Mechanism of Down-regulation of c-kit Receptor

ROLES OF RECEPTOR TYROSINE KINASE, PHOSPHATIDYLINOSITOL 3'-KINASE, AND PROTEIN KINASE C*

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The receptor tyrosine kinase Kit and Kit ligand (KL), encoded at the murine white spotting (W) and steel (Sl) loci, respectively, function in hematopoiesis, melanogenesis, and gametogenesis. To understand the mechanism of turnover of Kit in mast cells, mutant receptors generated in vitro were heterologously expressed in W53/S1 mast cells lacking endogenous c-kit expression, and the effects of mutations on W53-induced internalization and ubiquitination/degradation of Kit were studied. Upon binding of KL, Kit receptor complexes were rapidly internalized, and the turnover was accelerated by ubiquitin-mediated degradation. Inactivation of the Kit kinase resulted in a reduced rate of internalization of KL-Kit complexes, degradation of kinase-inactive receptor complexes was relatively slow, and receptor ubiquitination was absent. But abolishment of KL-induced receptor association and activation of phosphatidylinositol 3'-kinase and of tyrosine 821 autophosphorylation did not affect KL-induced internalization and ubiquitination/degradation of Kit. Furthermore, Kit receptors can be down-regulated by proteolytic cleavage induced by either activation of protein kinase C or by isopropyl alcohol. In summary, KL-induced internalization of KL-Kit complexes and ubiquitination/degradation require an active kinase. By contrast, proteolytic cleavage of Kit mediated by protein kinase C activation is independent of kinase activity.

Ligand-induced down-regulation is an important aspect of the normal physiology of cell surface receptors. Upon ligand binding, receptor-ligand complexes are thought to be endocytosed via clathrin-coated pits. The receptor may then be recycled to the cell surface or targeted to lysosomes for degradation. Moreover, the receptor may be subjected to nonlysosomal degradation involving polyubiquitination of the protein. Consequently, cell surface levels of receptor are down-regulated. Understanding of the molecular steps involved in mediating receptor internalization and degradation has been facilitated using mutant receptors generated by in vitro mutagenesis. To elucidate the mechanism of ligand-induced receptor down-regulation, the roles of kinase activity and receptor autophosphorylation have been studied for several receptor tyrosine kinases. These include the platelet-derived growth factor receptor (PDGFR)* (Eschbo et al., 1988; Mori et al., 1992; Sor-kin et al., 1991), epidermal growth factor receptor (Prywes et al., 1986; Chen et al., 1987; Honegger et al., 1987), colony stimulating factor-1 receptor (CSF-1R) (Downing et al., 1989; Carberg et al., 1991), and insulin receptor (Russell et al., 1987; McClain et al., 1987; Backer et al., 1989). Although kinase activity is essential for mediating most of the biological effects, a role for kinase activity in receptor down-regulation is not universal and differs depending on the receptor system and the cell type. In addition, most often these analyses were done in nonphysiological cell systems which normally lack endogenous expression of the receptors analyzed. Therefore, caution should be taken when interpreting the physiological significance of results.

c-kit is allelic with the murine W locus (Chabot et al., 1988; Geissler et al., 1988) and encodes a transmembrane receptor tyrosine kinase belonging to the PDGFR receptor family (Besmer et al., 1986; Yarden et al., 1987; Qiu et al., 1988). The ligand of the c-kit encoded receptor (Kit), Kit ligand (KL), also known as steel factor, is encoded at the Sl locus (Nocka et al., 1990a, 1990b; Williams et al., 1999; Zseo et al., 1990; Copeland et al., 1990). Studies in bone marrow-derived mast cells (BMMC) show that Kit receptor activation initiates a cascade of molecular events. KL induces receptor dimerization and kinase activation. Subsequently, receptor autophosphorylation and phosphorylation/activation of downstream signaling molecules occur (Rotapel et al., 1991; Reith et al., 1991). Moreover, Kit can be down-regulated by KL, activation of protein kinase C, and calcium ionophores through independent mechanisms (Yee et al., 1993). KL accelerates the turnover of Kit by inducing receptor internalization and degradation. An active kinase has been shown to be essential for Kit to mediate its pleiotropic effects, but it is not known whether receptor kinase activity and receptor autophosphorylation are required for mediating ligand-induced down-regulation of Kit. Also, how activation of protein kinase C affects cleavage of Kit remains unclear.

To understand the mechanism of down-regulation of Kit, the roles of kinase activity and autophosphorylation in KL-induced receptor internalization and degradation were investigated. This was accomplished by using mutant Kit receptors in which: 1) the receptor kinase was inactivated by substitution of the conserved aspartic acid residue 790 with asparagine, known from the W53 allele (Tan et al., 1990); 2) tyrosine 719 and tyrosine 821 were substituted by the structurally similar phenylalanine. Tyrosine 719 within the kinase insert is essential for KL-induced association of the p85 regulatory subunit of phosphatidylinositol 3'-kinase (PI-3'-kinase) with Kit and activa-

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1 The abbreviations used are: PDGFR, platelet-derived growth factor receptor; CSF-1R, colony stimulating factor-1 receptor; Kit, Kit ligand; BMMC, bone marrow-derived mast cells; PI, phosphatidylinositol; rmKL, recombinant murine KL; TPA, 12-O-tetradecanoylphorbol-13-acetate; FITC, fluorescein isothiocyanate; PAGE, polyacrylamide gel electrophoresis.
tion of PI-3'-kinase (Serve et al., 1994); whereas tyrosine 821 is a potential autophosphorylation site. A myeloproliferative sarcoma virus-based retroviral expression vector (pGD) and Wpx/Wpx mutant BMMC lacking endogenous c-kit expression were used for expression of normal and mutant Kit receptors in mast cells (Tano et al., 1992; Duttlinger et al., 1993). Inactivation of the Kit kinase reduced the rate of internalization and degradation of KL. Kit complexes and inhibited receptor ubiquitination. Abolishment of KL-mediated receptor association and activation of PI-3'-kinase and of autophosphorylation of tyrosine 821 had no effect on KL-induced internalization and ubiquitination/degradation of Kit. In addition, the mechanism underlying protein kinase C-induced cleavage of Kit was examined.

EXPERIMENTAL PROCEDURES

Most Cell Cultures and Materials—C57Bl/6 (+/+ ) mice were purchased from The Jackson Laboratory. Wpx/Wpx mice were provided by Drs. Regina Duttlinger and Katia Manova. BMMC were cultured as described (Yee et al., 1993). Recombinant murine KL (rmKL) was prepared as described (Yee et al., 1993). 12-0-Tetradecanoylphorbol-13-acetate (TPA, Sigma) was dissolved in dimethyl sulfoxide (Me,SO), and the stock solution was stored at -20 °C. All other chemicals were purchased from The Jackson Laboratory.

Radiolabeling, Internalization Assays, and Ligand Binding—rmKL was radiolabeled with Na22P (17.4 Ci/mg, DuPont NEN) using chloramine T as described (Shieh et al., 1991). Internalization and ligand binding were assayed as described (Yee et al., 1993). The rate constant for internalization of 125I-KL (Kint) was determined by using the equation: d/dt = -Kint[S] where I = internalized 125I-KL and S = surface 125I-KL at time t.

For Scatchard analysis, BMMC (1.5 x 10^6/ml) were incubated with 125I-KL (3 x 10^8/i) in 80 µl of binding buffer (phosphate-buffered saline supplemented with 0.5% (w/v) bovine serum albumin and 0.03% (w/v) sodium azide) in a 96-well plate (Corning) for 1 h at room temperature. The cells were then layered onto calf serum and centrifuged at 400 x g for 5 min at room temperature. γ-Emission from the cell pellets and supernatants (containing free 125I-KL) was measured by a Beckman Gamma 5600 counter. Specific binding of 125I-KL was determined by using the following equation: specific binding = total binding (radioactivity bound in the absence of unlabeled KL) - nonspecific binding (radioactivity bound in the presence of 500-fold excess of unlabeled KL).

The average nonspecific binding accounts for less than 1.4% of total radioactivity added. Scatchard analysis was performed by plotting the 125I-KL bound/free ratio as a function of 125I-KL bound. The correlation coefficients (r) for the resulting linear regression lines are >0.98.

Metabolic Labeling and Immunoprecipitation Analysis—Metabolic labeling, immunoprecipitations, and SDS-PAGE were done as described (Yee et al., 1993) except that RIPA lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 10 mM EDTA, 1% deoxycholate) was used instead. For immunoblotting, the proteins were transferred from the gel to the nitrocellulose blot (TransBlot medium, Bio-Rad) at 60 V
overnight at 4 °C. The blot was incubated in Tris-buffered saline, pH 7.6 (containing 5% bovine serum albumin) at 37 °C for 1 h; subsequently, anti-Kit serum (1:100) or anti-ubiquitin serum (1:10) (Sigma) was added, and incubation was continued for 1 h at room temperature. The blot was washed twice with Tris-buffered saline containing 0.1% Tween 20 and incubated in Tris-buffered saline, pH 8.2, and proteins were detected by autoradiography (Molecular Dynamics) and are expressed as percent of those at time 0.

RESULTS

KL-induced Internalization and Ubiquitination (Degradation of Kit)—To determine the effect of rmKL on the cell surface level of Kit in bone marrow-derived mast cells (BMMC), the level of Kit-ab binding sites was analyzed by FACS. The loss of Kit-ab binding sites followed first-order kinetics (Fig. 1A). Within the initial 10 min following binding of KL, the Kit-ab binding sites decreased rapidly at a rate of 3.3% min⁻¹, and the rate constant was 1.4 min⁻¹. Next, the rate of ligand internalization after binding to Kit was determined. ¹²⁵I-KL was internalized at an initial rate of 3.8% min⁻¹, and the internalization rate constant (Kᵣ) was 1.5 x 10⁻³ s⁻¹ (Fig. 1B). After reaching a peak at 15 min, the percentage of internalized ¹²⁵I-KL decreased. Therefore, KL-induced loss of cell surface Kit-ab binding sites and the ligand itself was internalized.

To determine the kinetics of degradation of Kit, pulse-chase experiments were done by metabolic labeling of BMMC with [³⁵S]methionine/cysteine and incubating the labeled cells in the presence of excess unlabeled methionine to chase the ³⁵S-labeled receptors to the cell surface. The total amount of cellular Kit was analyzed by immunoprecipitation and SDS-PAGE. The half-life (t½) of Kit in the absence of ligand was 1–2 h (data not shown). Following incubation with KL, the level of Kit decreased with first-order kinetics and a t½ of 26.8 min (Fig. 2, A and B). The smear above Kit might represent a high molecular weight form of Kit of total cell lysate determined by immunoblotting using anti-Kit serum. B, the presence of polyubiquitin was identified by immunoblotting using anti-ubiquitin serum.

Mechanism of Ligand-induced Down-regulation of Kit: Requirement of Receptor Kinase, Autophosphorylation at Tyrosine 821, and Kit-associated Phosphatidylinositol 3'-Kinase—To determine whether receptor kinase activity and tyrosine autophosphorylation play a role in the down-regulation of Kit, mu-
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Fig. 5. Kinetics of KL-induced internalization of Kit+, KitW42, KitY719F, and KitY821F expressed in W%/W% BMMC. Kit-ab binding sites on KL-pretreated BMMC were determined by FACS and are expressed as percent of that at time 0. Linear regression analyses of Kit-ab binding sites within the initial 15 min indicated: \( r = 1.0 \) (Kit'), \( r = 0.92 \) (KitW42) (A) and \( r = 0.91 \) (KitY719F), \( r = 1.0 \) (KitY821F) (B). Same results were obtained in a repeated trial.

Fig. 6. Kinetics of \(^{125}\)I-KL internalization in BMMC expressing Kit' and KitW42. A, the amount of cell-associated \(^{125}\)I-KL being internalized is expressed as percent of that at time 0. B, linear regression analyses of \(^{125}\)I-KL (internalized/surface) versus time indicated \( r = 0.99 \) (Kit') and \( r = 1.0 \) (KitW42). C, Scatchard analysis of \(^{125}\)I-KL binding in BMMC expressing Kit' and KitW42.

 tant forms of Kit had been constructed using the c-kit cDNA. A Kit receptor lacking kinase activity contained a substitution of aspartic acid 790 with asparagine. Tyrosine 719 and adjacent amino acids located in the kinase insert contain the binding site for the regulatory subunit p85 of PI-3' kinase (Serve et al., 1994). Tyrosine 821, located within the C-terminal kinase domain, is a conserved residue for autophosphorylation in most receptor tyrosine kinases (Hanks et al., 1987). Both tyrosine 719 and tyrosine 821 had been changed to phenylalanine by in vitro mutagenesis (Serve et al., 1994). Subsequently, the mutant constructs were cloned into the myeloproliferative sarcoma virus-based retroviral expression vector (pGD) containing a neomycin resistance gene, and E86 packaging cells were transfected with the constructs to generate virus-producing cell lines. W%/W% BMMC lacking endogenous c-kit expression were infected with pGD-Kit viruses and infected cells were selected by G418 and then sorted by FACS based on cell surface expression of Kit. To determine whether the exogenously expressed receptors were appropriately expressed, the FACS-sorted cells were metabolically labeled with \[^{35}\text{S} \]methionine/
cysteine and then analyzed for Kit by immunoprecipitation and SDS-PAGE. Wild-type BMMC were included for comparison. The total cellular amounts of Kit+, KitW42, KitY71F, and KitYR21F were determined by immunoprecipitation and SDS-PAGE. The amounts of Kit+, KitW42, KitY71F, and KitYR21F expressed in the infected cells were comparable with each other but relatively low compared to that in wild-type BMMC (not shown). Furthermore, flow cytometric analysis indicated that the mean level of Kit-specific fluorescence on the infected cells was about 75% of that on wild-type BMMC (Fig. 4). The lack of kinase activity of KitW42 was confirmed by in vitro kinase assay and KL-stimulated tyrosine autophosphorylation in vivo (data not shown). Abolishment of KL-induced association of Kit with PI-3'-kinase and PI-3'-kinase activation for KitY71F was confirmed as well. BMMC expressing wild-type and mutant Kit receptors were then used to study receptor internalization, ubiquitination, and degradation in response to KL, as well as protein kinase C-induced proteolytic cleavage.

First, the role of Kit tyrosine kinase in ligand-induced receptor internalization was studied. KL-induced loss of cell-surface Kit-ab binding sites on W57/66 BMMC expressing Kit+ occurred at an initial rate of 3.0% min⁻¹ (Fig. 5A). In BMMC expressing KitW42, Kit-ab binding sites were overall maintained at a relatively constant level throughout the incubation period. Consistent with this finding, the internalization of 125I-KL proceeded at a relatively low initial rate of 2.0% min⁻¹ with a Kₘ of 4.3 × 10⁻⁴ s⁻¹ in BMMC expressing KitW42, as compared to that in cells expressing Kit+ (6.1% min⁻¹ and 2.0 × 10⁻³ s⁻¹, respectively) (Fig. 6, A and B). By contrast, KitY71F and KitYR21F did not affect the rate of internalization of 125I-KL (not shown). As indicated by the Scatchard analysis (Fig. 6C), the ligand binding affinity was essentially the same for Kit+ and KitW42. Thus, it is unlikely that the impaired ligand-induced internalization of KitW42 is due to different binding affinity. Next, the rates of ligand-induced internalization of KitY71F and KitYR21F receptors were determined. The initial rates of decrease in Kit-ab binding sites on BMMC expressing KitY71F and KitYR21F were similar to that for Kit+ (2.7% min⁻¹ and 2.9% min⁻¹, respectively, versus 3.0% min⁻¹) (Fig. 5B). Therefore, the tyrosine kinase activity of Kit is essential for ligand-induced internalization of the KL-Kit complex. However, abolishment of KL-induced PI-3'-kinase activation and association with Kit and abolishment of autophosphorylation at tyrosine 821 did not significantly affect this process.

We then examined the effects of kinase inactivation and mutations at tyrosine 719 and tyrosine 821 on ligand-induced receptor degradation. Following treatment of BMMC with KL, Kit+ rapidly decreased with a half-life (t½) of 21.7 min (Fig. 7, A and B). In contrast, degradation of KitW42 was relatively slow (t½ = 52.1 min). Only slightly reduced rates of degradation were observed for KitY71F (t½ = 30.3 min) and KitYR21F (t½ = 28.3 min). Therefore, receptor kinase activity is required for efficient degradation of PI-3'-kinase with Kit and autophosphorylation at tyrosine 821 are not essential for these processes.
TPA decreased the cell surface level as well as the total cellular amount of Kit, and this was associated with the release of the extracellular domain of Kit (ED100 kDa protein) into the medium. In an attempt to understand the mechanism underlying TPA-induced cleavage of Kit, a panel of protease inhibitors with different substrate specificities was used to characterize the cleavage process in BMMC. Only EDTA inhibited the release of the ED100 kDa protein to background level as in non-TPA-treated cells (Fig. 9A), suggesting that TPA-induced cleavage of Kit involves a protease whose activity depends on divalent cation. By contrast, isopropyl alcohol alone is sufficient to elicit release of the ED100 kDa protein (Fig. 9B) possibly by exposing Kit to a surface protease. Moreover, a synergistic effect was achieved by using a combination of isopropyl alcohol and TPA. However, protein kinase C-induced release of the ED100 kDa protein occurred in TPA-treated BMMC expressing Kit(R21F), Kit(Y219F), or Kit(Y821F) at least as efficiently as in the cells expressing Kit(+) (Fig. 9C). Therefore, proteolytic cleavage of Kit can be accomplished by TPA or isopropyl alcohol, each of which may act by independent mechanisms.

DISCUSSION

Ligand-induced down-regulation of receptors serves as an attenuation mechanism for growth factor-mediated signaling (Wells et al., 1990; Mori et al., 1993). KL binding to its receptor Kit accelerates the turnover of Kit by inducing internalization of receptor-ligand complexes, followed by polyubiquitination and degradation of Kit. We studied the mechanism of down-regulation of Kit by using mutant receptors expressed in W(F) Wh/BMMC lacking endogenous c-kit expression. Since these cells contain the normal machinery for endocytosis and degradation of Kit, our results provide information about Kit receptor desensitization in a unique relevant cellular context.

The Kit receptor in the W(F) allele contains a single amino acid substitution (D790N) and lacks kinase activity (Tan et al., 1990). Aspartic acid 790 of Kit is analogous with aspartic acid 184 of cyclic adenosine monophosphate-dependent protein kinase and is thought to play a role as the catalytic base in the phosphate transfer reaction (Knighton et al., 1991). KL-induced internalization of Kit(W(F)) was shown to be defective, and this was reflected in a slower rate of degradation of Kit(W(F)) and lack of polyubiquitin modification of Kit(W(F)) receptor. Similarly, kinase-inactive CSF-1 receptor does not undergo accelerated ligand-induced receptor turnover (Downing et al., 1989; Carlberg et al., 1991). Nevertheless, KL still facilitated degradation of Kit(W(F)), although to a lesser extent than the normal receptor, suggesting the existence of alternate pathways for receptor degradation such as lysosomal degradation known from studies with PDGF receptor down-regulation (Sorkin et al., 1991). The slower turnover of Kit(W(F)) may at least in part contribute to the dominant phenotype of the W(F) allele (Ray et al., 1991). Under such conditions, a net accumulation of Kit(W(F)) homodimers and Kit(R21F)/Kit(+) heterodimers at the cell surface in response to KL would be expected. In contrast, an EGF receptor mutation, in which receptor internalization is defective, was previously shown to produce an amplified mitogenic signal (Chen et al., 1989; Wells et al., 1990).

The requirement of an active receptor kinase for efficient down-regulation of Kit implies a role for tyrosine phosphorylation of the receptor itself and/or cellular substrates. In previous studies with CSF-1 receptor, deletion of the kinase insert was shown to affect ligand-induced receptor degradation but not receptor internalization (Carlberg et al., 1991). But, phenylalanine substitutions of tyrosines 699 and 708 in the kinase insert region and tyrosine 809 of the CSF-1 receptor had no...
were treated with or without TPA (100 ng/ml) in the presence of various the presence or absence of TPA for natants were analyzed for the ED100 kDa protein by immunoprecipi-

BMMC expressing Kit+, Kit'v42, KitY519F, and KitYR21F were incubated in incubation (Roussel nase) in intracellular protein transport had been suggested in agreement with this, mutation of the analogous tyrosine 821 of Kit did not affect KL-induced internalization and degradation of the receptor. By contrast, CSF-1R by-1 binding site mutations expressed in porcine aortic endothelial cells blocked growth factor-induced ubiquitin modification (Mori et al., 1993). It remains to be determined whether a similar mechanism mediates ubiquitin modification of Kit.

Activation of protein kinase C induces down-regulation of Kit receptors, and this down-regulation is associated with proteolytic cleavage of Kit, releasing the extracellular domain of the receptor (ED100 kDa protein) into the medium (Yee et al., 1993). While protein kinase C activity is essential for this effect to occur, tyrosine kinase activity of Kit is dispensable, and this finding agrees with that for CSF-1R (Downing et al., 1989). While the protease facilitating down-regulation of Kit requires divalent cation, isopropyl alcohol induces proteolytic cleavage of Kit, and isopropyl alcohol-induced cleavage is enhanced with TPA. This may suggest that exposure of Kit to the protease may be facilitated by isopropyl alcohol, possibly by modifying membrane fluidity, although the involvement of protein kinase C in isopropyl alcohol-induced cleavage cannot be excluded. Recently, Koike et al. (1993) showed that KL activated phospholipase D that led to the formation of 1,2-diacylglycerol, a physiological activator of protein kinase C. Moreover, protein kinase C activation increases serine/threonine phosphorylation of Kit resulting in inhibition of autophosphorylation of Kit (Blume-Jensen et al., 1993). Taken together, KL may activate protein kinase C and, thus, down-regulate Kit as a negative feedback mechanism by 1) cleavage of Kit to reduce ligand-binding capacity and 2) by impairment of autophosphorylation of Kit. Henceforth, a diversity of means for receptor down-regulation exists, and this may be important as alternative mechanisms to ensure receptor desensitization.

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Carlgberg, K., Tapley, P., Haysfield, C., and Schrader, L. (1981) EMBO J. 10, 877-883

Chabot, B., Stepheenson, D. A., Chapman, V. M., Besmer, P., and Bernstein, A. (1980) Nature 285, 88-89

Chen, W. S., Lazar, C. S., Poenie, M., Tsien, R. Y., Gill, G. N., and Rosenfeld, M. G. (1987) Nature 325, 820-823

Chen, W. S., Lazar, C. S., Lund, K. A., Welsh, J. R., Chang, C.-P., Walton, G. M., Der, C. J., Wiley, H. S., Gill, G. N., and Rosenfeld, M. G. (1989) Cell 59, 33-43

Copeland, N. G., Gilbert, D. J., Cho, B. C., Donovan, P. J., Jenkins, N. A., Coisman, D. Anderson, D., Lyman, S. D., and Williams, D. E. (1990) Cell 63, 175-185

Dastych, J., and Metcalf, D. D. (1994) J. Immunol. 153, 213-219

Downing, J. R., Roussel, M. F., and Sherr, C. J. (1989) Mol. Cell. Biol. 9, 2890-2896

Duttlinger, R., Manara, K., Chu, T.-Y., Gyssler, C., Bachvarova, N., and Besmer, P. (1988) Development 118, 755-777

Escobedo, J. A., Barr, P. J., and Williams, L. T. (1986) Mol. Cell. Biol. 6, 5126-5131

Geissler, E. N., Ryan, M. A., and Houman, D. E. (1986) Cell 45, 185-192

Hanks, S. K., Quinn, A. M., and Hunter, T. (1987) Science 234, 45-52

Harlow, E., and Lane, D. (1988) Antibodies: A Laboratory Manual. p. 354, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York

Hayashi, S., Kunisada, K., Ogawa, M., Yamaguchi, K., and Nishikawa, S. (1991) Nucleic Acids Res. 19, 1297-1271

Hosegger, A. M., Dull, T. J., Felder, S., Van Obberghen, E., Belfot, F., Szapary, D., Schmidt, A., Ullrich, A., and Schlessinger, J. (1987) Cell 51, 199-209

Huang, E., Nocke, K., Reier, D. R., Chu, T.-Y., Buck, J., Laemmer, H.-W., Wellner, D., Leider, P., and Besmer, P. (1990b) Cell 63, 225-235

Joly, M., Karlanlagas, A., Fay, F. S., and Corvera, S. (1994) Science 263, 684-687

Knighton, D. R., Zheng, J., Ten Eyck, F. A., Ashford, V. A., Xuong, N.-H., Taylor, S. S., and Sowadski, J. M. (1991) Science 253, 407-414

Koike, T., Hirai, K., Morita, Y., and Nogawa, Y. (1995) J. Immunol. 161, 359-366

Kong, S.-W., and Chock, P. B. (1992) J. Biol. Chem. 267, 14189-14192

McClain, D. A., Maegawa, H., Lee, J., Dull, T. J., Ulrich, A., and Olefsky, J. M. (1995) J. Biol. Chem. 269, 14063-14071

Mori, S., Heldin, C.-H., and Claesson-Welsh, L. (1992) J. Biol. Chem. 267, 6429-6434

Mori, S., Heldin, C.-H., and Claesson-Welsh, L. (1992) J. Biol. Chem. 268, 577-583

Mori, S., Ronnstrand, L., Claesson-Welsh, L., and Heldin, C.-H. (1994) J. Biol. Chem. 269, 4917-4921

Nagata, S., Kusakabe, M., Yoshina, K., Ogasawara, M., Hayashi, S., Kunisada, T., Ern, T., Sakakura, T., and Nishikawa, S.-I. (1991) EMBO J. 10, 2111-2118

Nocka, K., Buck, J., Levi, R., and Besmer, P. (1990a) EMBO J. 9, 3287-3294

Nocka, K., Tan, J. C., Chu, E., Chu, T. Y., Ray, P., Cervone, M., Bernstein, A., and Besmer, P. (1996a) EMBO J. 9, 1805-1815

Panayotou, G., and Waterfield, M. D. (1992) Trends Cell Biol. 2, 358-360

Prywes, R., Livneh, E., Ulrich, A., and Schlessinger, J. (1986) EMBO J. 5, 2179-2190

Qiu, F., Ray, P., Brown, K., Barker, P. E., Shanks, S., Rudle, F. H., and Besmer, P. (1988) EMBO J. 7, 1033-1041

Ray, P., Higgins, K. M., Tan, J. C., Chu, T. Y., Yee, N. S., Nguyen, H., Lacy, E., and Besmer, P. (1991) Genes & Dev. 5, 2285-2273

Rechsteiner, M. (1987) Annu. Rev. Cell Biol. 3, 1-30

Reich, A. D., Ellis, C., Lyman, S. D., Anderson, D. M., Williams, D. E., Bernstein, A., and Pawson, T. (1991) EMBO J. 10, 2451-2459

Rottapel, R., Reedijk, M., Williams, D. E., Lyman, S. D., Anderson, D. M., Pawson, T., and Bernstein, A. (1991) Mol. Cell. Biol. 11, 3045-3051

Roussel, M. F., Shurtleff, S. A., Downing, J. R., and Sherr, C. J. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 6738-6742

Russell, D. S., Gherzi, R., Johnson, E. L., Chou, C.-K., and Rosen, O. M. (1987) J. Biol. Chem. 262, 11833-11840

Serce, H., Hsu, Y.-C., and Besmer, P. (1994) J. Biol. Chem. 269, 6026-6030

Shieh, J.-H., Peterson, R. H., and Moore, M. A. (1991) J. Immunol. 146, 2648-2653

Sorkin, A., Westermark, B., Heldin, C.-H., and Claesson-Welsh, L. (1991) J. Cell Biol. 112, 499-548

Tan, J. C., Nocka, K. H., Ray, P., Traktman, P., and Besmer, P. (1990) Science 247, 209-212

Tao, T., Tsuchimura, T., Koshibu, U., Kanagai, T., Adachi, S., Isonoki, K., Nishikawa, S., Morimoto, M., Nishimune, Y., Nomura, S., and Kitamura, Y. (1992) Blood 80, 1445-1450

Wells, A., Welsh, J. B., Lazar, C. S., Wales, H. S., Gill, G. N., and Rosenfeld, M. G. (1990) Science 247, 962-964

Wells, A., Welsh, J. B., Lazar, C. S., Wales, H. S., Gill, G. N., and Rosenfeld, M. G. (1990) Science 247, 962-964

Williams, D. E., Eisenman, J., Baird, A., Rauch, C., Van Ness, K., March, C. J., Paek, E. S., Martin, U., Motchukio, D. Y., Boswell, H. S., Burgess, G. S., Coisman, D. and Lymans, S. D. (1990) Cell 63, 167-174

Yarden, Y., Kuang, W. J., Yang, F. T., Coussens, L., Munemitsu, S., Dull, T. J., Chen, E., Schlessinger, J., Friacek, U., and Ullrich, A. (1987) EMBO J. 6, 3451-3451

Yee, N. S., Langen, H., and Besmer, P. (1990) J. Biol. Chem. 265, 14189-14208

Zabeo, K. M., Williams, D. A., Geiser, E. N., Broudy, V. C., Martin, F. H., Atkins, H. L., Nac, R. Y., Birckett, N., Okino, K. H., Mardock, D. C., Jacobson, P. W., Langley, K. E., Smith, R. A., Takeishi, T., Cattanas, B. M., Galli, S. J., and Suggs, S. V. (1990) Cell 63, 213-224