assembled into signaling complexes via interaction between its tandem Src homology 2 (SH2) domains and a tyrosine phosphorylated activation motif found in receptors of the immune system or their associated chains. The motif, termed the immunoreceptor tyrosine-based activation motif (ITAM), has the amino acid sequence YXXL/IxYXXL/I (13), and is phosphorylated on the conserved tyrosine residues by a member of the Src kinase family upon receptor activation.

We recently proposed (12) that a collagen receptor or an associated protein may contain an ITAM motif to allow interaction with Syk. In this report we show that stimulation of platelets with collagen induces tyrosine phosphorylation of the Fc receptor γ-chain (FcγRIIA), which contains an ITAM motif, and that this promotes an association between the FcγRIIA and Syk. This allows the construction of a model linking collagen receptor ligation to activation of Syk and a tyrosine kinase signaling cascade.

EXPERIMENTAL PROCEDURES

Materials—Anti-FcγRIIIa antibody (14) was kindly provided by Dr. J.-P. Kinet (Beth Israel Hospital, Boston, MA), the monoclonal antibody (mAb) 6F1 was a gift from Dr. B. Coller (Mount Sinai School of Medicine, New York, NY), and the rabbit polyclonal antibody to the T cell γ-chain was kindly donated by Dr. D. Cantrell (ICRF, London). Collagen fibers, as Harm collagen, a suspension of type I fibers from equine tendon, were obtained from Nycomed (Munich, Germany). A collagen-related peptide (CRP: GCP*(GPP*)10GCP*G; single amino acid code P* = hydroxyproline) was synthesized and cross-linked as described previously (15). Anti-phosphotyrosine mAb 4G10 was from Upstate Biotechnology (TCS Biologicals Ltd., Botolph Claydon, Bucks, UK), anti-Syk antibodies: Syk(LR) was from Santa Cruz Biotechnology Inc. (Santa Cruz, CA) and mAb 101 from Wako GmbH (Neuss, Germany), and anti-FcγRIIIa mAb IV.3 was purchased from Medarex Inc. (Annandale, NJ). Other reagents were from previously described sources.

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1 The abbreviations used are: PLCγ2, phospholipase Cγ2; SH2, Src homology region 2; ITAM, immunoreceptor tyrosine-based activation motif; CRP, collagen-related peptide; WGA, wheat germ agglutinin; mAb, monoclonal antibody; GST, glutathione S-transferase; GST-Syk SH2, glutathione S-transferase fusion protein containing tandem SH2 domains of Syk; FcγRIIA and FcγRIIIa, low affinity immunoglobulin G receptors; TBS-T, Tris-buffered saline containing Tween 20; FcγRI, high affinity immunoglobulin G receptor; FcγRII, high affinity immunoglobulin E receptor; PAGE, polyacrylamide gel electrophoresis.
Tyrosine Phosphorylation of FcR γ-Chain in Platelets

Preparation and Stimulation of Platelets—Platelets were prepared as described previously (12) and suspended in modified Tyrodes-Hepes buffer (134 mM NaCl, 0.34 mM NaHPO4, 2.9 mM KCl, 12 mM NaHCO3, 20 mM Hepes, 5 mM glucose, 1 mM MgCl2, pH 7.3) containing 1 mM EDTA and 10 μM indomethacin to a density of 8 × 10⁸ cells/ml. Mg⁶⁺ was omitted, and 1 mM EDTA was added to the buffer where stimulation was required. Stimulation of platelets (675 μg) with collagen (100 μg/ml, 90 s), collagen-related peptide (CRP, 3 μg/ml, 90 s), and wheat germ agglutinin (WGA, 100 μg/ml, 60 s) was performed at 37°C in an aggregometer with continuous stirring at 800 rpm. Stimulation by cross-linking of the low affinity receptor for immunoglobulin G (FcγRIIA) was performed by preincubation of platelets for 60 s with monoclonal antibody IV.3 (1:10000 diluted) followed by stimulation for 60 s by addition of anti-mouse IgG F(ab')2 (30 μg/ml).

Immunoblotting Studies—Platelet stimulation was terminated by addition of an equal volume of Laemmli sample treatment buffer. Proteins were separated by SDS-PAGE on 10–18% gradient slab gels and transferred to polyvinylidene difluoride membranes. Membranes were then blocked by incubation in 10% (w/v) bovine serum albumin dissolved in TBS-T (20 mM Tris, 137 mM NaCl, 0.1% (v/v) Tween 20, pH 7.6). Primary and secondary antibodies were diluted in TBS-T containing 2% (w/v) bovine serum albumin and incubated with Western blots for 1 h at room temperature. Blots were washed for 2 h in TBS-T following each incubation with antibodies, and then developed using an enhanced chemiluminescence (ECL) detection system. Anti-phosphotyrosine and anti-Syk immunoblots were performed using mAb 4G10 and mAb 101, respectively, at 1 μg/ml; anti-FcγRIIA and anti-γ-chain were diluted 1:10000. Horseradish peroxidase-conjugated secondary antibodies were diluted 1:100000.

Immunoprecipitation Studies—Platelet stimulation was terminated by addition of an equal volume of ice-cold lysis buffer (20 mM Tris, 300 mM NaCl, 10 mM EDTA, 2% (w/v) Nonidet P40, 1 mM phenylmethylsulfonyl fluoride, 2 mM Na3VO4, 10 μg/ml leupeptin, 10 μg/ml apro- tinin, 1 mM pepstatin A, pH 7.3). Detergent-insoluble debris was removed, and the lysates were preclarified by mixing with protein A-Sepharose for 1 h at 4°C (20 μl of a 50% (w/v) suspension of protein A-Sepharose in TBS-T). Preclarification was omitted in samples stimulated by cross-linking of FcγRIIA, since this would result in removal of FcγRIIA from the lysate. FcγRIIA was immunoprecipitated from 1.2 ml of lysate using 4 μl of rabbit antiseraum, and Syk was immunoprecipitated from 100 μl of lysate using 4 μl of a polyclonal antibody Syk(LR). Following rotation at 4°C for 1 h, 25 μl of a 50% (w/v) suspension of protein A-Sepharose was added to each sample, and mixing was continued for 1 h. The Sepharose pellet was washed in lysis buffer and then TBS-T, before addition of Laemmli sample treatment buffer. Proteins were separated by SDS-PAGE using 10–18% gradient slab gels and transferred to polyvinylidene difluoride membranes.

RESULTS

Tyrosine Phosphorylation of the Fcγ-Chain—In a strategy designed to identify ITAM-containing proteins which undergo phosphorylation following collagen stimulation, platelet lysates were incubated with the tandem SH2 domains of Syk expressed as a GST fusion construct (GST-Syk SH2). Precipitation and immunoblotting for tyrosine phosphorylation revealed a reproducible association of two bands of 11 and 13 kDa along with several higher molecular mass bands. Candidates for the tyrosine-phosphorylated doublet which are known to contain an ITAM include the T cell receptor-associated γ-chain and the FcγRIIA γ-chain. The γ-chain was not detected in whole platelet lysates or in GST-Syk SH2 precipitates following immunoblotting with a specific antibody. Immunoblotting for the FcγRIIA γ-chain detected its presence in whole cell lysates and in GST-Syk SH2 precipitates from cells stimulated with collagen.

Proteins precipitated by GST-Syk SH2 and separated by SDS-PAGE under reducing conditions were immunoblotted using anti-FcγRIIA chain antiserum (Fig. 1a(i)). Four proteins of 13, 11, 8.5, and 6.5 kDa were detected in samples precipitated from platelets stimulated with collagen, CRP, and WGA, but were not present in basal cells or cells stimulated by cross-linking FcγRIIA (Fig. 1a) or by thrombin (not shown). Reprobing with an anti-phosphotyrosine antibody demonstrated that only the upper two bands were tyrosine-phosphorylated (Fig. 1a(ii)), indicating that tyrosine phosphorylation of the FcγRIIA chain is associated with a mobility shift on SDS-PAGE. Two uncharacterized proteins of 65 and 70 kDa were also tyrosine-phosphorylated and precipitated from collagen-, CRP-, and WGA-stimulated platelets (Fig. 1a(iii)). Fig. 1a(ii) also demonstrates association of the tyrosine phosphorylated FcγRIIA with GST-Syk SH2 in cells stimulated by cross-linking of the receptor, as previously reported (12), and also by WGA. The absence of tyrosine-phosphorylated proteins other than those mentioned above in WGA-stimulated platelets suggests that platelets do not express other ITAM-containing proteins which undergo tyrosine phosphorylation on cross-linking of surface glycosylated proteins.

FcγRIIA γ-chain is expressed in cells as a homodimer linked by a disulfide bridge. Precipitation of non-tyrosine-phosphorylated FcγRIIA γ-chain with the SH2 domains of Syk may therefore be due to covalent association with a tyrosine-phosphorylated FcγRIIA γ-chain. To examine this, GST-Syk SH2 preciptates were subjected to SDS-PAGE under nonreducing conditions and immunoblotted for FcγRIIA γ-chain and for phosphotyrosine residues. Under nonreducing conditions, the FcγRIIA γ-chain protein remains dimerized and migrates a distance consistent with an approximate doubling of its molecular mass (Fig. 1b(i)). In accordance with this, there was an approximate doubling of the molecular mass of the tyrosine phosphorylated bands under nonreducing conditions (Fig. 1b(ii)).

The SH2 domains of Syk precipitated similar amounts of the tyrosine-phosphorylated and non-tyrosine-phosphorylated forms of the FcγRIIA γ-chain, suggesting that only 1 molecule in each pair becomes phosphorylated. Phosphorylation of one chain in each dimer may prevent phosphorylation of the other chain or, alternatively, one chain may not be accessible to the kinase. The existence of four bands under reduced conditions may reflect the fact that the FcγRIIA γ-chain has additional tyrosine residues to those of the ITAM motif and can also undergo phosphorylation on threonine and serine residues (16–18). The appearance of non-tyrosine-phosphorylated FcγRIIA γ-chain as a doublet on SDS-PAGE has been observed in other cells (16).

Time Course of Phosphorylation of FcγRIIA γ-Chain—FcγRIIA γ-chain was immunoprecipitated from lysates of basal and stimulated cells and the level of tyrosine phosphorylation examined by immunoblot analysis. A time course of FcγRIIA γ-chain phosphorylation in response to collagen revealed that tyrosine phosphorylation occurred within 20 s and reached a maximum by 90 s (Fig. 2(i)). The FcγRIIA γ-chain, along with the tyrosine kinase Syk (12), is one of the earliest proteins to undergo tyrosine phosphorylation in collagen-stimulated platelets.

Association of the FcγRIIA γ-Chain with Syk—While the above
Fig. 1. Association of tyrosine-phosphorylated FcR γ-chain with GST-Syk SH2 domains. Platelets were stimulated with collagen (100 μg/ml, 90 s), CRP (3 μg/ml, 90 s), WGA (100 μg/ml, 60 s), and by cross-linking FcγRIIA (60 s), and proteins were precipitated from cell lysates using 10 μg of GST-Syk SH2 or a molar equivalent of GST as described under "Experimental Procedures." Proteins were separated by SDS-PAGE under a, reducing, or b, nonreducing, conditions and immunoblotted for FcR γ-chain and phosphotyrosine residues. a(i) shows FcR γ-chain precipitated from collagen-, CRP-, and WGA-stimulated cells; a(ii) is the same blot reprobed for phosphotyrosine residues. b(i) shows the FcR γ-chain precipitated from collagen-, CRP-, and WGA-stimulated cells run under nonreducing conditions, which was subsequently reprobed for phosphotyrosine residues (b(ii)). In a(ii) and b(ii), the position of tyrosine-phosphorylated FcγRIIa from FcγRIIa- and WGA-stimulated cells is indicated.

Tyrosine Phosphorylation of FcR γ-Chain in Response to Collagen Is Not Dependent on the Integrin α2β1—Although platelets adhere to collagen via the integrin α2β1, phosphorylation of the FcR γ-chain by CRP, which does not bind to the α2β1 integrin (15), is also maintained in the absence of Mg²⁺. Tyrosine phosphorylation of the FcR γ-chain is observed in response to both collagen and CRP in the presence of an α3β1 integrin blocking mAb 6F1 (Fig. 2(iii)). The different levels of basal FcR γ-chain phosphorylation in 2(ii) and 2(iii) are due to the use of different platelet preparations.

**DISCUSSION**

The present study identifies a signaling protein which is involved in collagen-stimulated activation of platelets. The FcR γ-chain becomes tyrosine phosphorylated when platelets are stimulated with collagen enabling binding of Syk and the assembly of a signaling complex. Phosphorylation of the FcR γ-chain is rapid and detectable within 20 s of stimulation and reaches maximal levels by 90 s. This time course is similar to that observed for the onset of tyrosine phosphorylation in collagen-stimulated platelets and suggests an early involvement.
of the FcR γ-chain in collagen signaling.

The FcR γ-chain is recognized for its role in signaling by the high affinity receptors for IgE (FcεRI) (19–21) and IgG (FcγRI) (16, 22) and by the low affinity IgG receptor (FcγRII) (22). Clustering and activation of FcεRI, FcγRI, and FcγRII stimulates tyrosine phosphorylation of the ITAM on the FcγRI, enabling recruitment of Syk which becomes tyrosine-phosphorylated and activated (16, 19, 23, 24). The promiscuous nature of the FcγRI γ-chain is further supported by the recent finding that it forms a functional association with the IgA receptor (FceRII) in cells transfected to express both proteins (25). The FcγRI γ-chain is also vital for the assembly and surface expression of FcεRI and FcγRII, by preventing inappropriate degradation in the endoplasmic reticulum (26). The expression of FcεRI, FcγRI, FcγRII, and the FcγRI γ-chain in platelets has not been reported.

The kinase which is responsible for FcγRI γ-chain tyrosine phosphorylation in platelets requires identification. There is a precedent for the involvement of Src-like kinases in the phosphorylation of the ITAM (17, 27–29). The kinase assays shown in Fig. 3, which were performed on FcγRI γ-chain and Syk immunoprecipitates, indicate that a kinase activity capable of phosphorylating the FcγRI γ-chain is co-immunoprecipitated from collagen- and CRP-stimulated cells. It is not known whether Syk or a co-precipitated Src-family kinase is the source of this activity.

The identity of the collagen receptor which leads to tyrosine phosphorylation of the FcγRI γ-chain is not known. Several lines of evidence suggest that tyrosine phosphorylation of the FcγRI γ-chain is not dependent on the integrin αβ2. Tyrosine phosphorylation of FcγRI γ-chain is maintained in the absence of Mg2+ or presence of mAb 6F1, conditions which prevent the association of collagen with the integrin. In addition, CRP also stimulates tyrosine phosphorylation of FcγRI γ-chain, yet is not capable of binding to the integrin (15). This suggests that collagen stimulation of platelets involves an uncharacterized receptor which is linked to the FcγRI γ-chain. Candidates for this receptor include those proteins described in the Introduction. Collagen is the only physiological platelet agonist that has been identified to induce phosphorylation of the FcγRI γ-chain, suggesting that this event plays a unique role in collagen signaling. Platelets express a low affinity IgG receptor, FcγRIIA, which also contains an ITAM sequence, but its activation does not induce phosphorylation of the FcγRI γ-chain. Clustering of the platelet FcγRIIA receptors results in tyrosine phosphorylation of its integral ITAM and tyrosine phosphorylation of Syk and PLCγ2 (12, 30). The lectin WGA, which is recognized as a powerful stimulus of platelet activation (31–34), elicits tyrosine phosphorylation of Syk (35) and PLCγ2 (10), and also induces phosphorylation of FcγRI γ-chain and FcγRIIA. WGA therefore manifests its effects on platelets, at least in part, by activating components of both collagen and FcγRIIA pathways. Thrombin also stimulates tyrosine phosphorylation of Syk (36) but induces very weak or no phosphorylation of PLCγ2 (10) and does not induce tyrosine phosphorylation of the FcγRI γ-chain. This indicates that other pathways lead to phosphorylation of Syk in the platelet and it can be speculated that phosphorylation of an ITAM containing protein may be essential in leading to tyrosine phosphorylation of PLCγ2.

A model for collagen stimulation of platelets can be proposed in light of the results of this study. Under the physiological condition of flow, platelets adhere to extracellular matrix collagen via the integrin αβ2. This brings collagen into association with a separate cell surface receptor which is coupled to the FcγRI γ-chain. Receptor clustering leads to tyrosine phosphorylation of the FcγRI γ-chain, possibly by a Src-family kinase, enabling binding of Syk which becomes tyrosine phosphoryl-
ated and activated. This initiates a series of events which may involve other kinases and adaptor proteins leading to phosphorylation and activation of PLC. A defect in collagen signaling may contribute to the hemorrhaging in utero which has recently been reported in mice engineered to lack the tyrosine kinase Syk (37, 38).

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REFERENCES
1. Santoro, S. A., Rajpara, S. M., Staatz, W. D., and Woods, V., Jr. (1988) Biochem. Biophys. Res. Commun. 153, 217–223
2. Tandon, N. N., Kralisz, U., and Jamieson, G. A. (1989) J. Biol. Chem. 264, 7576–7583
3. Ryo, R., Yoshida, A., Sugano, M., Nakayama, K., Saiga, K., Adachi, M., Yamaguchi, N., and Okuma, M. (1992) Am. J. Hematol. 39, 25–31
4. Chiang, T. M., and Kang, A. H. (1982) J. Biol. Chem. 257, 7581–7586
5. Deckmyn, H., Van Houtte, E., and Vermylen, J. (1992) J. Clin. Invest. 84, 1440–1445
6. Moroi, M., Jung, S. M., Okuma, M., and Shinmyozu, K. (1989) J. Biol. Chem. 264, 2277–2279
7. Kehrel, B., Kronenberg, A., Rauterberg, J., Niesing-Bresch, D., Niehues, U., Kardoeus, J., Schwippert, B., Tschope, D., van de Loo, J., and Clemetson, K. J. (1993) Blood 82, 3364–3370
8. McKeown, L., Vail, M., Williams, S., Kramer, W., Hansmann, K., and Gralnick, H. (1994) J. Biol. Chem. 269, 257, 7581–7586
9. Daniel, J. L., Dangelmaier, C., Strouse, R., and Smith, J. B. (1994) J. Biol. Chem. 269, 22427–22432
10. Blake, R. A., Schieven, G. L., and Watson, S. P. (1994) FEBS Lett. 353, 212–216
11. Daniel, J. L., Dangelmaier, C., and Smith, J. B. (1994) Biochem. J. 302, 617–622
12. Yanaga, F., Poole, A., Asselin, J., Blake, R., Schieven, G., Clark, E. A., Law, C.-L., and Watson, S. P. (1995) Biochem. J. 311, 471–478
13. Reth, M. (1989) Nature 338, 383–384
14. Letourneur, O., Kennedy, I. C., Brini, A. T., Ortaldo, J. R., O'Shea, J. J., and Kinet, J. P. (1991) J. Immunol. 147, 2652–2656
15. Morton, L. F., Hargreaves, P. G., Farndale, R. W., Young, R. D., and Barnes, M. J. (1995) Biochem. J. 306, 337–344
16. Duchemin, A.-M., Ernst, L. K., and Anderson, C. L. (1994) J. Biol. Chem. 269, 12111–12117
17. Padini, R., Jouvin, M.-H., and Kinet, J.-P. (1993) Nature 365, 855–858
18. Durand, D. L., Rosen, H., and Cooper, J. A. (1994) Biochem. J. 299, 567–577
19. Shiue, L., Green, J., Green, O. M., Karas, J. L., Morgenstern, J. P., Ram, M. K., Taylor, M. K., Zoller, M. J., Zydowsky, L. D., Bolen, J. B., and Bugge, J. S. (1995) Mol. Cell. Biol. 15, 272–281
20. Padini, R., Renard, V., Vivier, E., Ochiai, K., Jouvin, M. H., Malissen, B., and Kinet, J. P. (1995) J. Exp. Med. 181, 247–255
21. Jouvin, M. H., Numerof, R. P., and Kinet, J. P. (1995) Semin. Immunol. 7, 29–36
22. Greenberg, S., Chang, P., and Silverstein, S. C. (1994) J. Biol. Chem. 269, 3897–3902
23. J. Johnson, S., Pleiman, C., Pao, L., Schnieringer, J., Hippen, K., and Cambier, J. (1995) J. Immunol. 155, 4596–4603
24. Benhamou, M., Ryba, N., Kihara, H., Nishikata, H., and Siraganian, R. (1993) J. Biol. Chem. 268, 23318–23324
25. Morton, H. C., van den Herik-Oudijk, I. E., Vosse fled, P., Snijders, A., Vanho een, A. J., Capel, P. J. A., and van de Winkel, J. G. J. (1995) J. Biol. Chem. 270, 29781–29787
26. Takai, T., Li, M., Sylvestre, D., Clynnes, R., and Ravetch, J. V. (1994) Cell 76, 519–529
27. Eiseman, E., and Bölen, J. B. (1992) Nature 355, 78–80
28. Kihara, H., and Siraganian, R. P. (1994) J. Biol. Chem. 269, 22427–22432
29. Jouvin, M. H., Adamczewski, M., Numerof, R., Letourneur, O., Valle, A., and Kinet, J. P. (1994) J. Biol. Chem. 269, 5918–5925
30. Chacko, G., Duchemin, A.-M., Coggeshall, K., Osborne, J., Brandt, J., and Anderson, C. (1994) J. Biol. Chem. 269, 32435–32439
31. Ganguly, C. L., Che lladurai, M., and Ganguly, P. (1985) Biochips. Biophys. Res. Commun. 132, 313–319
32. Higashihara, M., Takahata, K., Ohashi, T., Kariya, T., Kume, S., and Oka, H. (1985) FEBS Lett. 183, 433–438
33. Inazu, T., Taniguchi, T., Ohta, S., Miyasbo, S., and Yamamura, H. (1991) Biochips. Biophys. Res. Commun. 174, 1154–1158
34. Yatomi, Y., Ozaki, Y., Koike, Y., Satoh, K., and Kume, S. (1993) Biochips. Biophys. Res. Commun. 191, 453–458
35. Ohta, S., Taniguchi, T., Asahi, K., Takeuchi, F., Nakamura, S., and Yamamura, H. (1992) Biochips. Biophys. Res. Commun. 185, 1128–1132
36. Taniguchi, T., Kitagawa, H., Yasue, S., Yanagi, S., Sakai, K., Asahi, M., Ohta, S., Takeuchi, F., Nakamura, S., and Yamamura, H. (1993) J. Biol. Chem. 268, 2277–2279
37. Cheng, A., Rowley, B., Pao, W., Hayday, A., Bolen, J. B., and Pawson, T. (1995) Nature 378, 303–306
38. Turner, M., Mee, P., Costello, P., Williams, O., Price, A., Duddy, L., Furlong, M., Geahlen, R., and Tybulewicz, V. (1995) Nature 378, 298–302