Ski-interacting Protein Interacts with Smad Proteins to Augment Transforming Growth Factor-β-dependent Transcription*

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Gary M. Leong‡§, Nanthakumar Subramaniam‡, Jonine Figueroa¶, Judith L. Flanagan∥, Michael J. Hayman¶, John A. Eisman‡, and Alexander P. Kouzmenko‡

From the §Bone & Mineral Research Program, Garvan Institute of Medical Research, Darlinghurst, New South Wales 2010, Australia and the ¶Department of Molecular Genetics & Microbiology, State University of New York, Stony Brook, New York 11794

Transforming growth factor-β (TGF-β) signaling requires the action of Smad proteins in association with other DNA-binding factors and coactivator and corepressor proteins to modulate target gene transcription. Smad2 and Smad3 both associate with the c-Ski and Sno oncoproteins to repress transcription of Smad target genes via recruitment of a nuclear corepressor complex. Ski-interacting protein (SKIP), a nuclear hormone receptor coactivator, was examined as a possible modulator of transcriptional regulation of the TGF-β-responsive promoter from the plasminogen activator inhibitor gene-1. SKIP augmented TGF-β-dependent transactivation in contrast to Ski/Sno-dependent repression of this reporter. SKIP interacted with Smad2 and Smad3 proteins in vitro in yeast and in mammalian cells through a region of SKIP between amino acids 201–333. In vitro, deletion of the Mad homology domain 2 (MH2) domain of Smad3 abrogated SKIP binding, like Ski/Sno, but the MH2 domain of Smad3 alone was not sufficient for protein-protein interaction. Overexpression of SKIP partially overcame Ski/Sno-dependent repression, whereas Ski/Sno overexpression attenuated SKIP augmentation of TGF-β-dependent transcription. Our results suggest a potential mechanism for transcriptional control of TGF-β signaling that involves the opposing and competitive actions of SKIP and Smad MH2-interacting factors, such as Ski and/or Sno. Thus, SKIP appears to modulate both TGF-β and nuclear hormone receptor signaling pathways.

Transforming growth factor-β (TGF-β) superfamily members are multifunctional cell-cell signaling proteins, which include the TGF-βs, bone-morphogenetic proteins (BMPs), activins, and inhibins, mullerian-inhibiting substance and growth differentiation factors (1). Members of this superfamly mediate many key cellular events in growth and development and are evolutionarily conserved from Drosophila to mammals (2). TGF-β signaling requires the action of a family of DNA-binding proteins called Smads, including TGF-β-specific (Smad2 and Smad3), BMP-specific (Smad1, Smad5 and Smad8), a common Smad4, and anti-Smads (Smad6 and Smad7). TGF-β signals through sequential activation of two cell surface receptor serine-threonine kinases, which phosphorylate Smad2 and/or Smad3. Phosphorylated Smad2 or Smad3, together with Smad4, translocates into the nucleus where the Smad heterodimer binds Smad-binding elements (SBEs) in association with other nuclear factors in promoters of target genes (1, 3, 4).

Recently it has been shown that Smad proteins also interact with other nuclear factors such as c-Ski and the Ski-related novel (Sno) protein and nuclear hormone receptors, including the vitamin D receptor (VDR) to modulate TGF-β signaling (5–10). Ski and Sno are involved in oncogenic transformation and enhancement of muscle differentiation by blocking TGF-β signaling (11–14). The mechanism of Ski/Sno repression of TGF-β signaling appears to involve an interaction with a complex consisting of the nuclear corepressor (N-CoR) and a histone deacetylase enzyme (15, 16). N-CoR, and its related co-repressor silencing mediator for retinoic acid and thyroid receptors (SMRT), interact with a wide variety of other nuclear factors to mediate transcriptional repression (17–19). Interestingly, the Ski-interacting protein (SKIP) was initially identified in a two hybrid screen using v-Ski as a bait and was later independently identified as a VDR- and CBF1-interacting factor (20–22). Thus, the recent observation that SKIP modulates CBF1 and Notch-dependent signaling suggests that SKIP may play a role in the regulation of a number of different and distinct cellular signaling pathways (23).

As Ski and Sno can modulate TGF-β-dependent signaling, it was of interest to determine whether SKIP could also modulate the TGF-β-signaling pathway through interaction with the Smad proteins. In this study, in contrast to Ski- and Sno-mediated repression, SKIP augmented TGF-β-dependent transcription. A region of SKIP, aa 201–333, appeared to be required for the Smad interaction. SKIP interacted in vitro and in vivo with Smad3 and partially counteracted Ski- and Sno-dependent repression, while Ski/Sno attenuated SKIP transactivation of TGF-β signaling. These results suggest that SKIP may play an opposite role to Ski and Sno in the control of TGF-β-dependent transcription.

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Plasmid Constructs—SKIP wild-type cDNA was PCR cloned with the forward primer (5′-GGG AAT TCC CGG CTT GTA CAA CCA CCA CGG TGG CGC TCA CCA GCT TTT TTA-3′) and reverse primer (5′-GGG GGG TCC TGA CTT CTC CCT CTT CTT CTT-3′). The PCR product was ligated into pGEM-T Easy plasmid (Promega, Madison, WI) from which an EcoRI/BamHI insert was excised and subcloned into a modified GAL4AD pACTII plasmid (pACTIIb) and the vector pBSGS (Stratagene, La Jolla, CA). The pACTIIb plasmid was created by replacing the BglII polylinker fragment of pACTII (CLONTECH, Palo Alto, CA) with the double-stranded oligonucleotide: 5′-GAT CTG TGA ATT CCC GGG-3′ and 5′-GAT CCG TCG ACC TA-3′. The pBSGS plasmid was amplified by PCR using the forward primer 5′-CCT ATT TTC CAT TTC CAT TTT TG-3′ and reverse primer 5′-CGC TCA CCA GCT TTT TA-3′. Deletional analysis of the skipping domain was performed (CLONTECH Yeast Handbook, PT3024-1). Yeast ligand expression was performed in 24-well plates with the 3TP-lux reporter (250 ng/well) and the following expression plasmids as indicated: SKIP-pCGN and Smad2-, Smad3-, and Smad4-pcDNA3. The 3TP-Lux reporter contains three clusters of Smad-binding elements from the PAI-1 promoter and is predominantly a Smad3-responsive promoter reporter. Cells were treated with vehicle (□) or TGF-β ligand (solid square) (1 ng/ml). The total amount of transfected DNA was kept constant by use of respective empty vectors. The results are shown as the mean ± S.E. of three independent experiments performed in triplicate.

FIG. 1. SKIP augments TGF-β-dependent transactivation in mammalian cells. A, transient transfections of COS1 cells were performed in 24-well plates with the 3TP-lux reporter (250 ng/well) and the following expression plasmids as indicated: SKIP-pCGN and Smad2-, Smad3-, and Smad4-pcDNA3. The 3TP-Lux reporter contains three clusters of Smad-binding elements from the PAI-1 promoter and is predominantly a Smad3-responsive promoter reporter. Cells were treated with vehicle (□) or TGF-β ligand (solid square) (1 ng/ml). The total amount of transfected DNA was kept constant by use of respective empty vectors. The results are shown as the mean ± S.E. of three independent experiments performed in triplicate. B, SKIP interacts in vivo with Smad2 and Smad3 in a yeast two hybrid system through a domain between 201 and 333 aa. Deletional analysis of SKIP-GAL4-AD fusion proteins was performed as described previously (28). 35S-Galactosidase activity in protein lysates was measured with the Tropix Galactolight chemiluminescence assay (PerkinElmer Life Sciences) using the Promega lysis buffer. Luciferase assays were performed in triplicate with the firefly luciferase assay kit (Promega) and measured with a luminometer (Berthold). The pACTIIb plasmid was amplified by PCR using the forward primer 5′-GAT CTG TGA ATT CCC GGG-3′ and reverse primer 5′-GAT CCG TCG ACC TA-3′. Deletional analysis of the skipping domain was performed (CLONTECH Yeast Handbook, PT3024-1). Yeast ligand expression was performed in 24-well plates with the 3TP-lux reporter (250 ng/well) and the following expression plasmids as indicated: SKIP-pCGN and Smad2-, Smad3-, and Smad4-pcDNA3. The 3TP-Lux reporter contains three clusters of Smad-binding elements from the PAI-1 promoter and is predominantly a Smad3-responsive promoter reporter. Cells were treated with vehicle (□) or TGF-β ligand (solid square) (1 ng/ml). The total amount of transfected DNA was kept constant by use of respective empty vectors. The results are shown as the mean ± S.E. of three independent experiments performed in triplicate.

RESULTS

SKIP Augments TGF-β-dependent Transcription—As Ski and Sno interact directly with Smad proteins (Smad2 and Smad3) to repress TGF-β-dependent transcription, the effects
of SKIP on the 3TP-lux TGF-β-responsive reporter construct (27) were tested (Fig. 1). In COS1 cells this reporter responded to TGF-β with a 4-fold increase in reporter activity, consistent with these cells expressing endogenous Smad proteins (30). Smad3 alone, or Smad2 and Smad4 together (but neither alone) augmented both basal (2-fold) and TGF-β responses (10-fold) of this reporter activity. Smad3 co-transfection with Smad4 led to a 6-fold increase in basal and a 30-fold increase in ligand-dependent reporter activity. This augmentation was similar to that of SKIP alone on ligand-dependent reporter activity (Fig. 1). An interaction between SKIP and Smads was suggested in co-transfection studies, with the -fold increase of basal activity progressively increasing when SKIP was co-transfected with Smad2 (8-fold), Smad2 and 4 (20-fold), Smad3 (53-fold), and Smad3 and Smad4 (164-fold). The comparable increases in TGF-β induced activity were 39-, 116-, 96-, and 323-fold, respectively. These data are consistent with a functional interaction primarily occurring between SKIP and Smad3, with or without exogenous Smad4.

Mapping of SKIP-Smad Interaction Domains in Yeast and Mammalian Cells—SKIP interaction with Smad proteins was investigated by yeast two-hybrid interaction analysis. SKIP interacted with both Smad2 and Smad3 (Fig. 1B). Smad4 induced a high level of reporter activity, which was unaltered by co-expression of SKIP. However, Smad4, as expected, interacted strongly with v-Ski-GAL4-AD in yeast (data not shown). Domains of SKIP required for Smad interaction were examined using deletion constructs of SKIP (Fig. 1B). The C-terminally deleted aa 1–333 and N-terminally deleted aa 201–536 SKIP mutants had interaction with Smad2 comparable to that of wild-type SKIP. The interaction between these two mutants and Smad3 were about 50% and 25% of wild-type SKIP, respectively. No interaction of the SKIP N-terminal (aa 1–200) or C-terminal (aa 334–536) domain with Smad2 or Smad3 was observed. Thus, these results suggest that the region of SKIP between aa 201 and 333 interacts with Smad2 and Smad3.

The same SKIP deletion constructs were tested with the 3TP-lux reporter in the COS1 mammalian cell line (Fig. 1C). Co-expression of wild-type SKIP (aa 1–536) with Smad3 caused a synergistic 3-fold increase in reporter activity above SKIP or Smad3 alone. The N-terminal domain of SKIP (aa 1–200) had no effect on reporter activity, while the other SKIP constructs had comparable transactivation to that of wild-type SKIP. Western blot analysis of these deletion constructs showed comparable expression with wild-type, except for the aa 1–200 construct, which, despite its lack of transactivation, was 2–3 times more highly expressed (data not shown). Thus, these transfection data were consistent with the yeast interaction data and suggest that expression of the aa 201–333 region of SKIP with Smad3 is sufficient for near maximal transactivation of the 3TP-lux reporter. Surprisingly, the aa 334–536 SKIP construct was able to activate the 3TP-lux reporter with Smad3, even though no interaction was observed with Smad3 in yeast. This suggests that an additional C-terminal domain may also be transcriptionally functional and possibly recruits other Smad-interacting co-factors present in mammalian cells, but not yeast.

SKIP Interaction with Smad2 and Smad3 in Vitro—The potential direct physical interaction between the Smad proteins and SKIP was explored using a GST "pull-down" assay. GST-SKIP bound both Smad2 and Smad3 (Fig. 2A). In comparison there was minimal, if any, binding of Smad2 or Smad3 to GST-0 and no binding of luciferase to GST-SKIP.

To determine which domains of Smad3 may be involved in SKIP interaction, a GST-Smad3 binding assay was performed with 35S-labeled in vitro translated SKIP (Fig. 2B). GST-wild-type Smad3 bound SKIP and the positive control VDR (9). Deletion of the MH1 domain of Smad3 (aa 199–427) had no effect on SKIP binding, but, as expected, VDR binding was abolished. Both SKIP and VDR binding was lost when both the MH1 and MH2 domains of Smad3 were deleted (GST-Smad3 aa 199–405). However, no binding of SKIP was observed to a GST-MH2 construct, which expressed only the last 26 aa of hSmad3. This result was further supported by a lack of interaction between SKIP-GAL4-AD and a MH2-GAL4-DBD construct containing the same C-terminal 26 aa of hSmad3 in vitro in yeast (data not shown). These results indicate that, although deletion of the C-terminal MH2 domain abrogates SKIP binding, the MH2 domain alone is not sufficient for SKIP interaction.

To further support the existence of a direct protein-protein interaction in vitro, a Far Western assay was performed using mammalian cell nuclear extracts overexpressing HA-SKIP (upper panel) or Smad3 (middle panel) (Fig. 2C). In the Far Western analysis (lower panel), Smad3 detected by Western analysis anti-HA antibody detected a band co-migrating with Smad3, but not the negative control extract.
FIG. 3. SKIP augments TGF-β-dependent transcription and partially counters Ski- and Sno-dependent repression. Transient transfections of COS1 cells were performed with the 3TP-lux reporter (250 ng), Smad3-pcDNA3 (100 ng), and increasing amounts of wild-type SKIP-pCGN as indicated, with either c-Ski-pMT2 (A, 100 ng) or Sno-pCGN (B, 100 ng) expression plasmids. Cells were treated with vehicle (□) or TGF-β ligand (■) (1 ng/ml). The results are shown as the mean luciferase activity ± S.E. of triplicate wells relative to ligand-dependent reporter activity of Smad3 transfection alone set at 1, and are representative of three independent experiments.

As Ski/Sno interact with both Smad3 and SKIP, one alternative possibility other than a competitive interaction between these proteins is that they form a ternary complex. To address this question, a gel shift analysis was performed (Fig. 5). Using the PE-2 probe from the PAI-1 promoter, which binds a Smad3/4 complex, which presumably contained SKIP and Smad3/4 (lanes 3–6). The addition of Sno nuclear extracts also led to increased Smad3/4 binding with a similar mobility shift (lanes 7–10). Similar results were obtained using Ski-overexpressing nuclear extracts (data not shown).

FIG. 4. Ski and Sno attenuates SKIP augmentation of TGF-β-dependent transcription. Transient transfections of COS1 cells were performed with the 3TP-lux reporter (250 ng) and Smad3-pcDNA3 (100 ng), wild-type SKIP-pCGN (50 ng), and increasing amounts of either c-Ski-pMT2 (A) or Sno-pCGN (B) expression plasmids as indicated. Cells were treated with vehicle (□) or TGF-β ligand (■) (1 ng/ml). The results are shown as the mean luciferase activity ± S.E. of triplicate wells relative to maximal ligand-dependent reporter activity with SKIP and Smad3 co-transfection set at 10, and are representative of three independent experiments.

7–10). Similar results were obtained using Ski-overexpressing nuclear extracts (data not shown).

DISCUSSION

The Ski and Sno oncoproteins have been shown to negatively modulate TGF-β signaling through an interaction with a N-CoR repressor complex (15). As SKIP, a nuclear hormone receptor-interacting cofactor, also associates with both Ski and Sno, this study was undertaken to determine the potential role of SKIP in TGF-β signaling. In these studies SKIP augmented TGF-β-dependent transcription and exhibited a direct interaction with Smad proteins. This SKIP-Smad interaction was apparent both in vitro and in vivo, as demonstrated by GST pull-down assays, Far Western analysis, and yeast two hybrid protein-protein studies. The region between aa 201 and 333 within SKIP appeared to act as the Smad-interacting domain, while, SKIP, like Ski and Sno, interacted with the MH2 domain of Smad3. Moreover, Ski and Sno attenuated SKIP transactivation, while SKIP partially counteracted Ski- and Sno-mediated transcriptional repression.
The C-terminal MH2 domain of Smad2 and Smad3 has been reported to be a key region involved in multiple protein-protein interactions, including those with the coregulators CBP/p300 and the Smad repressors Ski and Sno (1). The N-terminal MH1 domain of the Smads confers only low affinity DNA binding to a consensus SBE (1). Although natural TGF-β-responsive promoters contain functional clusters of SBEs, other DNA-binding factors, such as FAST-1, TFE3, and AP-1, as well as non-DNA binding factors through protein-protein interaction with the C-terminal MH1-2 domain, are involved in determining the specificity and direction of Smad target gene action (29, 31, 32).

As such, SKIP appears to play a role in augmentation of TGF-β-specific Smad transcriptional activity via an interaction with the MH2 domain of Smad3. Our data also suggest, as SKIP was unable to interact with an isolated MH2 domain (last 26 aa) of Smad3 in GST binding assays and yeast two-hybrid studies, that other regions within the Smad proteins possibly within the context of the whole Smad protein may also modulate Smad-Skip interaction.

Although SKIP was able to interact with Smad2 and Smad3 in yeast, SKIP co-transfection with Smad3 with or without Smad4, led to the greatest increases in reporter activity in mammalian cells, presumably because the 3TP-lux reporter is Smad3-selective (27). Thus, as SKIP interacted with Smad2 in vivo and in vitro, it is also possible that SKIP in mammalian cells may be able to modulate TGF-β signaling through Smad2 in certain situations (30). Furthermore, in the transient transfections we observed that Smad3 augmented basal reporter activity, as previously described with this promoter (5), but this activity was further increased by SKIP. Further studies will be required to address the specific reasons for this effect of SKIP.

The deletional analysis of SKIP in yeast and mammalian cells suggests that the aa 201–333 region of SKIP is required for Smad interactions in vivo. However, some functional differences were observed between yeast and mammalian cells. Specifically, although the C-terminal SKIP construct (aa 334–536) did not interact with Smad2 or Smad3 in yeast, its transactivation activity in mammalian cells was comparable to wild-type SKIP. A C-terminal transactivation domain of SKIP that functions in mammalian cells, distinct from the Smad interaction domain, is consistent with the domain C-terminal to aa 437 of murine SKIP (NcoA-62) being involved in vitamin D-dependent transactivation (21).

As SKIP interacts with Ski/Sno and Smad3 and, in turn, Ski/Sno interact with Smad3, to address the possibility that these proteins form a ternary complex, we performed a gel shift analysis using the PE2 probe from the PAI-1 promoter, as used in the transient transfections. The EMSA clearly showed that both SKIP and Ski/Sno alone formed a slightly higher migrating complex with Smad3/4. However, we did not observe the formation of a ternary complex in the presence of all three proteins. Thus, these data are consistent with the transient transfection results, which suggests competition occurring between SKIP and Ski/Sno for Smad3 transactivation, but cannot exclude the presence of a ternary complex forming between these proteins.

In this study SKIP acted as a coactivator of TGF-β-dependent transcription. SKIP similarly acts as a coactivator of nuclear hormone receptor-dependent transcription, but also as a repressor of Notch signaling through its interaction with SMRT and associated histone deacetylase enzyme proteins (21, 23). These divergent effects of SKIP may depend on interaction of SKIP with other, possibly cell-specific nuclear factors. For example, SKIP converts CBP1 from a transcriptional repressor to activator through switching its interaction between the corepressor SMRT and Notch 1C (23). In our study SKIP and Ski/Sno modulated each other’s opposing transcriptional activities, raising the intriguing possibility that the relative cellular expression of SKIP versus Ski or Sno may play a regulatory role on TGF-β-dependent transcription and hence its effects on cell growth and differentiation. Interestingly, the Smad-interacting domain of SKIP (aa 201–333) appears to be also involved in interaction with Ski and Sno.2 These results and those showing that SKIP, like Ski/Sno, interacted with the MH2 domain of Smad3 suggest that the opposing transcriptional effects of SKIP and Ski/Sno may involve competition for Smad3 binding between SKIP and c-Ski/Sno, and/or other Smad3 MH2-interacting factors, such as with CBP/p300 (7, 33, 34). Thus, the modulatory effects of SKIP through the MH2 domain potentially increase the complexity and diversity of Smad-dependent transcriptional effects. Furthermore, as SKIP and Ski/Sno interact with each other and also with the related corepressors N-CoR/SMRT, an additional mechanism could involve SKIP-mediated derepression (1, 15, 20, 23). This may possibly occur via SKIP sequestration of corepressors such as SMRT or N-CoR from the Ski/Sno repressor complex, a mechanism similar to that suggested for Hoxc-8 and Smad1 (35). Whatever the molecular mechanism of SKIP action, it is nevertheless clear that SKIP plays a role in modulation of this important cellular and signaling pathway.

In summary, our results support a model in which SKIP positively modulates TGF-β-dependent transcription and potentially competes with other MH2-interacting factors, such as c-Ski and Sno, to determine the transcriptional outcome of a TGF-β-responsive target gene. This suggests a potential role for SKIP in the regulation of TGF-β effects on cell growth and differentiation.

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REFERENCES

1. Massague, J., and Wotton, D. (2000) EMBO J. 19, 1745–1754
2. Whitman, M. (1998) Genes Dev. 12, 2445–2462
3. Nakao, A., Imamura, T., Soucelhatskyi, S., Kawabata, M., Ishikawa, A., Oeda, E., Tamaki, K., Hanai, J., Heldin, C. H., Miyazono, K., and Tendjikpe, P. (1997) EMBO J. 16, 5353–5362
4. Wrana, J. L. (2000) Cell 100, 189–192
5. Sun, Y., Liu, X. D., Eaton, E. N., Lane, W. S., Lodish, H. F., and Weinberg, R. A. (1999) Mol. Cell. 4, 499–509
6. Stroschein, S. L., Wang, W., Zhou, S. L., Zhou, Q., and Luo, K. X. (1999) Science 286, 711–714
7. Akiyoshi, S., Inoue, H., Hanai, J., Kusunagi, K., Nenoto, N., Miyazono, K., and Kawabata, M. (1999) J. Biol. Chem. 274, 35269–35277
8. Xu, W. D., Angelis, K., Daniel pour, D., Haddad, M. M., Bischof, O., Campisi, J., Stavnezer, E., and Medrano, E. E. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 5924–5929
9. Yamagawara, J., Yanagi, Y., Masuhiro, Y., Suzawa, M., Watanabe, M., Kushiwagi, K., Toriyabe, T., Kawabata, M., Miyazono, K., and Kato, S. (1999) Science 283, 1317–1321
10. Subramaniam, N., Leong, G. M., Cook, T. A., Flanagan, J. L., Gong, C., Eisman, J. A., and Kozumpenko, A. P. (2001) J. Biol. Chem. 276, 15741–15746
11. Colmenares, C., and Stavnezer, E. (1999) Cell 99, 293–303
12. Berk, M., Desai, S. Y., Heyman, H. C., and Colmenares, C. (1997) Genes Dev. 11, 2629–39
13. Sut rave, P., Kelly, A. M., and Hughes, S. H. (1990) Genes Dev. 4, 1462–72
14. Colmenares, C., Sut rave, P., Hughes, S. H., and Stavnezer, E. (1991) J. Virol. 65, 4929–35
15. Luo, K. X., Sut rave, S. L., Wang, W., Chen, D., Martin, E., Zhou, S., and Zhou, Q. (1999) Genes Dev. 13, 2196–2206
16. Nomura, T., Khan, M. M., Kaul, S. C., Dong, H. D., Waddington, R., Colmenares, C., Kohn, I., and Ichii, S. (1999) Genes Dev. 13, 413–423
17. Horlein, A. J., Naar, A. M., Heinzel, T., Torchia, J., Goss, B., Kurokawa, R., Ryan, A., Kamel, Y., Soderstrom, M., Glass, C. K., and Rosenfeld, M. G. (1995) Nature 377, 397–404
18. Chen, J. D., and Evans, R. M. (1995) Nature 377, 454–457
19. Burke, L. J., and Banaimahad, A. (1876) FASEB J. 14, 1876–1888

2 J. Figueroa and M. J. Hayman, unpublished observations.
20. Dahl, R., Wani, B., and Hayman, M. J. (1998) *Oncogene* **16**, 1579–1586
21. Baudino, T. A., Kraichely, D. M., Jefcoat, S. C., Winchester, S. K., Partridge, N. C., and Macdonald, P. N. (1998) *J. Biol. Chem.* **273**, 16434–16441
22. Zhou, S. F., Fujimuro, M., Hsieh, J. J. D., Chen, L., and Hayward, S. D. (1999) *J. Virol.* **74**, 1939–1947
23. Zhou, S. F., Fujimuro, M., Hsieh, J. J. D., Chen, L., Miyamoto, A., Weinmaster, G., and Hayward, S. D. (2000) *Mol. Cell. Biol.* **20**, 2409–2410
24. Guan, K. L., and Dixon, J. E. (1991) *Anal. Biochem.* **192**, 262–267
25. Dahl, R., Kieslinger, M., Beug, H., and Hayman, M. J. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95**, 11187–11192
26. Sun, Y., Liu, X. D., Ng-Eaton, E., Lodish, H. F., and Weinberg, R. A. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96**, 12442–12447
27. Carcamo, J., Zentella, A., and Massague, J. (1995) *Mol. Cell. Biol.* **15**, 1573–1581
28. Leong, G. M., Wang, K. S., Marten, M. J., Blanco, J. C. G., Wang, I. M., Rolfs, R. J., Ozato, K., and Segars, J. H. (1998) *J. Biol. Chem.* **273**, 2296–2305
29. Hua, X. X., Miller, Z. A., Wu, G., Shi, Y. G., and Lodish, H. F. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96**, 13130–13135
30. Labbe, E., Silvestri, C., Hoodless, P. A., Wrana, J. L., and Attisano, L. (1998) *Mol. Cell* **2**, 109–120
31. Chen, X., Rubock, M. J., and Whitman, M. (1996) *Nature* **383**, 691–696
32. Zhang, Y., Feng, X. H., and Derynck, R. (1998) *Nature* **394**, 909–913
33. Feng, X. H., Zhang, Y., Wu, R. Y., and Derynck, R. (1998) *Genes Dev.* **12**, 2153–2163
34. Janknecht, R., Wells, N. J., and Hunter, T. (1998) *Genes Dev.* **12**, 2114–2119
35. Shi, X. M., Yang, X. L., Chen, D., Chang, Z. J., and Cao, X. (1999) *J. Biol. Chem.* **274**, 13711–13717