Identification of Important Regions in the Cytoplasmic Juxtamembrane Domain of Type I Receptor That Separate Signaling Pathways of Transforming Growth Factor-β*

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Proteins in the transforming growth factor-β (TGF-β) superfamily exert their effects by forming heteromeric complexes of their type I and type II serine/threonine kinase receptors. The type I and type II receptors form distinct subgroups in the serine/threonine kinase receptor family based on the sequences of the kinase domains and the presence of a highly conserved region called the GS domain (or type I box) located just N-terminal to the kinase domain in the type I receptors. Recent studies have revealed that upon TGF-β binding several serine and threonine residues in the GS domain of TGF-β type I receptor (TβR-I) are phosphorylated by TGF-β type II receptor (TβR-II) and that the phosphorylation of GS domain is essential for TGF-β signaling. Here we investigated the role of cytoplasmic juxtamembrane region located between the transmembrane domain and the GS domain of TβR-I by mutational analyses using mutant mink lung epithelial cells, which lack endogenous TβR-I. Upon transfection, wild-type TβR-I restored the TGF-β signals for growth inhibition and production of plasminogen activator inhibitor-1 (PAI-1) and fibronectin. A deletion mutant, TβR-I D11(Δ150–181), which lacks the juxtamembrane region preceding the GS domain, bound TGF-β in concert with TβR-II and transduced a signal leading to production of PAI-1 but not growth inhibition. Recombinant receptors with mutations that change serine 172 to alanine (S172A) or threonine 176 to valine (T176V) were similar to wild-type TβR-I in their abilities to bind TGF-β, formed complexes with TβR-II, and transduced a signal for PAI-1 and fibronectin. Similar to TβR-I D11(Δ150–181), however, these missense mutant receptors were impaired to mediate a growth inhibitory signal. These observations indicate that serine 172 and threonine 176 of TβR-I are dispensable for extracellular matrix protein production but essential to the growth inhibition by TGF-β.

The cell growth and differentiation in a multicellular organism are critically regulated by members of transforming growth factor-β (TGF-β) superfamily including TGF-β, activin/inhibin, bone morphogenetic protein (BMP), Müller inhibiting substance, and glial cell line-derived neurotrophic factor. TGF-β is a prototype in this superfamily of structurally related molecules and regulates cell proliferation, extracellular matrix formation, migration, adhesion, and many other cellular functions important for development and homeostasis (reviewed in Refs. 1–4).

Certain members of the TGF-β superfamily exert their biological actions through heteromeric complexes of two types (type I and type II) of transmembrane receptors with a serine/threonine kinase domain in their cytoplasmic region (5–8). To date, more than 15 receptor serine/threonine kinases have been cloned in flies through humans (reviewed in Refs. 4 and 9–12). Among them six different type I receptors have been identified in mammals (5, 8, 13–20), including one TGF-β type I receptor (TβR-I), two activin type I receptors (ActR-I and ActR-IB), two BMP type I receptors (BMPR-IA and BMPR-IB), and one additional type I receptor called activin receptor-like kinase-1 (also termed TGF-β superfamily receptor type I or R3) that has recently been shown to mediate certain signals in response to BMP-7 (osteogenic protein-1). The type I receptors have similar sizes (502–532 amino acid residues) and 60–90% amino acid sequence identities to each other in their kinase domains. In addition, type I receptors contain a conserved sequence known as the GS domain (also called type I box) in their cytoplasmic juxtamembrane region (10, 11). Type I receptors are more similar to each other than they are to the known type II receptors, including TGF-β type II receptor (TβR-II) and two activin type II receptors (ActR-II and ActR-IB), and thus form a subgroup of mammalian type I receptors in the family of receptor serine/threonine kinases.

TGF-β initiates the signaling of its multiple responses through formation of a heteromeric complex of TβR-I and TβR-II. TGF-β binds directly to TβR-II that is a constitutively active kinase, which then recruits TβR-I into the complex. TβR-II in the complex then phosphorylates the GS domain of TβR-I, resulting in propagation of further downstream signals (21, 22). The catalytic activities of the kinases of TβR-I and TβR-II are indispensable for signaling (22–25). Mutational analyses

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altering serine and threonine residues in the TβR-I GS domain have revealed that phosphorylation of certain serines and threonines by TβR-II is essential for TGF-β signaling, although its signaling activity does not appear to depend on the phosphorylation of any particular serine or threonine residue in the TSGS2SG sequence of the GS domain (22, 26, 27). In addition, recent identification of a constitutively active form of TβR-I that does not require TβR-II and TGF-β for signaling suggested that TβR-I acts as a downstream signaling molecule of TβR-II (27).

Despite the functional importance of the GS domain for initiating intracellular signals, little is known about how the signals are propagated after phosphorylation of the GS domain. Based on the knowledge of receptor tyrosine kinases, one possible mechanism could be that the phosphorylated serine and/or threonine residues in the GS domain may act as the binding sites for the intracellular substrate to be activated by signals propagated after phosphorylation of the GS domain. Initiating intracellular signals, little is known about how the phosphorylated serine and/or threonine residues in the GS domain (27). In fact, a TβR-I chimeric receptor substituting the GS domain of ActR-I for that of TβR-I still transduces the TGF-β-induced antiproliferative signal, which is not mediated through intact ActR-I (27, 28). Thus, certain region(s) other than the GS domain in the type I receptors may also be important for diverse signaling activities of the proteins in the TGF-β superfamily.

In the present study we focused on the role of the TβR-I juxtamembrane region preceding the GS domain, and serine 172 and threonine 176 within this region were found to be essential for signaling a TGF-β antiproliferative response but not plasminogen activator inhibitor-1 (PAI-1) and fibronectin induction. Identification of such cytoplasmic regions important only for a limited response may suggest that at least two different signals are specified through different cytoplasmic parts of TβR-I.

EXPERIMENTAL PROCEDURES

List and Sequences of the Oligonucleotides Used to Generate Expressions Constructs—The sequences of the oligonucleotide primers are presented in the 5′ to 3′ direction. Numbering is based on the nucleotide sequence of TβR-I (8), and restriction enzyme sites incorporated into the primers are underlined. The junction of a deletion primer RISdel5 is indicated by a hyphen. The sequences are: RIS-1-sma, GTTGCCGGGTGCTGACAAGCCACT (nucleotides 441–445); RISdel2-sma, GCCCGGTTGTTGCAATAAAAGGGCG (nucleotides 527–504); and RIS-1, ATAAAAGGGCGATCTAATGCAGG (nucleotides 514–492); RISdel2-sma, GCCCGGTTGTTGCAATAAAAGGGCG (nucleotides 527–504); S-3, GAGGGTACTGTGTTGAAAGAC (nucleotides 519–539); S-1, S-2, and S-3, respectively) and primer RIAS-not were used to amplify the 3′ fragment. PCR products were mixed in respective combinations and reamplified with primers RIS-hind and RIAS-not. For TβR-I/JM2 (S165A/S172A/T176V), primer RIS-1 as a template for the 3′ fragment and primer S-2 and RIAS-not were used to amplify the 3′ fragments. PCR products were mixed in respective combinations and reamplified with primers RIS-hind and RIAS-not. For TβR-I/JM2 (S165A/S172A/T176V), primer RIS-1 as a template for the 3′ fragment and primer S-2 and RIAS-not were used to amplify the 3′ fragments. PCR products were mixed in respective combinations and reamplified with primers RIS-hind and RIAS-not. For TβR-I/JM2 (S165A/S172A/T176V), primer RIS-1 as a template for the 3′ fragment and primer S-2 and RIAS-not were used to amplify the 3′ fragments.
with minor modifications (28, 32). Briefly, subconfluent cells in 6-well plates were incubated for 5 h with DMEM containing 0.2% FBS and 100 μM ZnCl₂. Cells were washed once with PBS and incubated for 4 h in methionine- and cysteine-free DMEM (ICN Biomedicals Inc.) containing 100 μM ZnCl₂, with or without 50 nM/ml of TGF-β1. During the final 2 h of incubation, 30 μCi/ml of [35S]methionine and [35S]cysteine mixture (Pro-mix cell labeling mix; Amersham Corp.) were added to the cells. The cells were then added with or without 50 ng/ml of TGF-β1, incubated for 20 h, and labeled with 50 Ci/ml [35S]methionine and [35S]cysteine mixture in methionine- and cysteine-free DMEM for the final 4 h. The labeled culture media were incubated with glutathione-Sepharose beads (glutathione-Sepharose 4B; Pharmacia) (5:1, v/v) for 1 h at 4°C. After extensive washing in PBS, the beads were subjected to phosphorylation assays.

Protein Kinase Assay—25 μl of glutathione-Sepharose beads that attached GST fusion proteins were washed once with kinase buffer (20 mM Hepes, pH 7.4, 100 mM NaCl, 10 mM MnCl₂, 0.5 mM DTT, 0.05% Triton X-100) and added with 25 μl of kinase buffer containing 1 μCi of [γ-³²P]ATP (Amersham Corp.). The beads were incubated for 15 min at 4°C. Proteins were resolved on SDS-10% polyacrylamide gel under reducing conditions and analyzed by Bio-Imaging Analyzer.

RESULTS

Generation of a TβR-I Deletion Mutant and Its Binding Ability to TGF-β—Based on the sequence comparison among the type I receptors, the cytoplasmic juxtamembrane region preceding the kinase domain of TβR-I can be divided into two subregions (Fig. 1). The C-terminal half of the juxtamembrane region (leucine 177 to valine 206), which is composed of 30 amino acids and rich in serine and threonine residues (three serines and four threonines), contains the highly conserved SGSGSG core sequence and other conserved amino acids among the type I receptors. Therefore, this region was previously designated GS domain (or type I box). Recent findings revealed that phosphorylation of several serine and threonine residues in this region is also rich in serine and threonine residues (two serines and three threonines out of 27 amino acids), and in this region is also rich in serine and threonine residues (two serines and three threonines out of 27 amino acids), and in contrast to the GS domain, the sequences of the corresponding region in the type I receptors are very divergent (Fig. 1A), so that this region might be involved in the specification of the downstream substrates that mediate diverse responses triggered by the proteins in the TGF-β superfamily. If this region
is essential in TβR-I, its deletion should prevent signaling. We deleted the 32 amino acids of TβR-I in this region, yielding TβR-I/JD1 (Δ150–181), as expected from its signaling activity of TGF-β1 is restored also in R4–2 cells transfected with the wild-type TβR-I but much less potently in the presence of ZnCl2, [3H]thymidine incorporation into the DNA was inhibited dose-dependently up to 97% (Fig. 4A), whereas TGF-β1 had no effect on the [3H]thymidine incorporation in the R4–2 cells transfected with the vector alone. When R4–2 cells transfected with the wild-type TβR-I were treated with TGF-β1 in the presence of ZnCl2, [3H]thymidine incorporation into the DNA was inhibited by 65–75%, whereas only a marginal inhibition was observed in the absence of ZnCl2. In contrast, R4–2 cells transfected with TβR-I/JD1 (Δ150–181) were refractory to TGF-β1 growth inhibition in the presence or the absence of ZnCl2 (Fig. 4, A and B). These results suggested that the N-terminal half of the cytoplasmic juxtamembrane domain of TβR-I was not required for signaling a PAI-1 response, whereas it was essential for signaling growth inhibitory activity.

Serine 172 and Threonine 176 Are Essential for Signaling Growth Inhibitory Activity—The inability of TβR-I/JD1 (Δ150–181) to mediate a growth inhibitory signal raised the possibility that the N-terminal half of the cytoplasmic juxtamembrane domain of TβR-I contains a site for interaction with downstream components that transduces a signal specific for growth inhibition. Alternatively, such a deletion might change the structural conformation, yielding a receptor that is unable to transduce signals even if the substrate interaction sites were preserved. To address these questions, missense mutations instead of deletion were introduced into certain serine and threonine residues in the TβR-I juxtamembrane region that was deleted in TβR-I/JD1 (Δ150–181). As an initial attempt, serine 165, serine 172, and threonine 176 were chosen because these serine and threonine residues were rather conserved among the type I receptors for the TGF-β superfamily (Fig. 1), especially in ActR-I/IB, which transduces growth inhibition and PAI-1 signals by activin A (28). Serine and threonine residues were mutated simultaneously or individually to alanine and valine residues, respectively, resulting in four different expression constructs including TβR-I/JM1 (M1/S165A/S172A/T176V), TβR-I/JM2 (M1/S165A), TβR-I/JM3 (S172A), and TβR-I/JM3 (T176V). These constructs were stably transfected into R4–2 cells, and their expression, TGF-β1 binding, and physical association with TβR-II were examined by affinity cross-linking with 125I-TGF-β1 followed by immunoprecipitation using anti-TβR-II antisera (Fig. 2). All the different receptor mutants were expressed on the cell surface and bound TGF-β1 in complex with TβR-II in a Zn2+-dependent manner.

To test the signaling activities of these missense mutant forms of TβR-I, the transfected cells were subjected to the analyses for extracellular matrix production and growth inhibition by TGF-β1. In PAI-1 and fibronectin assays, as wild-type TβR-I, all the constructs analyzed including TβR-I/JM1 (M1/S165A/S172A/T176V), TβR-I/JM1 (M1/S165A), TβR-I/JM2 (S172A), and TβR-I/JM3 (T176V) restored responsiveness to TGF-β1 (Fig. 3, A and B). With regard to TGF-β1 antiproliferative effect, the TβR-I/JM1 (S165A) construct mediated a growth inhibitory effect comparable with that mediated by the wild-type TβR-I, whereas TβR-I/JM2 (S172A), and TβR-I/JM3 (T176V) were unable to restore this activity (Fig. 4B). More than five different clones for TβR-I/JM1 (S165A), TβR-I/JM2 (S172A), and TβR-I/JM3 (T176V) were subjected to the growth inhibition assay, which gave essentially the same results (data not shown).

Kinase Activity of TβR-I and Its Mutant Derivatives in Vitro—The differences among TβR-I and its mutant derivatives in their ability to restore responsiveness to TGF-β1 might

To evaluate whether TβR-I/JD1 (Δ150–181) is able to restore TGF-β1 antiproliferative effect, DNA synthesis assay was performed by measuring the incorporation of [3H]thymidine into the DNA (Fig. 4, A and B). Upon treatment with TGF-β1, [3H]thymidine incorporation into the DNA of Mv1Lu cells was inhibited dose-dependently up to 97% (Fig. 4A), whereas TGF-β1 had no effect on the [3H]thymidine incorporation in the R4–2 cells transfected with the vector alone. When R4–2 cells transfected with the wild-type TβR-I were treated with TGF-β1 in the presence of ZnCl2, [3H]thymidine incorporation into the DNA was inhibited by 65–75%, whereas only a marginal inhibition was observed in the absence of ZnCl2. In contrast, R4–2 cells transfected with TβR-I/JD1 (Δ150–181) were refractory to TGF-β1 growth inhibition in the presence or the absence of ZnCl2 (Fig. 4, A and B). These results suggested that the N-terminal half of the cytoplasmic juxtamembrane domain of TβR-I was not required for signaling a PAI-1 response, whereas it was essential for signaling growth inhibitory activity.

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Kinase Activity of TβR-I and Its Mutant Derivatives in Vitro—The differences among TβR-I and its mutant derivatives in their ability to restore responsiveness to TGF-β1 might
be due to altered catalytic activity of their receptor kinase. To address this issue, kinase activity was determined by expressing the cytoplasmic regions of TβR-I in mammalian cells, like wild-type TβR-I, in the absence of ligand. The TβR-I kinase appears to be activated by formation of a hetero-oligomeric complex composed of TGF-β, TβR-II, and TβR-I. In the complex, several serine and threonine residues in the GS domain of TβR-I become phosphorylated by TβR-II, and the phosphorylation of GS domain is essential for TGF-β signaling (22, 26, 27); however, the functional role of phosphorylated serine and threonine residues in the GS domain as well as the mechanism of signaling after the phosphorylation are largely unknown. In addition, functional importance of the TβR-I cytoplasmic region other than the GS domain remains to be elucidated.

In the present communication, we studied the role of the N-terminally flanking region of the TβR-I GS domain by mutating this region and testing its ability to restore the signaling activity in TβR-I-defective R4–2 cells. When expressed in R4–2 cells, like wild-type TβR-I, the deletion mutant TβR-I/ J D1(Δ150–181) and other missense mutants including TβR-I/ J M123(S165A/S172A/T176V), TβR-I/J M1(S165A), and TβR-I/ J M2(S172A), and TβR-I/J M3(T176V) were all cross-linked with radioiodinated TGF-β and co-immunoprecipitated with TβR-II (Fig. 2), indicating that this region in TβR-I is dispensable at least for its expression and binding to TGF-β on the cell surface and forming a complex with TβR-II.

The signaling activities of these mutant TβR-I constructs were tested for some of the most characteristic responses to TGF-β; i.e., PAI-1 and fibronectin induction and growth inhibition. Wild-type TβR-I and all the missense mutants restored PAI-1 and fibronectin responses in R4–2 cells (Fig. 3, A and B), indicating that serine 165, serine 172, and threonine 176 of TβR-I are not needed to transduce a signal for PAI-1 and fibronectin induction.

Antiproliferative response was also restored by the wild-type TβR-I and one of the receptor mutants, TβR-I/J M1(S165A); however, the other mutants including TβR-I/J D1(Δ150–181), TβR-I/J M123(S165A/S172A/T176V), TβR-I/J M2(S172A), and TβR-I/J M3(T176V) were unable to rescue this response (Fig. 4, A and B). Because the wild-type TβR-I and all the mutant TβR-I were similar in their activities to bind TGF-β, form a complex with TβR-II, and phosphorylate themselves in vitro, the differences in their ability to restore the antiproliferative response does not seem to be at the level of ligand-receptor complex formation or basal kinase activity. Rather, TβR-I/J M123(S165A/S172A/T176V), TβR-I/J M2(S172A), and TβR-I/J M3(T176V) are likely to be impaired in interacting with a specific substrate that transduces antiproliferative response but not PAI-1 and fibronectin responses.

From our present data, it is not easy to deduce the mechanistic significance of serine 172 and threonine 176 of TβR-I in TGF-β signaling. Although it was reported that TGF-β-induced phosphorylation of these residues was not detected in vivo (22), it is still possible that TβR-II may phosphorylate these residues as minor phosphorylation site(s). Alternatively, these residues might be constitutively phosphorylated even in the absence of TGF-β, which would not be detected as the ligand-induced
phosphorylation sites. It is also possible that serine 172 and threonine 176 in TβR-I may not be themselves phosphorylated, but their integrity is essential to maintain the proper conformation of TβR-I to interact with its substrates.

It was reported that whereas Mv1Lu cells expressing SV40 T-antigen were refractory to the antiproliferative effect of TGF-β, TGF-β induced the expressions of junB mRNA and extracellular matrix proteins including PAI-1, fibronectin, and thrombospondin in these cells (34, 35). In addition, we have previously shown that growth inhibition and extracellular matrix production by TGF-β are sensitive and insensitive, respectively, to phorbol 12-myristate 13-acetate in prostatic carcinoma cells (36). These observations have suggested that the signals induced by TGF-β that lead to growth inhibition and to extracellular matrix production should differ at a certain step within the signaling cascade from the receptor to the nucleus. In this regard, the present study is of particular importance.

TβR-I mutants including TβR-I/JM123(S165A/S172A/T176V), TβR-I/JM2(S172A), and TβR-I/JM3(T176V) had signaling activity for extracellular matrix protein responses but not growth inhibition. Although other responses including expressions of junB and thrombospondin should be determined, identification of such mutant forms of TβR-I strongly suggests that the signal of TβR-I to interact with its substrates.

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nals for growth inhibition and extracellular matrix production are diverged closely at the receptor level. In conclusion, serine 172 and threonine 176 within the TβR-I juxtamembrane region preceding the GS domain are essential for signaling the TGF-β antiproliferative response and might be involved in the interaction with the downstream substrate responsible for growth inhibition.

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REFERENCES

1. Lyons, R. M., and Moses, H. L. (1990) Eur. J. Biochem. 187, 467–473
2. Sporn, M. B., and Roberts, A. B. (1992) J. Biol. Chem. 267, 1017–1021
3. Massagué, J., Attisano, L., and Wrana, J. L. (1994) Trends Cell Biol. 4, 172–178
4. Miyazono, K., ten Dijke, P., Ichijo, H., and Heldin, C.-H. (1994) Adv. Immunol. 55, 181–220
5. Attisano, L., Cárcamo, J., Ventura, F., Wei, F. M. B., Massagué, J., and Wrana, J. L. (1993) Cell 75, 671–680
6. Bassing, C. H., Yingling, J. M., Howe, D. J., Wang, T., He, W. W., Guatadson, M. L., Shah, P., Donahoe, P. K., and Wang, X.-F. (1994) Science 263, 87–89
7. Ebner, R., Chen, R.-H., Lawler, S., Zoncheck, T., and Derynck, R. (1993) Science 262, 900–902
8. Franzen, P., ten Dijke, P., Ichihi, H., Yamashita, H., Schulz, P., Heldin, C.-H., and Miyazono, K. (1993) Cell 75, 681–692
9. Lin, H. Y., and Lodish, H. F. (1993) Trends Cell Biol. 3, 14–19
10. Attisano, L., Wrana, J. L., López-Casillas, F., and Massagué, J. (1994) Biochim. Biophys. Acta 1222, 71–80
11. Kingsley, D. M. (1994) Genes & Dev. 8, 133–146
12. ten Dijke, P., Franzen, P., Yamashita, H., Ichijo, H., Heldin, C.-H., and Miyazono, K. (1994) Prog. Growth Factor Res. 5, 55–72
13. Ebner, R., Chen, R.-H., Shum, L., Lawler, S., Zoncheck, T. F., Lee, A., Lopez, A. R., and Derynck, R. (1993) Science 260, 1344–1348
14. He, W. W., Gustafson, M. L., Hirobe, S., and Donahoe, P. K. (1993) Dev. Dyn. 196, 133–142
15. ten Dijke, P., Ichijo, H., Franzen, P., Schulz, P., Saras, J., Toyoshima, H., Heldin, C.-H., and Miyazono, K. (1993) Oncogene 8, 2879–2887
16. Köning, B. B., Cook, J. S., Wolsing, D. H., Ting, J., Treece, J. P., Correa, P. E., Olson, C. A., Pecquet, A. L., Ventura, F., Grant, R. A., Chen, G.-X., Wrana, J. L., Massagué, J., and Rosenbaum, J. S. (1994) Mol. Cell. Biol. 14, 5961–5974
17. Matsuzaki, K., Xu, J., Wang, F., McKeehan, W. L., Krummen, L., and Kan, M. (1993) J. Biol. Chem. 268, 12719–12723
18. Suzuki, A., Thies, R. S., Yamei, N., Song, J. J., Wozney, J. M., Murakami, K., and Ueno, N. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 10225–10229
19. ten Dijke, P., Yamashita, H., Ichijo, H., Franzen, P., Laiho, M., Miyazono, K., and Heldin, C.-H. (1994) Science 264, 101–104
20. Tsuchida, K., Mathews, L. S., and Vale, W. W. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 11242–11246
21. Ventura, F., Doody, J., Liu, F., Wrana, J. L., and Massagué, J. (1994) EMBO J. 13, 5581–5589
22. Wrana, J. L., Attisano, L., Wieser, R., Ventura, F., and Massagué, J. (1994) Nature 370, 341–347
23. Cárcamo, J., Zentella, A., and Massagué, J. (1995) Mol. Cell. Biol. 15, 1573–1581
24. Wrana, R., Attisano, L., Wrana, J. L., and Massagué, J. (1993) Mol. Cell. Biol. 13, 7239–7247
25. Wrana, J. L., Attisano, L., Cárcamo, J., Zentella, A., Doody, J., Laiho, M., Wang, X.-F., and Massagué, J. (1992) Cell 71, 1003–1014
26. Franzen, P., Heldin, C.-H., and Miyazono, K. (1995) Biochem. Biophys. Res. Commun. 207, 682–689
27. Wieser, R., Wrana, J. L., and Massagué, J. (1995) EMBO J. 14, 2199–2208
28. Cárcamo, J., Wei, F. M. B., Ventura, F., Wieser, R., Wrana, J. L., Attisano, L., and Massagué, J. (1994) Mol. Cell. Biol. 14, 3810–3821
29. Laiho, M., Wei, F. M. B., and Massagué, J. (1996) J. Biol. Chem. 265, 18518–18524
30. Laiho, M., Wei, F. M. B., Boyd, F. T., Ignatza, R., and Massagué, J. (1991) J. Biol. Chem. 266, 9108–9112
31. Frolik, C. A., Wakefield, L. M., Smith, D. M., and Sporn, M. B. (1984) J. Biol. Chem. 259, 10995–11000
32. Ohno, M., and Massagué, J. (1992) Mol. Cell. Biol. 12, 261–265
33. Boyd, F. T., and Massagué, J. (1989) J. Biol. Chem. 264, 2272–2278
34. Laiho, M., Rönnstrand, L., Heino, J., Decaprio, J., A., Ludlow, J. W., Livingston, D. M., and Massagué, J. (1991) Mol. Cell. Biol. 11, 972–978
35. Zentella, A., Wei, F. M. B., Ralph, D. A., Laiho, M., and Massagué, J. (1991) Mol. Cell. Biol. 11, 4952–4958
36. Franzen, P., Ichijo, H., and Miyazono, K. (1993) Exp. Cell Res. 207, 1–7