Multiple Modes of Repression by the Smad Transcriptional Corepressor TGIF*

(Received for publication, July 23, 1999, and in revised form, October 7, 1999)

David Wotton, Roger S. Lo, Laurie-Anne C. Swaby, and Joan Massagué‡‡

From the Cell Biology Program, Howard Hughes Medical Institute, Memorial Sloan-Kettering Cancer Center, New York, New York 10021

TGIF is a DNA-binding homeodomain protein that has been demonstrated to play a role in transforming growth factor β-regulated transcription and implicated in the control of retinoid-responsive transcription. We investigated the intrinsic transcriptional activity of TGIF fused to a heterologous DNA-binding domain. Our results demonstrate that TGIF is a transcriptional repressor able to repress transcription from several different promoters. Repression by TGIF is insensitive to the distance at which it is bound from the promoter. Moreover, the wild type TGIF effectively represses transcription when bound to its cognate DNA-binding site via its homeodomain. Deletion analysis revealed the presence of at least two separable repression domains within TGIF. Repression by one of these is dependent on the activity of histone deacetylases (HDACs), whereas the other appears not to require HDAC activity. Finally, we demonstrate that TGIF interacts with HDACs via its carboxyl-terminal repression domain. Together, these results suggest that TGIF is a multifunctional transcriptional repressor, which acts in part by recruiting HDAC activity.

Homeodomain transcription factors play roles in both transcriptional repression and activation, and their action is critical for the correct regulation of diverse developmental processes (1). Homeobox genes, first discovered in Drosophila (2, 3), where they regulate segment identity, have now been identified in yeasts, plants, and many metazoans. The homeodomain is a conserved 60-amino acid DNA-binding domain consisting of three α-helices, the third of which is the major DNA recognition helix (4). In contrast, helices one and two appear to be less important for DNA binding but play a role in specific interactions with other proteins. Thus, the homeodomain proteins HoxB1 and Pbx1 have been shown to bind to DNA together, and they interact with each other primarily via helices one and two (5). In addition, the interaction of homeodomain proteins with components of the basal transcription machinery has also been shown to be mediated via the amino-terminal part of the homeodomain (6, 7).

Homeodomain proteins can be grouped into multiple subfamilies, based on such criteria as the primary sequence of the homeodomain, their flanking sequences, and organization into gene clusters (1). One subfamily of homeobox genes encodes proteins with an atypical homeodomain referred to as TALE (for three-amino acid loop extension) homeodomains (8, 9). This class of proteins has a three-amino acid loop between helices one and two of the homeodomain. This insertion does not appear to affect DNA binding but may play a role in determining specific interactions with other transcription factors (5). Members of this subfamily have been identified in diverse species (9) and include the yeast Mata2 protein (10) and the human proteins Pbx1, Meis1, and TGIF (8, 11–13).

Human TGIF was cloned by its ability to bind to a specific retinoid-responsive element, and its DNA binding specificity has been determined (8). TGIF mRNA has been shown to be present in a wide variety of adult human tissues, although expression levels vary greatly. Expression of TGIF appears to be most strictly regulated within the brain (8). Murine TGIF transcripts are absent from the early embryo and are first detectable at embryonic day 9.5. In situ analysis of TGIF expression in mouse embryos revealed expression in an extensive range of tissues, including the brain. However, during development, expression in the brain becomes restricted to specific areas of proliferating cells, such as the cerebellar neuroepithelium (14). Human TGIF maps to 18p11.3 (15), a region that contains a locus (HPE4) associated with holoprosencephaly (16), a prevalent congenital disorder of brain and craniofacial malformation (17).

Transcriptional repression can be brought about in various ways. One way is by competition for binding to DNA, thus displacing activators (18). In the case of TGIF, its binding to the retinoid X receptor response element within a reporter construct inhibits retinoid X receptor-dependent transcription in this manner (8). Transcriptional regulation can also be achieved by the remodeling of chromatin structure by histone acetyl transferases and histone deacetylases (HDACs)3 (19, 20). The recruitment of HDACs by transcriptional repressors to specific target genes results in the compaction of nucleosome structure, limiting accessibility of the DNA template to transcription factors (21–27). TGIF and the mediator of TGF-β signaling, Smad2, have recently been shown to interact following TGF-β receptor activation (28). This interaction results in the recruitment of TGIF to TGF-β response elements, repressing TGF-β-activated transcription. Repression of Smad-dependent transcription by TGIF correlates with the recruitment of HDAC instead of the coactivator p300 into the Smad complex (28–31). Together, these results suggest that TGIF acts to repress transcription. However, little is known about

* This work was supported by National Institutes of Health Grant CA34610 (to J. M. and the Memorial Sloan-Kettering Cancer Center) and National Institutes of Health MSTP Grant GM07739 (to R. S. L.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ An Investigator of the Howard Hughes Medical Institute.

§ To whom correspondence should be addressed: Box 116, Memorial Sloan-Kettering Cancer Center, 1275 York Ave., New York, NY 10021. Tel.: 212-639-8975; Fax: 212-717-3298; E-mail: j-massague@ski.mskcc.org.

1 The abbreviations used are: HDAC, histone deacetylase; TSA, trichostatin A; GBD, Gal4p DNA-binding domain; HA, hemagglutinin; CMV, cytomegalovirus; TGF, transforming growth factor; TK, thymidine kinase; RD, repressor domain.
FIG. 1. Transcriptional repression by GBD/TGIF. Full-length TGIF was fused to the GBD, creating GBD/TGIF. A, L17 cells were cotransfected with GBD/TGIF (50, 100, or 200 ng) and GBD alone (200 ng) and a reporter ((Gal)5-E1b-luc) in which luciferase gene transcription is under the control of five Gal4p binding sites and a minimal TATA element. 36 h after transfection, cells were assayed for luciferase activity, which is presented as the mean ± S.D. of triplicate transfections (in arbitrary units). B, as described for A, except that in (Gal)5-TK-luc, transcription of the luciferase gene is driven by the herpes simplex virus thymidine kinase promoter. C, as described for A, except that luciferase gene transcription is activated by the adeno virus major late promoter. D, GBD/TGIF (1–272) was targeted to five Gal4p binding sites located either adjacent to the SV40 promoter or separated from it by 450 base pairs or 2.9 kilobase pairs. Luciferase activity was assayed as described in A and is presented as the mean ± S.D. of triplicate transfections in which the activity of each reporter in the presence of a control expression vector has been set to 100%.

whether TGIF has intrinsic transcriptional repressor activity or whether TGIF is a context-dependent repressor. Here we show that TGIF is an active transcriptional repressor. This transcriptional repression appears to be dependent on multiple regions of the TGIF protein and, at least in part, requires interaction of TGIF with a histone deacetylase.

EXPERIMENTAL PROCEDURES

Cell Culture and Transfection—Mink lung epithelial L17 cells were cultured in minimum Eagle’s medium supplemented with 10% fetal bovine serum, and COS-1 cells were cultured in Dulbecco’s modified Eagle’s medium with 10% fetal bovine serum. L17 cells were transfected in 6-well plates using DEAE-dextran, as described previously (32).

Reporter Assays—Firefly luciferase was measured using Promega luciferase assay reagents, and Renilla luciferase was measured in 25 mM Tris (pH 7.5) and 100 mM NaCl with 0.09 μM coelenterazine (Bioyrsynth). PCMV-Renilla luciferase (Promega) was included in all transfections to control for transfection efficiency. Five Gal4p binding sites are present upstream of the E1b TATA box in (Gal)5-E1b-luc. The (Gal)5-AdMLP-luc, (Gal)5-SV40-luc, and its derivatives with intervening sequence between the Gal4p sites and the SV40 promoter are as described previously (33). (Gal)5-TK-luc has four Gal4p binding sites upstream of the TK promoter from pBLCAT2 (34), driving luciferase. (TG)5-TK-luc has five copies of a TGIF binding site (gatcctCGTCGAAATCA (TGIF binding site in uppercase letters)) inserted into the BamHI site of TK-luc. L17 cells were transfected with the indicated reporters (1000 ng/6-well plate) together with the indicated expression constructs. Total amounts of DNA were kept constant with either pCMV5 lacking an insert or a pCMV5-farnesyl transferase construct. TSA (100% ethanol) was added to the indicated concentrations 18 h before cell lysis.

Expression Constructs—TGIF expression constructs were created within a modified pCMV5 containing a single Flag epitope or two copies of a HA epitope. Deletion constructs were created by polymerase chain reaction or by using existing restriction sites within the TGIF sequence. HDAC expression vectors are as described previously (35). GBD fusions were created within a Gal4p DNA-binding domain vector (pM; CLONTECH).

Immunoprecipitation and Western Blotting—COS-1 cells were transfected using Lipofectin (Life Technologies, Inc.), according to the manufacturer’s instructions. Thirty-six to 48 h after transfection, cells were washed in phosphate-buffered saline and lysed in LSLD (50 mM HEPES, pH 7.4, 50 mM NaCl, 0.1% Tween 20, and 10% glycerol) supplemented with 50 mM NaF, 0.3 mM Na3VO4, 20 mM β-glycerophosphate, and complete protease inhibitor mixture (Boehringer). Proteins were immunoprecipitated using M2 Flag-agarose (Sigma), separated by SDS-polyacrylamide gel electrophoresis, and transferred to Immobilon-P membranes (Millipore). Proteins were detected with a HA-specific monoclonal antibody (12CA5; Roche Molecular Biochemicals) or the M2 Flag monoclonal antibody (Sigma) and horseradish peroxidase-conjugated goat anti-mouse Ig (Pierce) and enhanced chemiluminescence (Amersham).

RESULTS

TGIF Represses Transcription—To assess the role of TGIF in transcriptional regulation, we fused its entire coding sequence to the GBD. This expression construct was transfected into mink lung epithelial (L17) cells, together with various luciferase reporter constructs. When coexpressed with a reporter in which transcription of the luciferase gene was driven by a minimal TATA element and multiple Gal4p binding sites, GBD/TGIF failed to activate transcription. In contrast, GBD/TFG repressed transcription from this reporter when compared with the activity obtained in the presence of a control plasmid or GBD alone (Fig. 1A). To determine whether TGIF was able to repress transcription from more complex promoters, GBD/TGIF was targeted via multiple Gal4p sites to reporter constructs in which transcription was activated by the herpes simplex virus TK promoter or the adenovirus major late promoter. In addition to a TATA element, binding sites for Sp1 and NF-1 are present within the TK promoter, and the adenovirus major late promoter contains a CCAAT box and a binding site for USF (34, 36). The activities of both the adenovirus major late and TK promoters were dramatically repressed by targeting of TGIF (Fig. 1, B and C). In contrast, little effect of GBD alone was observed.

Repression from a Distance—To determine whether this repression was dependent on the distance of the binding sites from the promoter region, TGIF was targeted to multiple Gal4p sites at different distances from the SV40 promoter. As shown in Fig. 1D, luciferase expression driven by the SV40 promoter was strongly repressed by GBD/TGIF. In addition, when the Gal4p sites were separated from the promoter region by either 450 base pairs or 2.9 kilobase pairs, repression by GBD/TGIF was essentially unchanged. Thus, TGIF appeared to be able to repress transcription from a distance.

TGIF Contains Multiple Repression Domains—To localize the repression domain within TGIF, a series of GBD/TGIF fusions were coexpressed with the TK-luciferase reporter containing Gal4p binding sites. As shown in Fig. 2A, both the amino- and carboxyl-terminal halves of TGIF (amino acids 1–137 and 138–272) repressed transcription from the TK pro-
Transcriptional Repression by TGIF

GBD/TGIF fusions were tested for their ability to repress luciferase
vertical stripes
diagonal stripes
the amino-terminal repression domain (schematically, with the homeodomain (C
ning the carboxyl-terminal region of TGIF.

GBD fusions to a series of amino-terminal TGIF deletion
of TGIF. GBD fusions to a series of amino-terminal TGIF deletion
domains of repression domains within the amino- and carboxyl-terminal regions

TGIF Represses Transcription when Bound Directly to
DNA—Five copies of the TGIF binding site (CTGTCAA; Ref. 8)
were inserted upstream of the TK promoter within TK-luc
(creating (TG)5-TK-luc). As shown in Fig. 3A, expression of
increasing amounts of TGIF in L17 cells reduced the activity of
(TG)5-TK-luc by up to 80%. Little effect of TGIF expression
on the TK-luc reporter lacking TGIF sites was observed. Thus,
TGIF is able to repress transcription when bound directly to
DNA via its homeodomain.

Coexpression of a series of TGIF deletion constructs with
(TG)5-TK-luc revealed that the presence of the homeodomain is
critical for repression by TGIF via its own site (Fig. 3B). However,
expression of the homeodomain alone (construct 31–137) did not result in repression of transcription from (TG)5-TK-luc.

All other TGIF deletion mutants containing an intact home-
domain repressed transcription to some degree, although some differences in transcriptional repression were observed
(Fig. 3B).

To more carefully compare the contribution of each domain
to transcriptional repression by TGIF when bound to DNA via
its homeodomain, we tested five TGIF expression constructs
that were expressed at similar levels (Fig. 3C). Increasing
amounts of each expression vector were cotransfected with
a TK-luciferase reporter containing two TGIF sites. In this case, repression by TGIF was less potent, due to the reduced number
of TGIF binding sites. With the maximum amount of TGIF(1–
272), activity was repressed to about 35% of that with a control
vector (Fig. 3C). Carboxyl-terminal deletion to amino acid 192
had no effect on repression by TGIF. However, removal of an
additional 28 amino acids (construct 1–164), leaving only the
amino-terminal repression domain fully intact, dramatically
reduced repression (Fig. 3C). A small internal deletion (con-
struct 1–147:177–272) decreased transcriptional repression,
suggesting that this deletion was targeting an important do-
main of TGIF. However, TGIF(31–192) was also unable to
repress transcription as efficiently as the wild type protein,
suggesting that the other domains of TGIF contribute to re-
pression (Fig. 3C).

Repression by the Carboxyl-Terminal Half of TGIF Is De-
pendent on Histone Deacetylase Activity—Repression of tran-
scription by many proteins has been shown to involve HDAC
activity. Repression of transcription from the TK promoter by
GBD/TGIF(1–272) was examined in the presence of increasing
concentrations of the inhibitor of deacetylase activity, TSA. As
shown in Fig. 4, GBD/TGIF(1–272) repressed transcription to
less than 5% of that seen with a control plasmid, and TSA had
little effect. Because this fusion protein contains multiple do-
 mains capable of repressing transcription, the effect of TSA on
one domain may be masked by the presence of a second repres-
domain. We therefore tested the effect of TSA on repression
by GBD/TGIF(1–137) and GBD/TGIF(138–272). TSA was
unable to relieve repression by the isolated amino-terminal
repression domain. However, transcriptional repression medi-
atated by the carboxyl-terminal half of TGIF was significantly
reduced by increasing concentrations of TSA (Fig. 4). These
results suggest that the carboxyl-terminal region of TGIF may
repress transcription by recruitment of a histone deacetylase,
whereas the amino-terminal repression domain appears to be
independent of this activity.

TGIF Interacts with Histone Deacetylases—To determine
whether TGIF can interact with histone deacetylases, COS-1
cells were cotransfected with full-length TGIF tagged at its
amino terminus with two copies of a HA epitope, together with
Flag-tagged HDAC1. As shown in Fig. 5A, TGIF was present in
Flag immunoprecipitates from cells expressing both HA-TGIF
and Flag-HDAC1. In contrast, no TGIF precipitated in the
absence of coexpressed HDAC. To identify the HDAC-interact-
ing region within TGIF, a series of HA-tagged TGIF deletion
constructs was coexpressed with Flag-HDAC1. Carboxyl-termi-

nal deletion to amino acid 230 or amino acid 192 did not affect
the interaction with HDAC. Similarly, removal of the amino-
terminal 107 amino acids had no effect on the interaction (Fig.
5A). Deletion within the central repression domain of TGIF

FIG. 2. TGIF contains multiple repression domains. A series of
GBD/TGIF fusions were tested for their ability to repress luciferase
transcription from (Gal)5-TK-luc, as described in Fig. 1A. Identification
of repression domains within the amino- and carboxyl-terminal regions
of TGIF. GBD fusions to a series of amino-terminal TGIF deletion
constructs revealed a domain at the extreme amino terminus of TGIF that
can repress transcription. B, targeting of multiple subfragments span-
ning the carboxyl-terminal region of TGIF. C, TGIF is represented
schematically, with the homeodomain (HD) in black. The positions of
the amino-terminal repression domain (diagonal stripes) and the two
carboxyl-terminal repression domains (vertical stripes) are indicated.
abolished the interaction with HDAC1 (compare constructs 1–230 and 1–147:177–230, Fig. 5A). Additionally, no interaction of amino acids 1–164 of TGIF with HDAC1 was observed. These results place the HDAC-interacting domain of TGIF between amino acids 108 and 192, suggesting that the central repression domain of TGIF acts by recruiting HDACs.

As shown in Fig. 5B, TGIF was clearly detectable in Flag immunoprecipitates from cells expressing Flag-HDAC1. Similarly, HA-TGIF coprecipitated with the related proteins (35) HDAC2 and HDAC3, suggesting that TGIF was able to interact with all three HDACs tested.

**DISCUSSION**

TGIF is a member of the atypical TALE family of homeodomain proteins (8, 9). Members of this family of proteins have been shown to play roles in both transcriptional activation and repression. In the case of proteins such as Prep1, effects on transcription appear to be context-dependent. Thus, Prep1 is able to participate in either transcriptional repression or activation, depending on the other DNA-binding proteins with which it associates. Prep1 can displace HoxB1 and bind to DNA together with Pbx1, repressing transcription relative to a Pbx1/HoxB1 complex. However, recruitment of Prep1 by a DNA-
TGIF contains multiple functional domains with roles in recruitment to DNA and transcriptional repression. TGIF interacts with DNA and with TGF-β-activated Smads and is able to repress transcription in a HDAC-dependent and -independent way. See the text ("Discussion") for a detailed description.

bound complex of Pbx1 and HoxB1, without a requirement for DNA binding by Prep1, results in increased transcriptional activity (37, 38). In contrast, TGIF appears to be an obligate transcriptional repressor. We have been unable to show transcriptional activation by TGIF, regardless of whether it was bound directly to DNA, tethered via a heterologous DNA-binding domain, or recruited to DNA by an activated Smad complex (28). However, we cannot rule out the possibility that interaction of TGIF with a specific protein would mask its ability to repress transcription. Under such circumstances, it is conceivable that TGIF could aid another protein in transcriptional activation, perhaps by stabilizing its binding to DNA.

TGIF appears to contain at least two functionally separable repression domains. The amino-terminal domain, including part of the homeodomain, represses transcription independent of HDAC activity. Repression by the amino-terminal region of TGIF is unlikely to be due to competition with activator proteins for binding to specific response elements because it is able to repress as a Gal4 fusion. Together with the fact that TGIF repressed several unrelated promoters, this also suggests that TGIF is not acting specifically on one transcriptional activator, but rather that repression by TGIF is at the level of more general factors.

Several homeodomain proteins repress transcription via interactions with components of the general transcription machinery. The Drosophila Eve protein represses transcription by interacting directly with the TATA-binding protein, preventing it from binding to the TATA element (39). It has been demonstrated that part of the homeodomain of human Max-1 (termed the amino-terminal arm), which precedes helix one, interacts with TATA-binding protein and represses transcription (7). Similarly, repression by HoxA7 is mediated by the homeodomain and is modulated by the amino-terminal arm (6). The amino-terminal repression domain of TGIF may also repress transcription by interacting with TATA-binding protein or other general transcription factors. Alternatively, TGIF may interact with and recruit a second repressor protein, which confers the ability of this domain to repress transcription.

Within the carboxyl-terminal half of TGIF, two regions appear to contribute to transcriptional repression (see Fig. 6). The central region of the protein interacts with HDAC, whereas we have been unable to demonstrate an interaction of HDAC with the extreme carboxyl terminus of the protein alone. Experiments with an inhibitor of HDAC activity (TSA) demonstrated that, in contrast to the amino-terminal repression domain, repression by the entire carboxyl-terminal half of the protein is dependent on HDAC activity. One interpretation of this result is that both repression domains within this half of the protein contribute to the recruitment of HDAC. In isolation, the carboxyl-terminal region of TGIF can repress transcription in the context of a GBD fusion, but it does not stably interact with HDAC to allow detection by co-immunoprecipitation. If this region represents an entirely separate repression domain, it is likely that it would mask the effect of TSA on the central region, as the amino-terminal repression domain appears to do. However, we cannot rule out the possibility that TGIF contains three repression domains, only one of which is dependent on HDAC.

When bound to DNA via its homeodomain, full transcriptional repression by TGIF appears to require the central HDAC-interacting repression domain. Thus, a major way in which TGIF represses transcription may be by the recruitment of HDAC activity. However, the relative importance of these domains may be skewed by deletion analyses.

We have demonstrated that TGIF actively represses transcription from multiple TGIF binding sites. A tandem array of five TGIF-like binding sites is present within the retinoid X receptor response element from the cellular retinol-binding protein II promoter (8). Binding of bacterially expressed TGIF to this response element has been demonstrated, and this binding has been suggested to repress retinoid-dependent transcription. Our results demonstrate that such an element would be the target of active repression by TGIF. However, four of these TGIF-like sites are degenerate, suggesting that repression of this element by TGIF may require other factors. Interactions with other DNA-bound factors could modify the DNA binding specificity of TGIF. In light of numerous other homeodomain proteins that bind DNA cooperatively with other transcription factors (40, 41), such a mechanism seems likely. Interestingly, many of these interactions involve multiple DNA-binding homeodomain proteins (5, 37, 38, 42).

TGIF is able to repress Smad2/Smad3-dependent, TGF-β-activated transcription, and it appears that both the amino- and carboxyl-terminal repression domains are required for this corepressor activity (28). TGIF may play two separate roles within the cell: (a) acting as a specific corepressor for TGF-β-activated Smads, thereby limiting the effect of TGF-β signaling; and (b) repressing the transcription of a distinct set of genes. Thus, repression of transcription by TGIF, when bound directly to DNA, may have no functional overlap with its role in TGF-β-activated transcription.

As shown in Fig. 6, TGIF appears to contain two separate recruitment domains: one that binds directly to DNA (8) and another, which binds TGF-β-activated Smad proteins (28). Three other functional domains are present within TGIF: an amino-terminal, HDAC-independent repression domain (repression domain 1 (RD-1)), and two regions (RD-2a and RD-2b) within the carboxyl terminus of the protein that repress transcription in a HDAC-dependent manner. Although RD-2a alone is capable of interacting with HDAC, RD-2b may be part of the same carboxyl-terminal repression domain and may contribute to the formation of a TGIF/HDAC complex.

In summary, we have demonstrated that TGIF is an active transcriptional repressor. This repression is dependent, at least in part, on HDAC activity, suggesting that TGIF may regulate transcription by remodeling chromatin. TGIF represses transcription in a context-independent manner, suggesting that any promoter to which TGIF is recruited will be repressed. It will therefore be of great interest to identify the regulatory mechanisms controlling the level or activity of TGIF within the cell.

Acknowledgments—We thank Dr. E. Seto for the HDAC expression vectors, Dr. J. Milbrandt for luciferase reporters, Dr. D. W. Russell for pCMV5, and J. Doody for pCMV-farnesyl transferase. We also thank members of the Massagué laboratory for helpful discussions.

REFERENCES
1. Gehring, W. J., Affolter, M., and Burglin, T. (1994) Annu. Rev. Biochem. 63, 487–526
2. McGinnis, W., Levine, M. S., Hafen, E., Kuroiwa, A., and Gehring, W. J. (1984)
3. McGinnis, W., Garber, R. L., Wirz, J., Kurisu, A., and Gehring, W. J. (1984) *Cell* 37, 403–408
4. Gehring, W. J., Qian, Y. Q., Biller, M., Furukubo-Tokunaga, K., Schier, A. P., Resendez-Perez, D., Affolter, M., Otting, G., and Wuthrich, K. (1994) *Cell* 78, 211–223
5. Piper, D. E., Ratchefor, A. H., Chang, C.-P., Cleary, M. L., and Wolberger, C. (1999) *Cell* 96, 587–597
6. Schnabel, C. A., and Abate-Shen, C. (1996) *Mol. Cell. Biol.* 16, 2678–2688
7. Zhang, H., Catron, K. M., and Abate-Shen, C. (1996) *Proc. Natl. Acad. Sci. U. S. A.* 93, 1764–1769
8. Bertolino, E., Reimund, B., Wildt-Perinic, D., and Clerc, R. (1995) *J. Biol. Chem.* 270, 31178–31188
9. Burglin, T. R. (1997) *Nucleic Acids Res.* 25, 4173–4180
10. Astell, C. R., Ahlstrom-Jonasson, L., Smith, M., Tatchell, K., Nasmyth, K. A., and Hall, B. D. (1981) *Cell* 27, 15–23
11. Nourse, J., Mellentin, J. D., Galili, N., Wilkinson, J., Stanbridge, E., Smith, S. D., and Cleary, M. L. (1990) *Cell* 60, 535–545
12. Kamps, M. P., Murre, C., Sun, X. H., and Baltimore, D. (1990) *Cell* 60, 547–555
13. Moskow, J. J., Bullrich, F., Huebner, K., Daar, I. O., and Buchberg, A. M. (1995) *Mol. Cell. Biol.* 15, 5434–5443
14. Bertolino, E., Wildt, S., Richards, G., and Clerc, R. G. (1996) *Dev. Dyn.* 205, 410–420
15. Edwards, M. C., Liegenis, N., Herecka, J., DePinho, R. A., Sprague, G. F., Jr., Tyers, M., and Elledge, S. J. (1997) *Genetics* 147, 1063–1076
16. Overhauser, J., Mitchell, H. F., Zuckai, E. H., Tick, D. B., Rojas, K., and Muenke, M. (1995) *Am. J. Hum. Genet.* 57, 1080–1085
17. Struhl, K. (1998) *Science* 272, 371–372
18. Liu, C., and Manley, J. L. (1998) *Mol. Cell. Biol.* 18, 3771–3781
19. Mann, R. S., and Chan, S. K. (1996) *Trends Genet.* 12, 258–262
20. Mann, R. S., and Affolter, M. (1998) *Curr. Opin. Genet. Dev.* 8, 423–429
21. Jacob, W., Schnabel, C. A., and Cleary, M. L. (1999) *Mol. Cell. Biol.* 19, 5134–5143
22. Steinbuch, C. A., and Cleary, M. L. (1999) *Nature* 387, 43–48
23. Kadosh, D., and Struhl, K. (1997) *Cell* 89, 341–347
24. Bertolino, E., Reimund, B., Wildt-Perinic, D., and Clerc, R. (1995) *J. Biol. Chem.* 270, 11178–11188
25. Mogil, R., Jiang, Y., Zhang, H., and Abate-Shen, C. (1996) *Proc. Natl. Acad. Sci. U. S. A.* 93, 1764–1769
26. Zhang, H., Catron, K. M., and Abate-Shen, C. (1996) *Proc. Natl. Acad. Sci. U. S. A.* 93, 1764–1769
27. Zhang, Y., Iritani, R., Erdjument-Bromage, H., Tempst, P., and Reinberg, D. (1997) *Cell* 89, 357–364
28. Wotton, D., Lo, R. S., Lee, S., and Massague, J. (1999) *Cell* 97, 29–39
29. Pouponnot, C., Jayaraman, L., and Massague, J. (1998) *J. Biol. Chem.* 273, 22685–22689
30. Janneke, R., Wells, N. J., and Hunter, T. (1998) *Genes Dev.* 12, 2114–2119
31. Yen, X.-H., Zhang, Y., Wu, R.-Y., and Derfen, R. (1998) *Genes Dev.* 12, 2153–2163
32. Hata, A., Lo, R. S., Wotton, D., Lagna, M., and Massague, J. (1997) *Nature* 388, 82–86
33. Swirnoff, A. H., Apel, E. D., Svanen, J., Sevetsen, B. R., Zimonjic, D. B., Popescu, N. C., and Milbrandt, J. (1998) *Mol. Cell. Biol.* 18, 512–524
34. Luckow, B., and Schutz, G. (1987) *Cell* 54, 357–364
35. Yang, W.-M., Yao, Y.-L., Sun, J.-M., Davie, J. R., and Seto, E. (1997) *Mol. Cell. Biol.* 18, 512–524
36. Moncollin, V., Miyamoto, N. G., Zheng, X. M., and Egly, J. M. (1986) *EMBO J.* 5, 22865–22868
37. Berthelsen, J., Zappavigna, V., Mavilio, F., and Blasi, F. (1998) *EMBO J.* 17, 1423–1433
38. Berthelsen, J., Zappavigna, V., Ferretti, E., Mavilio, F., and Blasi, F. (1998) *EMBO J.* 17, 1423–1433
39. Li, C., Schnabel, C. A., and Cleary, M. L. (1999) *Mol. Cell. Biol.* 19, 5134–5143
40. Mann, R. S., and Affolter, M. (1998) *Curr. Opin. Genet. Dev.* 8, 423–429