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Role of focal adhesion kinase in MAP kinase activation by insulin-like growth factor-I or insulin

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Abstract Integrin-induced focal adhesion kinase (FAK) phosphorylation as well as insulin-like growth factor-I (IGF-I) and insulin activate MAP kinase. Since IGF-I or insulin have been suggested to affect FAK phosphorylation, we analyzed the role of FAK in IGF-I- or insulin-induced MAP kinase activation. Although MAP kinase was stimulated by IGF-I or insulin, FAK tyrosine phosphorylation remained unchanged in fibroblasts expressing normal or transiently elevated levels of IGF-I and insulin receptors. Further analysis in FAK deficient fibroblasts suggested that FAK impedes MAP kinase activation by IGF-I or insulin.

Key words: Focal adhesion kinase; Insulin; Insulin-like growth factor; Mitogen-activated protein kinase; Src

1. Introduction

The insulin-like growth factor-I receptor (IGF-IR) and the insulin receptor (IR) are related heterotetrameric protein tyrosine kinases [1]. It is generally accepted that the main role of the IR is to regulate glucose homeostasis, whereas the IGF-IR is essential for cell proliferation, growth and differentiation [2–5].

After binding of ligand to the extracellular subunit of the IGF-IR or the IR, the receptors become activated resulting in the phosphorylation of various substrates, including IRS-1, IRS-2 and Shc. Tyrosine-phosphorylated substrates subsequently bind SH2 domain-containing proteins, including the p85 subunit of PI 3′ kinase, the adaptor proteins Grb2 and Nck, the tyrosine phosphatase Syp and the C-terminal Src kinase Csk [6–14]. Shc and Grb2 binding to the activated receptor leads to the activation of the Ras-MAP kinase signaling pathway [15,16].

The focal adhesion kinase (FAK) is a non-receptor protein tyrosine kinase that plays an important role in cell adhesion and motility [17,18]. In response to integrin activation, FAK becomes phosphorylated on several tyrosine residues and binds the SH2 domains of Src and Fyn [19,20]. These kinases phosphorylate FAK on tyrosine 925, which then binds the Grb2 SH2 domain, thereby linking the integrin signaling pathway to the Ras-MAP kinase pathway [21–23].

Previous studies of the effects of insulin and IGF-I on FAK tyrosine phosphorylation have produced differing results. Several studies showed that insulin induced a decrease in FAK tyrosine phosphorylation [12,24–28], whereas CHO cells expressing endogenous levels of IR displayed negligible FAK dephosphorylation following insulin stimulation [29]. With regard to the effect of IGF-I on FAK phosphorylation, Leventhal et al. [30] found that IGF-I increased phosphorylation of FAK, whereas Pillay et al. [26] observed no effect of IGF-I. The differing results, although not necessarily incompatible, indicate that the effects of IGF-I and insulin on FAK phosphorylation deserve further study.

Because IGF-I and insulin may induce changes in FAK tyrosine phosphorylation and since FAK phosphorylation can lead to activation of the Ras-MAP kinase pathway, we studied whether IGF-I- or insulin-induced FAK phosphorylation contributes to MAP kinase activation. Although FAK tyrosine phosphorylation did not change following IGF-I or insulin stimulation in adherent fibroblasts, MAP kinase activity was stimulated. Similar results were observed after transient overexpression of the IGF-IR or the IR. However, fibroblasts stably expressing high levels of IGF-IR showed 2- to 3-fold increases in FAK tyrosine phosphorylation after IGF-I stimulation. In this situation the time courses of MAP kinase activation and FAK phosphorylation were not compatible with a direct effect of FAK on MAP kinase activation. Moreover, in cells lacking FAK protein, MAP kinase activity was not reduced but rather increased by IGF-I or insulin. Thus, IGF-I- or insulin-induced MAP kinase stimulation is independent of FAK phosphorylation and activation.

2. Materials and methods

2.1. Cell lines and vectors

pCLXSN-hIGF-IR and pCLXSN-hIR were constructed by subcloning human IGF-IR cDNA from pRLDN vector and human IR cDNA from pBluescript-SK$^-$ into the pCLXSN vector. Both vectors, pRLDN-IGF-IR and pBluescript-SK$^+$-hIR, were kindly provided by Dr. J.M.G. Olefsky (University of California, San Diego).

The Src −/− mouse fibroblast cell line, Src 1S, and its wild-type counterpart, Src 8S, both spontaneously immortalized [31,32], were kindly provided by Dr. P. Soriano (Fred Hutchinson Cancer Research Center). The FAK −/− mouse fibroblast cell line and its wild-type counterpart were kindly provided by Dr. D. Schlaepfer (The Scripps Research Institute).

2.2. Antibodies

Anti-FAK rabbit polyclonal antibody was kindly provided by Dr. D. Schlaepfer (The Scripps Research Institute). Antiphosphotyrosine antibody (4G10) was from UBI (New York), the hybridoma for the monoclonal antibody to the hemagglutinin epitope tag (anti-HA, MAb12CA5) was kindly provided by Dr. I. Wilson (The Scripps

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Abbreviations: FAK, focal adhesion kinase; GST, glutathione *S*-transferase; IGF-I, insulin-like growth factor-I; IGF-IR, IGF-I receptor; IR, insulin receptor; MAP kinase, mitogen-activated protein kinase

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Research Institute), anti-IR β-subunit antibody was from Santa Cruz Biotechnology, Inc., the hybridoma for the monoclonal peptide antibody directed against amino acids 2–17 of vSrc was from Microbiological Associates, and anti-Src hybridoma MAb327 was a generous gift from Joan Brugge [33].

Antibody to the IGF-IR was prepared by immunizing New Zealand white rabbits with a peptide corresponding to the C-terminal 14 amino acids of the β-subunit of the human IGF-IR (KNERALPLPQSSTC), cross-linked to keyhole limpet haemocyanin with glutaraldehyde. Affinity-purified antibodies were prepared using a column containing the peptide covalently coupled to cyanogen bromide-activated Sepharose beads (Pharmacia). The rabbit polyclonal antibody raised against the last 14 amino acids of the β-subunit of the human IGF-IR immunoprecipitated a protein of about 97 kDa, the expected size of the human IGF-IR β-subunit, from human 293 cells (data not shown). The specificity of the β-subunit antibody was verified by peptide competition (data not shown).

2.3. Immunoprecipitation

Cells were incubated in Dulbecco's modified Eagle's medium supplemented with 0.1% calf serum for 18 h before stimulation with LR3-IGF-I (JRH Biosciences) or insulin (Sigma). Cells were lysed in RIPA buffer [34] modified by the addition of 10 U/ml aprotinin, 50 mM NaF, 1 mM phenylmethylsulfonyl fluoride, 20 μg/ml leupeptin, 1 mM sodium orthovanadate, 0.5 mM dithiothreitol at 4°C. Lysates were precleared with formalin-fixed *Staphylococcus aureus* (Pansorbin) (Calbiochem) by centrifugation at 4°C for 20 min at 14000 rpm. Similar amounts of lysate (about 1 ml), adjusted to contain equal amounts of protein (0.5–1.0 mg), were incubated with 1 μg of antibody for 2 h at 4°C. Protein A-agarose (Regpligen) was added for 1 h at 4°C and washed three times with lysis buffer at 4°C. Immune complexes were boiled and separated on a 10% SDS acrylamide gel.

2.4. Immunoblotting

After gel electrophoresis, proteins were transferred to an Immobilon polyvinylidene fluoride membrane (Millipore). The membrane was blocked overnight at 4°C in TBS-T (50 mM Tris-HCl pH 8, 150 mM NaCl, 0.05% Tween 20) containing 5% bovine serum albumin. The membrane was incubated with primary antibodies at room temperature for 2 h, washed and incubated with horse-radish peroxidase-conjugated secondary antibody or ^{125}I-protein A for 1 h. Antibodies were detected by enhanced chemiluminescence (Amersham Pharmacia Biotech) or autoradiography.

2.5. In vitro kinase assay

FAK in vitro kinase assays were performed as previously described [23]. In vitro kinase assays of HA-tagged p44-Erk1 MAP kinase were done as previously reported [35].

3. Results and discussion

3.1. MAP kinase activation by IGF-I and insulin is independent of FAK

To test the responses of the Src 8S fibroblasts to IGF-I and insulin, MAP kinase activity was assayed. Cells transfected with an HA-tagged Erk1-MAP kinase gene were serum-starved and then stimulated with IGF-I or insulin. Erk1-MAP kinase was immunoprecipitated from cell lysates, and the kinase activity was assayed in vitro using the C-terminus of the transcription factor Elk-1, fused to GST, as a substrate [35]. As shown in Fig. 1A, both IGF-I and insulin stimulated MAP kinase activity 2- to 3-fold.

In addition, we analyzed the level of tyrosine phosphorylation of FAK following stimulation with IGF-I or insulin. FAK was immunoprecipitated from lysates of stimulated and unstimulated cells and immunoblotted with the 4G10 antiphosphotyrosine antibody. As shown in Fig. 1B, the relative levels of FAK tyrosine phosphorylation were unchanged by stimulation with IGF-I or insulin. To assess FAK tyrosine kinase activity, FAK immunoprecipitates were tested using an in vitro kinase assay. As shown in Fig. 1C, the incorporation of ^{32}P into FAK was unchanged by stimulation with IGF-I or insulin. Thus, in these adherent mouse fibroblasts, FAK activation, indicated by increased tyrosine phosphorylation or tyrosine kinase activity, is not involved in IGF-I- or insulin-induced MAP kinase activation.

3.2. FAK phosphorylation is increased by IGF-I or insulin stimulation of fibroblasts stably but not transiently overexpressing the IGF-IR or the IR

Previous studies showed that treatment with insulin induced FAK dephosphorylation in cell lines expressing high levels of IR [12,24–29]. To test whether the response of FAK to IGF-I or insulin stimulation was different in cells expressing elevated levels of the receptors, we overexpressed the IGF-IR and the IR using retroviral infection. Human 293 cells were cotransfected with the pCLXSN-IGF-IR or IR vectors and the ecotropic packaging vector, pCLeco. Retrovirus-containing medium was harvested 48 h after transfection and transferred to the 8S mouse fibroblasts. Infection efficiency varied from 50 to 95% and the level of receptor expression increased dramatically after infection (data not shown).

We then analyzed FAK tyrosine phosphorylation after stimulation with IGF-I or insulin in the cells transiently over-

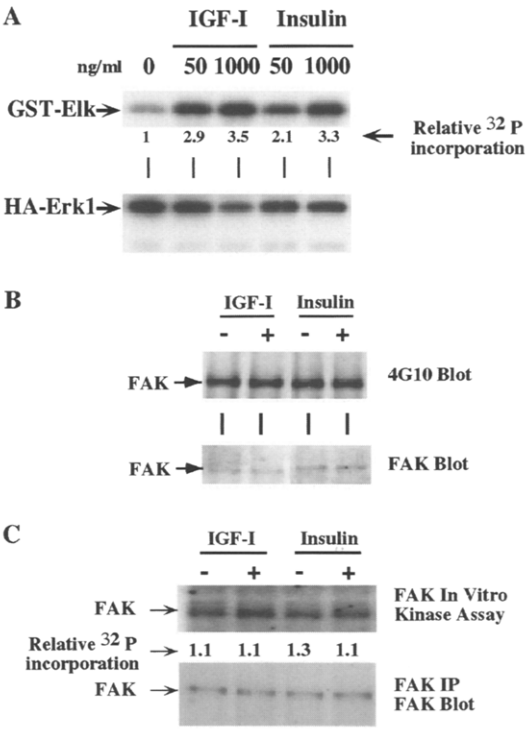

Fig. 1. MAP kinase and FAK activation after IGF-I and insulin stimulation. A: Fibroblasts were treated for 5 min with the indicated amounts of IGF-I or insulin. Immunoprecipitated Erk1-MAP kinase was assayed by an in vitro kinase assay (upper panel). MAP kinase activity is given as incorporation of ^{32}P into GST-Elk. HA-Erk1-MAP kinase was revealed by immunoblotting with anti-HA antibody (lower panel). B: Serum-starved fibroblasts were stimulated with IGF-I or insulin for 4 min. Immunoprecipitated FAK was revealed by immunoblotting with antiphosphotyrosine antibody 4G10 (upper panel), and with the affinity-purified anti-FAK antibody (lower panel). C: In vitro kinase assay of FAK immunoprecipitates (upper panel) and FAK Western blot using ^{125}I-protein A (lower panel).

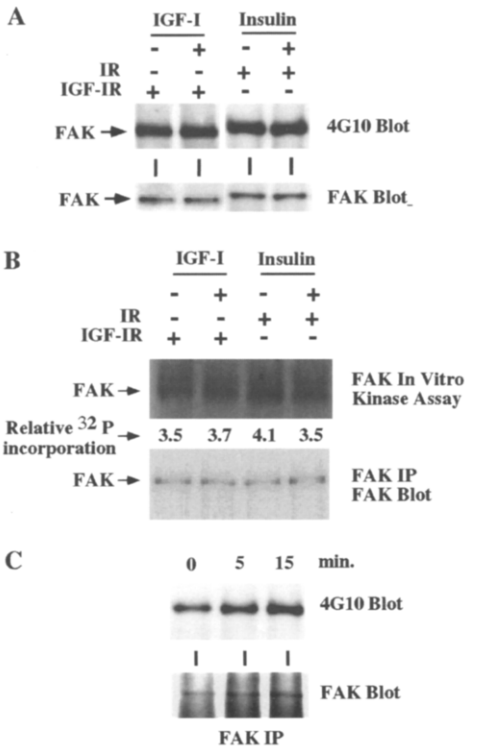

Fig. 2. FAK phosphorylation in fibroblasts transiently and stably overexpressing the IGF-IR or the IR. A: Immunoprecipitated FAK from fibroblasts transiently overexpressing the IGF-IR or the IR was revealed by immunoblotting with antiphosphotyrosine antibody 4G10 (upper panel), and with the affinity-purified anti-FAK antibody (lower panel). B: In vitro kinase assay of FAK immunoprecipitates from the same cells as in A (upper panel) and FAK Western blot using ^{125}I-protein A (lower panel). C: Immunoprecipitated FAK from fibroblasts stably overexpressing the IGF-IR was revealed by immunoblotting with antiphosphotyrosine antibody 4G10 (upper panel), and with the affinity-purified anti-FAK antibody (lower panel).

expressing the receptors. As shown in Fig. 2A, the level of FAK tyrosine phosphorylation was unchanged after stimulation with IGF-I or insulin. We further tested FAK autophosphorylation activity in the overexpressing cells. As shown in Fig. 2B, FAK autophosphorylation activity was unchanged by stimulation with IGF-I or insulin.

Leventhal et al. [30] observed that IGF-I increased FAK tyrosine phosphorylation in SH-SY5Y human neuroblastoma cells stably overexpressing the IGF-IR. To study whether mouse cells stably overexpressing the IGF-IR would behave differently from cells expressing normal levels, or transiently overexpressed levels of the IGF-I receptor, we tested the effect of IGF-I on NIH 3T3 mouse fibroblasts stably overexpressing the IGF-IR. Western blotting confirmed that the human IGF-IR was indeed overexpressed in these cells (data not shown). We then immunoprecipitated FAK after stimulation for either 5 or 15 min with IGF-I and analyzed tyrosine phosphorylation by Western blotting with 4G10 antibody. As shown in Fig. 2C, although FAK protein levels remained similar during the experiment, an increase in FAK tyrosine phosphorylation was apparent at 5 and 15 min (2- and 3-fold, respectively).

We conclude that FAK tyrosine phosphorylation in response to IGF-I or insulin was different depending on whether fibroblasts were transiently or stably overexpressing the IGF-IR or the IR. The lack of a change in FAK tyrosine phosphorylation following stimulation was somewhat surprising in view of previous reports that FAK was dephosphorylated following insulin and phosphorylated following IGF-I treatment in several cell types. However, our results are in agreement with a report by Konstantopoulos et al. [29] who observed differences in FAK phosphorylation in cells expressing endogenous or stably elevated levels of IR.

3.3. *Src does not affect FAK phosphorylation following IGF-I or insulin stimulation*

Src is required in vivo for the phosphorylation of FAK tyrosine 925 [22], the Grb2 SH2 domain binding site, suggesting a Src-dependent MAP kinase activation after integrin stimulation [23]. Therefore, to analyze whether Src is required for FAK tyrosine phosphorylation after IGF-I or insulin, we tested MAP kinase activity and FAK tyrosine phosphorylation and activity following IGF-I and insulin stimulation in fibroblasts with a targeted disruption of the Src gene [31,32]. These Src −/− fibroblasts were expressing either physiological levels or transiently overexpressed IGF-IR or IR, and FAK tyrosine phosphorylation and kinase activity were tested as described above. We observed that increasing concentrations of IGF-I or insulin stimulate MAP kinase activity in Src −/− fibroblasts (Fig. 3A) as we previously described for the wild-type control fibroblasts (see Fig. 1A). Again, FAK tyrosine phosphorylation and kinase activity remained unchanged after IGF-I or insulin stimulation (Fig. 3B and C). Moreover, we observed that the levels of FAK phosphorylation and FAK kinase activity were similar to the ones we observed in fibroblasts expressing Src. Thus, there is no difference in FAK tyrosine phosphorylation and activation between Src deficient and normal fibroblasts following hormone stimulation. This suggests that Src does not affect FAK phosphorylation and MAP kinase activation following IGF-I or insulin.

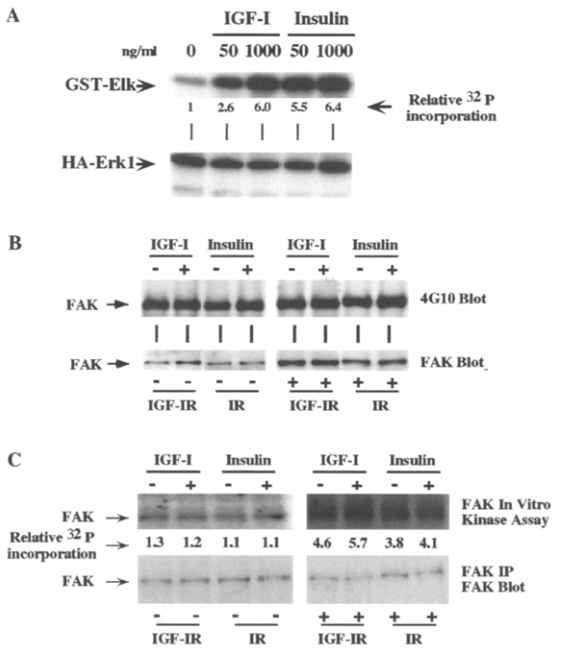

Fig. 3. Role of Src in FAK phosphorylation and activity after stimulation with IGF-I or insulin. Src deficient fibroblasts were used for these experiments. A: Same experiment as Fig. 1A done in parallel. B and C: Same experiments as Fig. 1B and Fig. 2A, and Fig. 1C and Fig. 2B, done in parallel.

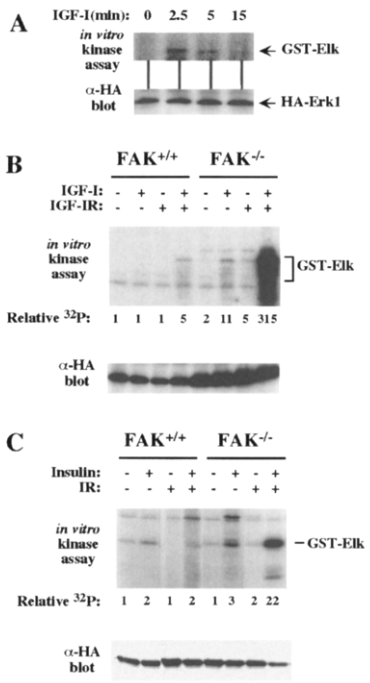

Fig. 4. Map kinase activation after IGF-I stimulation. A: Erk1-MAP kinase was assayed by an in vitro kinase assay using NIH 3T3 fibroblasts stably overexpressing the IGF-IR (upper panel). HA-Erk1 protein was revealed by immunoblotting with anti-HA antibody (lower panel). B: The same experiment as in A was done using FAK deficient cells or their wild-type control.

3.4. Analysis of FAK −/− cells and fibroblasts stably overexpressing the IGF-IR

To determine whether the time course of IGF-I-induced FAK phosphorylation following treatment of cells stably overexpressing the IGF-IR as described above was compatible with a role for FAK in MAP kinase activation, we compared the time course of FAK tyrosine phosphorylation with the time course of MAP kinase activation. NIH 3T3 fibroblasts stably overexpressing the IGF-IR were transfected with MAP kinase. Then, MAP kinase was immunoprecipitated from cells stimulated for various times with IGF-I. MAP kinase activity was tested in an in vitro kinase assay using GST-Elk as substrate. GST-Elk phosphorylation was maximal at 2.5 min of stimulation and returned to a basal level after 15 min (Fig. 4A). In contrast, FAK phosphorylation increased between 5 and 15 min after IGF-I stimulation (see Fig. 2C). These differences in kinetics of FAK phosphorylation and MAP kinase activation suggest that FAK does not play a direct role in MAP kinase activation.

Finally, to further confirm that FAK does not contribute to MAP kinase activation by IGF-I and insulin, we measured MAP kinase activity following IGF-I (Fig. 4B) and insulin (Fig. 4C) stimulation of FAK deficient fibroblasts and their wild-type control. Surprisingly, MAP kinase activity is not decreased but dramatically increased after IGF-I as well as insulin stimulation in these cells. This observation suggests that FAK could repress MAP kinase activation by IGF-I and insulin. To confirm that FAK represses IGF-I- and insulin-induced MAP kinase activity, we tried to re-introduce FAK into the FAK deficient cells either transiently or by stable expression. Unfortunately, we were unable to observe a reproducible decrease in MAP kinase activity in these experiments. Although we could not confirm that FAK might repress MAP kinase activity, we can conclude from our results that FAK does not contribute to IGF-I- or insulin-induced MAP kinase activation.

3.5. Conclusion

Integrin activates MAP kinase in a FAK-dependent manner [21]. IGF-I and insulin have been observed to produce different effects on FAK tyrosine phosphorylation [12,24–29], and both hormones activate MAP kinase after receptor activation. Accordingly, we studied the involvement of FAK tyrosine phosphorylation in MAP kinase activation after IGF-I or insulin stimulation of mouse fibroblasts. Although MAP kinase was activated after IGF-I and insulin stimulation, the levels of FAK tyrosine phosphorylation and kinase activity did not change following stimulation. However, in cells stably overexpressing the IGF-IR, we observed an increase in FAK tyrosine phosphorylation, in accordance with a previous report [30]. This may be an indirect effect, since the IGF-IR autophosphorylation was maximal after 5 min and decreased by 15 min, whereas FAK tyrosine phosphorylation was maximal at 15 min.

We observed similar differences in the time courses of FAK phosphorylation and MAP kinase activation in cells stably overexpressing the IGF-IR: MAP kinase activity was maximal after 2.5 min and decreased by 15 min, whereas FAK phosphorylation was maximal at 15 min. If FAK was situated upstream of MAP kinase in the signaling events following IGF-I stimulation, one would expect that FAK activation would precede MAP kinase activation. These kinetics suggest that FAK is not involved directly in MAP kinase activation following stimulation by IGF-I.

The absence of contribution of FAK in MAP kinase activation following IGF-I and insulin is further confirmed by the results observed with the FAK deficient fibroblasts. If FAK participated in insulin- or IGF-I-induced MAP kinase activation, one would expect that MAP kinase activation was decreased in these cells following stimulation in comparison to their control fibroblasts. This was not the case, and we even observed an increase in MAP kinase activation in the FAK deficient cells, suggesting that FAK may repress insulin- and IGF-I-induced MAP kinase activity. However, further studies are needed to better characterize this cell line and analyze whether this increase is due to a constitutive MAP kinase activation suggested by the higher MAP kinase activity before stimulation (see Fig. 4B), or due to a FAK-specific mechanism of MAP kinase downregulation.

In summary, we conclude that FAK does not contribute to MAP kinase activation following IGF-I or insulin stimulation indicating that FAK is not involved in the physiological signaling through the IGF-IR or the IR in fibroblasts.

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References

[1] van der Geer, P., Hunter, T. and Lindberg, R.A. (1994) Annu. Rev. Cell. Biol. 10, 251–337.
[2] Ullrich, A., Gray, A., Tam, A.W., Yang-Feng, T., Tsubokawa,

M., Collins, C., Henzel, W., Le Bon, T., Kathuria, S., Chen, E., Jacobs, S., Francke, U., Ramachandran, J. and Fujita-Yamaguchi, Y. (1986) EMBO J. 5, 2503–2512.
[3] Liu, J.-P., Baker, J., Perkins, A.S., Roberston, E.J. and Efstratiadis, A. (1993) Cell 75, 59–72.
[4] Joshi, R.L., Lamothe, B., Cordonnier, N., Mesbah, K., Monthioux, E., Jami, J. and Bucchini, D. (1996) EMBO J. 15, 1542–1547.
[5] LeRoith, D., Adamo, M., Werner, H. and Roberts Jr., C.T. (1991) Trends Endocrinol. Metab. 2, 134–139.
[6] Backer, J.M., Myers, M.G., Shoelson, S.E., Chin, D.J., Sun, X.J., Miralpeix, M., Hu, P., Margolis, B., Skolnik, Y., Schlessinger, J. and White, M.F. (1992) EMBO J. 11, 3468–3479.
[7] Baltensperger, K., Kozma, M.L., Cherniack, A.D., Klurlund, J.K., Chawla, A., Banerjee, U. and Czech, M.P. (1993) Science 260, 1950–1952.
[8] Kuhne, M.R., Pawson, T., Lienhard, G.E. and Feng, G.S. (1993) J. Biol. Chem. 268, 11479–11481.
[9] Li, N., Batzer, A., Daly, R., Yajnik, V., Skolnik, E., Chardin, P., Bar-Sagi, D., Margolis, B. and Schlessinger, J. (1993) Nature 363, 85–88.
[10] Skolnik, E.Y., Lee, C.H., Batzer, A.G., Vincentini, L.M., Zhou, M., Daly, R.J., Myers, M.G., Backer Jr., J.M., Ullrich, A., White, M.F. and Schlessinger, J. (1993) EMBO J. 12, 1929–1936.
[11] Tobe, K., Matuoka, K., Tamemoto, H., Ueki, K., Kaburagi, Y., Asai, S., Noguchi, T., Matuda, M., Tanaka, S., Hattori, S., Fukui, Y., Akanuma, Y., Yazaki, Y., Takenawa, T. and Kadowaki, T. (1993) J. Biol. Chem. 268, 11167–11171.
[12] Tobe, K., Sabe, H., Yamamoto, T., Yamauchi, T., Asai, S., Kaburagi, Y., Tamemoto, H., Ueki, K., Kimura, H., Akanuma, Y., Yazaki, Y., Hanafusa, H. and Kadowaki, T. (1996) Mol. Cell. Biol. 16, 4765–4772.
[13] Tartare-Deckert, S., Murdaca, J., Sawka-Verhelle, D., Holt, K.H., Pessin, J.E. and Van Obberghen, E. (1996) Endocrinology 137, 1019–1024.
[14] Sasaoka, T., Rose, D.W., Juhn, B.H., Saltiel, A.R., Draznin, B. and Olefsky, J.M. (1994) J. Biol. Chem. 269, 13689–13694.
[15] Denton, R.M. and Tavare, J.M. (1995) Eur. J. Biochem. 227, 597–611.
[16] Sasaoka, T., Ishiki, M., Sawa, T., Ishihara, H., Takata, Y., Imamura, T., Usui, I., Olefsky, J.M. and Kobayashi, M. (1996) Endocrinology 137, 4427–4434.
[17] Clark, E.A. and Brugge, J.S. (1995) Science 268, 233–239.
[18] Schwartz, M.A., Schaller, M.D. and Ginsberg, M.H. (1995) Annu. Rev. Cell Dev. Biol. 11, 549–599.
[19] Hanks, S.K. and Polte, T.R. (1997) Bioessays 19, 137–145.
[20] Cobb, B.S., Schaller, M.D., Leu, T.-H. and Parsons, J.T. (1994) Mol. Cell. Biol. 14, 147–155.
[21] Schlaepfer, D.D., Hanks, S.K., Hunter, T. and van der Geer, P. (1994) Nature 372, 786–791.
[22] Schlaepfer, D.D. and Hunter, T. (1996) Mol. Cell. Biol. 16, 5623–5633.
[23] Schlaepfer, D.D., Broome, M.A. and Hunter, T. (1997) Mol. Cell. Biol. 17, 1702–1713.
[24] Knight, J.B., Yamauchi, K. and Pessin, J.E. (1995) J. Biol. Chem. 270, 10199–10203.
[25] Yamauchi, K., Milarski, K.L., Saltiel, A.R. and Pessin, J.E. (1995) Biochemistry 92, 664–668.
[26] Pillay, T.S., Sasaoka, T. and Olefsky, J.M. (1995) J. Biol. Chem. 270, 991–994.
[27] Ouwens, D.M., Mikkers, H.M.M., Van der Zon, G.C.M., Stein-Gerlach, M., Ullrich, A. and Maassen, J.A. (1996) Biochem. J. 318, 609–914.
[28] Van der Zon, G.C.M., Ouwens, D.M., Dorrestijn, J. and Maassen, J.A. (1996) Biochemistry 35, 10377–10382.
[29] Konstantopoulos, N. and Clark, S. (1996) J. Biol. Chem. 271, 28960–28968.
[30] Leventhal, P.S., Shelden, E.A., Kim, B. and Feldman, E.L. (1997) J. Biol. Chem. 272, 5214–5218.
[31] Soriano, P., Montgomery, C., Geske, R. and Bradley, A. (1991) Cell 64, 693–702.
[32] Thomas, J.E., Aguzzi, A., Soriano, P., Wagner, E.F. and Brugge, J.S. (1993) Oncogene 8, 2521–2529.
[33] Lipsich, L.A., Lewis, A.J. and Brugge, J.S. (1983) J. Virol. 48, 352–360.
[34] Cartwright, C.A., Simantov, R., Kaplan, P.L., Hunter, T. and Eckhart, W. (1987) Mol. Cell. Biol. 7, 1830–1840.
[35] Janknecht, R. and Hunter, T. (1997) J. Biol. Chem. 272, 4219–4224.