A proline-rich TGF- β -responsive transcriptional activator interacts with histone H3

Athanassios Alevizopoulos,¹ Yves Dusserre,¹ Monika Tsai-Pflugfelder,^{1,3} Thierry von der Weid,^{1,4} Walter Wahli,^{1,2} and Nicolas Mermod^{1,5}

¹Institute of Animal Biology, University of Lausanne, CH-1015 Lausanne, Switzerland; ²Glaxo Institute for Molecular Biology, CH-1228 Plan-les-Ouates, Geneva, Switzerland

The molecular mechanisms involved in the regulation of gene expression by transforming growth factor- β (TGF- β) have been analyzed. We show that TGF- β specifically induces the activity of the proline-rich *trans*-activation domain of CTF-1, a member of the CTF/NF-I family of transcription factors. A TGF- β -responsive domain (TRD) in the proline-rich transcriptional activation sequence of CTF-1 was shown to mediate TGF- β induction in NIH-3T3 cells. Mutagenesis studies indicated that this domain is not the primary target of regulatory phosphorylations, suggesting that the growth factor may regulate a CTF-1-interacting protein. A two-hybrid screening assay identified a nucleosome component, histone H3, as a specific CTF-1-interacting protein in yeast. Furthermore, the CTF-1 *trans*-activation domain was shown to interact with histone H3 in both transiently and stably transfected mammalian cells. This interaction requires the TRD, and it appears to be upregulated by TGF- β in vivo. Moreover, point mutations in the TRD that inhibit TGF- β induction also reduce interaction with histone H3. In vitro, the *trans*-activation domain of CTF-1 specifically contacts histone H3 and oligomers of histones H3 and H4, and full-length CTF-1 was shown to alter the interaction of reconstituted nucleosomal cores with DNA. Thus, the growth factor-regulated *trans*-activation domain of CTF-1 can interact with chromatin components through histone H3. These findings suggest that such interactions may regulate chromatin dynamics in response to growth factor signaling.

[Key Words: TGF- β ; growth factors; transcriptional regulation; CTF/NF-I; chromatin; histones]

Received August 17, 1995; revised version accepted October 18, 1995.

The transforming growth factor- β (TGF- β) superfamily comprises a large number of growth factors including TGF- β s, activins, and bone¹ morphogenetic proteins. Family members regulate numerous biological activities, such as gene expression and cell growth and differentiation in organisms ranging from *Caenorhabditis elegans* to man (for review, see Kingsley 1994). TGF- β binds specific receptors at the cell surface, which leads to receptor activation through unidirectional receptor chain transphosphorylation on serine and threonine residues (Wrana et al. 1992, 1994).

In fibroblastic cells, TGF- β initiates a complex gene expression program that promotes cell adhesion and intense accumulation of extracellular matrix components, including collagens and their receptors. These effects may play important roles in morphogenesis and remodeling of embryonic tissues during development and in wound healing (Massagué 1990). The importance of col-

lagen upregulation in TGF- β action is emphasized by the identification of certain fibrotic diseases associated with aberrant TGF- β -dependent accumulation of extracellular matrix, such as in nephritis (Border et al. 1990). TGF- β regulates the expression of collagen genes at the level of transcription initiation (Ignatz et al. 1987; Mauviel et al. 1994). Moreover, activation of the collagen $\alpha 2(I)$ promoter by TGF- β has been proposed to involve binding sites for the transcription factors CTF/NF-I (CCAAT box-binding transcription factor-nuclear factor I) and Sp1 (Rossi et al. 1988; Inagaki et al. 1994). However, the mechanism by which TGF- β regulates the activity of transcription factors is currently unknown.

Here, we have analyzed the molecular mechanisms involved in the regulation of the CTF/NF-I proline-rich transcription factors (Santoro et al. 1988; Mermod et al. 1989) by TGF- β . This family of sequence-specific DNA-binding proteins can activate or repress transcription of several cellular and viral genes (Jones et al. 1987). How CTF/NF-I activates transcription is at present uncertain, but it may facilitate the assembly of a functional transcriptional initiation complex (Dusserre and Mermod 1992). For instance, CTF/NF-I may interact with components of the basal transcriptional initiation machin-

Present addresses: ³Swiss Institute for Experimental Cancer Research, CH-1066 Epalinges, Switzerland; ⁴DNAX Institute of Molecular and Cellular Biology, Palo Alto, California 94304 USA.

⁵Corresponding author.

ery, including TFIIB (Kim and Roeder 1994), TATA-binding protein (TBP) (Xiao et al. 1994), the TBP-associated factor TAF55 (Chiang and Roeder 1995), and with poorly characterized cofactors (Dusserre and Mermod 1992). Moreover, CTF/NF-I proteins participate in chromatin-regulated transcriptional control, as in the case of the mouse mammary tumor virus (MMTV) and the tissue-specific vitellogenin B1 gene promoters. Steroid-mediated activation of these genes requires synergistic cooperation of CTF/NF-I and other factors with steroid receptors, which may be brought about by precisely positioned nucleosomes (for review, see Lewin 1994; Wolffe 1994).

All these events may be regulated by growth factors. TGF- β may, for example, regulate the interaction of CTF/NF-I with components of the basal transcriptional machinery. Alternatively, TGF- β may help CTF/NF-I overcome the inhibitory effect of chromatin, which often restricts the access of the transcriptional machinery to regulatory promoter sequences *in vivo*. Finally, the expression of CTF/NF-I itself or that of accessory proteins may serve as potential targets for TGF- β signaling pathways.

In this paper we identify a portion of the CTF-1 proline-rich domain that mediates transcriptional induction in response to TGF- β . We show that this domain of CTF-1 interacts with a nucleosome component, histone H3, in yeast and in mammalian cells. *In vitro*, purified CTF-1 is capable of interacting with previously assembled nucleosomal octamers on DNA. Point mutations in the CTF-1 proline-rich domain reduce TGF- β induction and the efficiency of interaction with histone H3. Thus, our results imply histone H3 in the TGF- β -dependent transcriptional activation by CTF-1.

Results

Identification of a TGF- β -responsive domain on transcription factor CTF-1

Previous studies had indicated that CTF/NF-I binding sites in the $\alpha 2(I)$ collagen promoter mediate TGF- β induction (Rossi et al. 1988). Consistent with the finding that TGF- β induces the $\alpha 2(I)$ collagen promoter in the absence of *de novo* protein synthesis (Ignatz et al. 1987), we have found no effect of TGF- β on CTF/NF-I expression in mouse fibroblasts (N. Mermod, unpubl.). This implies that TGF- β may regulate the *trans*-activation function of CTF/NF-I rather than its expression. We therefore sought to identify putative protein determinants responsible for the TGF- β regulation of CTF-1, a specific member of the human CTF/NF-I family (Mermod et al. 1989). We constructed a panel of expression vectors by fusing various portions of the CTF-1 cDNA to the sequences encoding the DNA-binding and dimerization domains of the yeast GAL4 protein (amino acids 1–147, hereafter referred to as GAL-DBD), downstream of the SV40 early promoter. These GAL-CTF constructs were transfected in mouse NIH-3T3 cells together with a reporter construct containing five GAL4 binding sites in front of the chloramphenicol acetyltransferase (CAT) re-

porter gene. The prototype of the GAL4 fusion proteins GAL(399–499), containing the entire proline-rich *trans*-activation domain of CTF-1 (amino acids 399–499), moderately activated transcription in NIH-3T3 cells. However, the transcriptional activity of GAL(399–499) was significantly increased by TGF- β (Fig. 1A). Induction was specific for the CTF-1 proline-rich *trans*-activation domain (TAD), as the TADs of other known transcription factors, such as AP2, Oct2, Sp1, CTF-2, Myc, GAL4, and VP16, all failed to confer TGF- β inducibility when fused to GAL-DBD (Fig. 1A; data not shown). GAL(1–399), a GAL4 fusion encoding a CTF-1 derivative without the proline-rich TAD was not induced by TGF- β , whereas a chimera containing the minimal GAL-DBD (amino acids 1–93) fused to the CTF-1 TAD activated transcription and it was induced by TGF- β (not shown). Control experiments demonstrated that GAL(399–499) did not activate transcription when the GAL4 binding sites were deleted from the reporter and that TGF- β had no effect on the activity of the SV40 promoter used to express the GAL4 fusions. Consistently, gel mobility-shift assays demonstrated that TGF- β did not modulate the DNA-binding affinity of the GAL4 fusion proteins or their expression levels (Fig. 1B). Taken together, these results indicate that the proline-rich domain of CTF-1 is sufficient to confer TGF- β regulation when tethered to an heterologous DNA-binding domain.

Progressive deletion from the carboxy-terminal end of the CTF-1 TAD eliminated both basal transcriptional activity and TGF- β inducibility [Fig. 1A, GAL(399–472) and GAL(399–438)], whereas the amino-terminal portion was dispensable for TGF- β responsiveness [Fig. 1A, GAL(438–472), GAL(438–499), and GAL(472–499); Fig. 1C, GAL(479–499) W.T.]. Lack of TGF- β regulation did not result from low levels of fusion proteins, as all these GAL-CTF derivatives were expressed at comparable levels (data not shown; see also Fig. 1B). These results thus identify a TGF- β -responsive domain (TRD) at the extreme carboxy-terminal portion of the CTF-1 TAD.

Inspection of the TRD sequence of CTF-1 (Fig. 1C) revealed the presence of three potential phosphoacceptor sites, namely tyrosines 491 and 497 and serine 495 (hereafter referred to as Y491, Y497, and S495, respectively). To test whether TGF- β may induce the activity of the CTF-1 TAD through direct phosphorylation of these amino acids, we have mutated them to neutral or negatively charged amino acids (Fig. 1C; data not shown). GAL-CTF variants harboring a triple mutation of their TRD (where the tyrosines are mutated to phenylalanines and the serine to alanine) were similarly induced by TGF- β , as compared with their parental constructs. Consistently, immunoprecipitation of transiently expressed GAL(399–499) from NIH-3T3 cells metabolically labeled with orthophosphate indicated that GAL(399–499) phosphorylation is not detectably modulated by TGF- β (data not shown). These results demonstrate that TGF- β does not regulate CTF-1 through direct phosphorylation of its TAD. Thus, the TGF- β regulatory effect might be exerted on other factors, which interact and cooperate with the CTF-1 proline-rich domain to activate transcription.

A TGF- β -responsive transcription factor interacts with H3

Figure 1. TGF- β regulates the CTF-1 transcriptional activation domain in NIH-3T3 cells. **(A)** Deletion mapping of the TGF- β -responsive determinants of CTF-1. NIH-3T3 cells were transfected with expression vectors for the indicated GAL-DBD fusion proteins, (the various GAL-CTF1 derivatives, GAL-AP2, or GAL-Oct2), the internal control plasmid CMV β gal, and the G5BCAT reporter construct driven by five GAL4 binding sites, as described in Materials and methods. Five hours after transfection, cells were induced with ethanol vehicle or 5 ng/ml of TGF- β . CAT activities were determined 40 hr later and normalized to β -galactosidase activity. Results are expressed as normalized CAT activity relative to that obtained with GAL(399-499) in the absence of TGF- β 1, which corresponds to a mean 50-fold activation over the G5BCAT basal level, and was arbitrarily set to 100. **(B)** TGF- β does not affect the DNA-binding activity and expression of the GAL4 fusion proteins. NIH-3T3 cells were transfected with the indicated expression vectors and treated with or without TGF- β for 40 hr, as in A. Equal protein amounts of whole-cell lysates prepared from transfected cells (lanes 1-8) or lysate extraction buffer (lane 9) were analyzed for GAL4 DNA-binding activity in a gel mobility-shift assay, as described in the Materials and methods (see also Fig. 2C). (N.S.) A nonspecific DNA-binding activity. **(C)** TGF- β does not regulate CTF-1 through direct phosphorylation of the TRD. NIH-3T3 cells were transfected with the indicated GAL-CTF expression vectors [coding for either the wild-type (W.T.) or mutated (3xM) full-length CTF-1 TAD (399-499) or for the CTF-1 TRD (479-499)], the G5BCAT and CMV β gal reporter constructs, as described in the legend to A. Normalized CAT activities are expressed relative to that of GAL(399-499) in the absence of TGF- β , which was set to 100. The primary sequence of the last 20 amino acids of CTF-1 representing the TRD is displayed at the bottom, and amino acids mutated in the 3xM derivatives are indicated below the wild-type sequence.

Interaction of the CTF-1 TAD with histone H3

CTF-1 is a relatively weak transcriptional activator in yeast (M. Tsai-Pflugfelder, S.M. Gasser, and W. Wahli, in prep.). Thus, we used the yeast two-hybrid system to identify proteins that interact with the proline-rich TAD of CTF-1 and that might be relevant for TGF- β regulation. Yeast cells that mediate galactose-inducible CTF-1 expression and possess a CTF-1-responsive β -galactosidase reporter gene were constructed. These yeast cells

were transformed with a HeLa cell cDNA expression library tagged with the activation domain of the herpes simplex virus VP16 (Dalton and Treisman 1992; see Materials and methods for details). Screening of one million transformants identified 96 positive blue-staining clones. Of six galactose-dependent blue-staining colonies, only three had a phenotype that was dependent on the expression of both the cDNA library insert and CTF-1. The library inserts were rescued from these positive yeast colonies, cloned, and sequenced. Each corre-

sponded to a different cDNA. The first cloned sequence coded for the transcription factor USF (upstream stimulating factor). Interestingly, synergy between CTF/NF-I and USF binding sites has been shown previously in the induction of the promoter of the plasminogen activator inhibitor-1 (PAI-1) gene, in response to TGF- β (Riccio et al. 1992). The second cDNA encoded a proteasome component, which puts CTF-1 in a growing list of activators that may bind these poorly characterized putative cofactors (Ohana et al. 1993). These two clones will not be discussed further in this paper, but we will focus on the relevance of the third insert, which surprisingly was found to code for most of a human histone H3 variant (amino acids 10–136 of human H3.3; Wells et al. 1987).

Given the long-known implication of CTF/NF-I in chromatin-mediated transcriptional control (Archer et al. 1992; Schild et al. 1993), we decided to address the possible occurrence of an interaction between CTF/NF-I and histone H3 in mammalian NIH-3T3 cells. We first asked whether CTF/NF-I proteins could interact with the fusion protein consisting of the VP16 TAD and the 127 carboxy-terminal amino acids of histone H3.3 (hereafter referred to as VP-H3). Such an interaction between CTF/NF-I and the fusion protein would be expected to recruit the potent VP16 TAD to the promoter, which should lead to higher levels of transcription in transfection experiments. A CTF/NF-I-responsive reporter monitored a strong, dose-dependent interaction of CTF/NF-I polypeptides with VP-H3 in NIH-3T3 cells (Fig. 2A; p α CAT- Δ 87–3xAd), an effect that we call “superactivation”. Superactivation required both the VP16 TAD and H3 moieties (Fig. 3B), and no superactivation occurred when the CTF/NF-I binding sites were deleted from the reporter (Fig. 2A, p α CAT- Δ 55). These results implied that endogenous mouse CTF/NF-I, similarly to CTF-1 expressed in yeast, may also interact with the VP-H3 fusion protein in mammalian cells.

We then assessed the specificity of the VP-H3 effect on a panel of GAL4-fusion derivatives. VP-H3 efficiently superactivated GAL(399–499) in NIH-3T3 cells (ninefold; Fig. 2B). Under identical conditions, GAL-AP2 was only weakly superactivated (twofold), whereas the transcriptional activity of the synthetic proline-rich transactivator GAL-P₃₄ (Seipel et al. 1992) and GAL-DBD alone were not. VP-H3 did not superactivate GAL4 fusions encoding CTF-1 derivatives truncated of the TAD. Similarly, a minimal VP16 TAD derivative engineered to possess moderate transcriptional activity, as well as the wild-type VP16 TAD, its amino- and carboxy-terminal deletions or a set of other acidic chimeric transactivators did not mediate superactivation either (Fig. 2B; data not shown). In addition, gel mobility-shift analysis demonstrated that VP-H3 had no significant effect on the expression of the GAL4 derivatives (Fig. 2C). Thus, these results demonstrate the specificity of the CTF-1 TAD interaction with VP-H3 in NIH-3T3 cells.

A battery of other VP16-histone fusions was constructed to further substantiate the specificity of this interaction. Expression of VP-H2A, VP-H2B, and VP-H1 fusion proteins did not increase GAL(399–499) and

GAL-AP2 transcriptional activities in NIH-3T3 cells (Fig. 3A). In contrast, VP-H4 induced GAL-DBD, GAL(399–499), and GAL-AP2 activities (Fig. 3A), suggesting that it might interact with the GAL-DBD independently of the TAD fused to it. Although this finding was not analyzed further, it is consistent with the binding of GAL-DBD to chromatin-reconstituted DNA and nucleosome removal in vitro (Taylor et al. 1991; Morse 1993), and it may provide a rationale for the observed effects of H4 mutations on GAL4 transcriptional activity in vivo (Fisher-Adams and Grunstein 1995). Finally, fusions of VP16 TAD with TBP, TFIIB, and the PC4 cofactor, which have been proposed to interact with CTF-1 in vitro, all failed to mediate superactivation (Fig. 3B). Taken together, these data indicate that histone H3 specifically interacts with the TAD of CTF-1 in vivo.

To map the domain of histone H3 that mediates interaction with the CTF-1 TAD, we constructed a set of VP16-tagged H3 derivatives (Fig. 3B). Removal of the histone amino-terminal tail (amino acids 1–30) did not prevent superactivation of GAL(399–499) in NIH-3T3 cells. We conclude that the globular domain of histone H3 is sufficient for efficient interaction with CTF-1 in vivo.

CTF-1 interacts with histone H3 in vitro

To further analyze the proposed interaction, we assessed whether CTF-1 interacts with histone H3 in vitro, using glutathione S-transferase (GST) pull-down assays. Glutathione-Sepharose beads were used to collect bacterially expressed proteins, such as GST alone or GST fused to full-length wild-type CTF-1 or AP2 (GST-CTF1 and GST-AP2, respectively). The beads were then mixed with in vitro translated and radiolabeled VP16-histone fusions. After washes, bound proteins were eluted and resolved by SDS-PAGE and autoradiography.

GST-CTF1 beads efficiently retained histone H3, whereas GST beads alone did not (Fig. 4A, panel “H3”). In a converse experiment, we detected specific binding of in vitro-translated CTF-1 on immobilized GST-H3 (data not shown). GST-AP2 beads interacted very weakly with histone H3 in vitro, consistent with the weak superactivation of GAL-AP2 by VP-H3 observed in vivo (Fig. 2B). To exclude the possibility that the interaction of CTF-1 with histone H3 occurred because both proteins were bound to contaminating nucleic acids present in the preparations, we used a CTF-1 point mutant that is defective in DNA binding (CTF1-mut6; Armentero et al. 1994). GST-CTF1-mut6 beads could still recruit histone H3 with high efficiency. Moreover, deletion of the proline-rich domain of CTF-1 essentially abolished interaction with H3 (GST-CTF1 Δ). Thus, these data demonstrate that the TAD of CTF-1 is necessary for efficient interaction with histone H3 in vitro and that this interaction occurs independently of DNA binding.

Other core histones (H4, H2A, and H2B) and the linker histone H1 did not interact efficiently with the GST-CTF1 beads (Fig. 4A), which is consistent with the lack of CTF-1-dependent interaction in the superactivation assay. As histones H3 and H4 are known to form multi-

A TGF- β -responsive transcription factor interacts with H3

meric complexes in solution, we tested whether mixing of H3 and H4 may enable the latter to bind CTF-1. H3-H4 complexes were capable of interacting with GST-CTF1 and GST-CTF1-mut6 but not with control proteins (Fig. 4A, panel "H3 + H4"). Because a tetramer of histones H3 and H4 forms the building block of a nucleosome, these results suggested that the CTF-1 TAD may interact directly with higher order histone-DNA complexes, such as nucleosomes.

The proline-rich domain of CTF-1 interacts with nucleosomal particles in vitro

We then asked what might be the functional relevance of the interaction of CTF-1 with histone H3 and H4 complexes. We used a fragment of the estrogen receptor (ER)-regulated vitellogenin B1 (vitB1) promoter shown previously to possess a sequence that favors positioning of a nucleosome within a region close to a high-affinity CTF/

Figure 3. The globular domain of H3 mediates interaction with the proline-rich domain of CTF-1 in vivo. (A) NIH-3T3 cells were cotransfected with expression vectors encoding the indicated GAL4 derivatives (activator), the VP16 TAD fusions to the indicated histones (superactivator), and the G5BCAT and CMVβgal reporter constructs, as described in the legend to Fig. 2 and in the text. CAT activities were determined 18 hr after transfection. Results are expressed relative to the normalized CAT activity obtained with GAL(399–499) in the presence of VP, which was set to 100. (B) NIH-3T3 cells were transfected with G5BCAT, CMVβgal, and the expression vector for GAL(399–499) as well as with plasmids driving the expression of either no protein (none) or of the following VP16 derivatives: VP16 TAD alone (VP) or fused to TBP, TFIIB, PC4, or portions of histone H3. CAT activities were determined 18 hr after transfection, and results are expressed relative to the normalized CAT activity obtained with GAL(399–499) in the presence of VP, which was set to 100.

NF-I binding site (Schild et al. 1993; Cardinaux et al. 1994). This nucleosome is thought to create a static loop that may facilitate cross talk of CTF/NF-I and/or other factors with ER and lead to synergistic transcriptional activation upon steroid induction (Schild et al. 1993). We employed fast salt-removal procedures to enforce the rapid reconstitution of nucleosome cores (octamers) from purified histones on the naked vitB1 promoter DNA. These reconstituted templates were then challenged with purified CTF-1, followed by DNase I footprinting (Fig. 4B). In the absence of CTF-1, the reconstituted templates were digested in a similar fashion as compared with naked DNA, although a general protection of the upstream region of the promoter was observed (Fig. 4B, cf. lanes 3 and 5 with lane 1). Addition of CTF-1 resulted in the protection of the regions that correspond to its binding sites, including the high-affinity site at position –110, both on naked and on reconstituted templates [lanes 2,4,6]. This is expected from the previous

finding that full-length CTF-1 is capable of binding to the vitB1 promoter when the latter is reconstituted at physiological histone/DNA ratios (Schild et al. 1993). CTF-1 did not affect DNase I cleavage of upstream promoter sequences on naked DNA (positions –150 to –300, lanes 1,2). However, the digestion pattern in this region of the reconstituted vitB1 promoter revealed major differences, after incubation with CTF-1 (Fig. 4B, cf. lanes 1 and 2 with lanes 5 and 6). The CTF-1-treated reconstituted templates contributed a set of hypersensitive DNase I cleavage sites (e.g., at positions –168 and –208, cf. lanes 5 and 6), and the overall digestion pattern displayed a characteristic 10-bp periodicity. In contrast, the intensity of intermediate bands within the nucleosomal region was decreased as compared with unreconstituted DNA, as exemplified by protection between positions –230 and –240 or between –198 and –208 (cf. lanes 2 and 6), indicating that these promoter sequences are not bare DNA. This pattern is similar to that ob-

Figure 4. CTF-1 interacts with histone H3 and nucleosomal particles in vitro. (A) Histone H3 and CTF-1 interact in vitro. 35 S-labeled in vitro-translated VP fusions of the indicated histones were mixed with equal quantities of immobilized GST protein alone (GST) or GST fused to either full-length CTF-1 (GST-CTF1), a full-length CTF-1 mutant defective for DNA binding (GST-CTF1-mut6; Armentero et al. 1994), to CTF-1 truncated of its proline-rich TAD (GST-CTF1 Δ), or full-length AP2 (GST-AP2). After extensive washing, bound proteins were eluted and resolved by SDS-PAGE and autoradiography. One-tenth amount of the in vitro-translated input proteins were loaded in lanes labeled In. (M) Molecular mass markers (200, 97, 69, 46, 30, and 14 kD). (B) Evidence for direct interaction of CTF-1 with nucleosomal particles. A 437-bp end-labeled vitB1 promoter reconstituted previously with octamer cores at the indicated histone to DNA mass ratios was incubated with or without purified CTF-1 (30 ng) or a CTF-1 derivative lacking the proline-rich domain (CTF1- Δ , 80 ng) for 30 min at 30°C. The reaction mixture was then treated with DNase I and analyzed on a sequencing gel. The promoter region previously shown to favor translational positioning of the single vitB1 nucleosome (from positions $\sim$ -300 to -140; Schild et al. 1993) is bracketed, and the CTF-1 binding sites are indicated by shaded ellipses, whose size corresponds to the binding affinity for CTF/NF-1 (Cardinaux et al. 1994). In the region from positions -300 to -140, positions corresponding to the DNase I hypersensitive sites appearing at a characteristic 10-bp periodicity of the CTF-1-treated, core-reconstituted templates are indicated with dashes, whereas the arrowhead points to the mid position of the sequence showing this hypersensitive cleavage site pattern.

served previously with rotationally positioned chromatin on the vitB1 promoter (Schild et al. 1993). In contrast, the naked templates (lane 2) and the reconstituted templates incubated in the absence of CTF-1 (lane 5) contributed no such pattern, whereas the CTF-1-treated templates that had been reconstituted at lower histone to DNA ratios (lane 4) mediated intermediate chromatin redistribution pattern. Control experiments using agarose gel shift assays demonstrated that under these conditions >90% of the templates were reconstituted with nucleosomal cores and that CTF-1 did not detectably remove nucleosomal octamers from the template (data not shown). Thus, the incubation of reconstituted DNA with CTF-1 appears to assist reconfiguration of chromatin on the promoter. To examine whether this effect required the presence of the CTF-1 TAD, we used a truncated CTF-1 derivative lacking this domain, CTF1- Δ .

This protein bound naked DNA as expected; however, it was unable to bind reconstituted DNA with high efficiency, even when added in large excess (Fig. 4B, lanes 7–12). This implies that the histone H3-interacting domain is required for efficient CTF-1 binding to nucleosomal DNA. In contrast to wild-type CTF-1, CTF1- Δ did not significantly affect the DNase I cleavage pattern of the vitB1 cores. Thus, our results argue for a direct interaction of the CTF-1 TAD with nucleosomal cores on DNA, and they further suggest that CTF-1 may affect the interaction of nucleosomal particles with the vitB1 promoter in vitro.

Interactions of CTF-1 with histone H3 on reporter promoters stably integrated into cellular chromatin

Assays in vitro and in transiently transfected cells indi-

cated an interaction of CTF-1 with VP-H3. We wondered whether transfected GAL(399–499) may also interact with nucleosomal histone H3 *in vivo*, on cellular templates. Upon transient transfection of cultured cells, most exogenously introduced plasmid DNA remains episomal and may not be properly assembled in chromatin. Therefore, we investigated whether the interaction of GAL(399–499) with VP-H3 may also occur on reporter promoters stably integrated in cellular chromatin, as GAL(399–499) might be expected to interact with endogenous nucleosomes located nearby on the promoter. We generated three NIH-3T3 cell lines consisting of pools of transformant cells stably expressing the CAT gene under the control of a minimal promoter driven by five, one, or no GAL4 binding sites (designated G5/3T3, G1/3T3, and G0/3T3, respectively). Transient expression of GAL(399–499) in G5/3T3 cells resulted in moderate activation of transcription (Fig. 5A, upper panel), indicating that this GAL-CTF derivative is capable of binding nucleosomal DNA, as proposed previously for other GAL4 fusion proteins (Taylor et al. 1991; Vettese-Dadey et al. 1994). Expression of VP-H3 alone resulted in low, yet detectable, levels of transcriptional activation. However, coexpression of VP-H3 and GAL(399–499) in G5/3T3 cells resulted in a dramatic (>38-fold) potentiation of GAL(399–499)-activated transcriptional activity. As expected, no interaction of GAL(399–499) with histone H3 could be observed in G0/3T3 cells that lack GAL4 binding sites (Fig. 5A). We conclude that GAL(399–499) readily interacts with VP-H3 in the chromatin-reconstituted template DNA of G5/3T3 cells, as noted with transiently transfected cells (Fig. 2B).

Similarly, GAL(399–499) detectably stimulated transcription from one GAL4 binding site when transiently expressed in G1/3T3 stable transfectants (fivefold over basal level), whereas expression of VP-H3 alone again resulted in some weak activation (twofold, Fig. 5A). Strikingly, VP-H3 failed to increase transcriptional activity upon coexpression with GAL(399–499), suggesting that interaction of the two proteins may not occur on the stably transfected promoter containing only one GAL4 binding site. To rule out the possibility that this defect was a consequence of the cell pool used, a second polyclonal G1/3T3 cell pool was generated. However, once again VP-H3 failed to significantly interact with GAL(399–499) in these cells (data not shown). Thus, GAL(399–499) is apparently unable to interact with VP-H3 in cells stably expressing a reporter with a single GAL4 binding site.

Two possible explanations may account for the failure of VP-H3 to interact with GAL(399–499) in G1/3T3 cells. First, it is possible that GAL(399–499) is unable to bind the G1BCAT promoter. However, this possibility is inconsistent with the finding that GAL(399–499) activates transcription in G1/3T3 cells. Second, it is possible that the proline-rich domain of GAL(399–499) cannot recruit VP-H3 because it already interacts with endogenous histone H3 in nucleosomes bound to the G1BCAT promoter (Fig. 5B). In that way, the basal transcriptional activity of GAL(399–499) would not be significantly in-

creased upon VP-H3 coexpression (no superactivation). This suggested that superactivation of the GAL(399–499) transcription might be restored in nucleosome-free templates, such as transiently transfected G1BCAT templates. To test this hypothesis, we transiently transfected parental NIH-3T3 cells with the G1BCAT reporter and expression vectors for GAL(399–499) and/or VP-H3 (Fig. 5A, lower panel). Both fusion proteins activated transcription very weakly, if at all, when expressed individually. However, the transcriptional activity of GAL(399–499) was elevated almost sevenfold by coexpressed VP-H3, indicating that templates with a single GAL4 binding site support GAL(399–499) superactivation by VP-H3 as long as they are not incorporated in cellular chromatin. Taken together, these results imply the interaction of the CTF-1 TAD with chromatin *in vivo*, on stably propagated promoters (Fig. 5B).

Specific proline-rich and glutamine-rich trans-activators interact with histone H3 in vivo

Our results suggest that one major function of the proline-rich TAD of CTF-1 *in vivo* is to interact with nucleosomes by binding directly to the globular domain of histone H3. Interestingly, it has been proposed that the ubiquitous transcription factor Sp1 may regulate chromatin redistribution and create nucleosomal-free regions, by as yet unknown mechanisms (for review, see Workman and Buchman 1993). It was therefore of interest to determine whether the glutamine-rich TAD of Sp1 may also interact with histone H3 *in vivo*. Figure 6 shows that similarly to GAL(399–499), two glutamine-rich chimeric activators, GAL-Sp1 and GAL-Oct2, were efficiently superactivated by VP-H3 in NIH-3T3 cells, whereas GAL-DBD was not (Fig. 2B). Moreover, control experiments demonstrated that VP-H3 had no effect on the expression of all these activators (Fig. 2C). Thus, we find that specific proline-rich and glutamine-rich (but as yet no acidic) transcription factors interact with histone H3 *in vivo*.

Regulation of the interaction of CTF-1 with histone H3 by TGF- β

To further map the portion of CTF-1 that interacts with histone H3 *in vivo*, we performed superactivation assays using transiently transfected NIH-3T3 cells (Fig. 7A). Coexpression of VP-H3 with individual GAL-CTF deletion mutants revealed that mutants that respond poorly to TGF- β interacted correspondingly very weakly with VP-H3 [Fig. 7A, GAL(399–438), GAL(399–472), and GAL(399–486)]. In contrast, all TGF- β -responsive mutants were efficiently superactivated by VP-H3 [GAL(399–499), GAL(438–499), GAL(472–499), and GAL(486–499)]. Thus, the TAD of CTF-1 coincides with the histone H3-interacting domain, suggesting a functional link between the two activities.

We subsequently examined whether TGF- β might regulate the interaction of individual GAL-CTF mutants with VP-H3. Figure 7A shows that superactivation me-

A TGF- β -responsive transcription factor interacts with H3

Figure 5. Interaction of the CTF-1 proline-rich domain with VP-H3 on stably transfected promoters. (A) Superactivation of GAL(399–499) transcription by VP-H3 depends on the chromatin state of a promoter containing one GAL4 site. (Top) NIH-3T3 cells were stably transfected with the G0BCAT, G1BCAT, or G5BCAT reporter promoters, driven by either zero, one, or five GAL4 binding sites, yielding the G0/3T3, G1/3T3, and G5/3T3 cell line pools, respectively. These cell lines were then transiently transfected either with expression vector alone or with expression vectors for either VP-H3, GAL(399–499), or both proteins, as indicated. (Bottom) Parental NIH-3T3 cells were transiently transfected with the G1BCAT reporter promoter driven by a single GAL4 binding site, together with the indicated expression vectors. CAT activities were determined 20 hr later and normalized to β -galactosidase activity. The normalized

CAT activity obtained with GAL(399–499)-expressing G1/3T3 cells that was set to 100, and results are expressed relative to this value. (B) Summary and interpretation of the results presented in A. Promoter DNA is shown as a continuous line except for the region of the GAL4 binding sites, which are represented by hatched boxes. For simplicity, chromatin is schematically depicted as a single nucleosome shown as the open circle labeled N, whereas the arrows depict relative levels of transcriptional initiation obtained for each pool cell line. In this model, no significant interaction of GAL(399–499) (drawn as two connected stippled ellipses) with VP-H3 (hatched circle fused to the closed ellipse) is detected using G0/3T3 cells that lack GAL4 binding sites. However, in the case of the G1/3T3 stable transfectants, a single GAL(399–499) dimer can invade the chromatin structure deposited over the promoter and may then interact with endogenous histone H3 within adjacent nucleosomes. Thereby, the CTF-1 TAD becomes sequestered by the nucleosome and is now unable to efficiently recruit exogenous VP-H3, thus preventing superactivation. In contrast, in G5/3T3 cells, five GAL(399–499) dimers may simultaneously bind the promoter (Vettese-Dadey et al. 1994). At least one of these activators may interact with adjacent nucleosomes, as above. However, other proline-rich TADs are left free to recruit exogenous VP-H3 molecules to the promoter, resulting in potent superactivation of transcription. Finally, in transiently transfected NIH-3T3 cells, no proper chromatin is deposited on the promoter, and thus, the interaction of GAL(399–499) with VP-H3 is freely detected on both G1BCAT and G5BCAT templates.

diated by the four TRD-containing fusions is increased in the presence of TGF- β . This effect is specific, as TGF- β did not upregulate the interaction of VP-H3 with GAL-CTF fusions deleted of the TRD (Fig. 7A) or with GAL-AP2 or GAL-Sp1 (Fig. 7B). Importantly, a GAL4 fusion protein containing the entire proline-rich domain of CTF-2, a CTF/NF-I variant that naturally lacks the CTF-1 TRD as a result of alternative splicing events (Mermod et al. 1989), did not activate transcription efficiently or mediate TGF- β induction and interacted poorly with VP-H3 in the superactivation assay, and TGF- β did not further upregulate this interaction [Fig. 7B, GAL(399–430)]. Thus, these results imply that TGF- β regulates the interaction of the CTF-1 TRD with histone H3 and further argue for a functional link between CTF/NF-I transcriptional activity, TGF- β regulation, and histone H3 interaction.

To further strengthen this correlation, we generated a set of amino acid point mutations in the CTF-1 TRD. Individual point mutations of either tyrosine 497 or tyrosine 491 to aspartate were found to reduce basal transcriptional activity by 80% and to abolish TGF- β induc-

tion. Strikingly, both GAL(399–499) Y497D and GAL(399–499) Y491D mutant proteins failed to interact strongly with VP-H3, and TGF- β did not increase this weak superactivation [Fig. 7B, GAL(399–499) Y497D; data not shown]. In contrast, proteins carrying control mutations of the tyrosines to phenylalanine [e.g., in GAL(399–499) Y497F; Fig. 7B] activated transcription efficiently, mediated TGF- β induction, and correspondingly retained efficient interaction with VP-H3. Band-shift experiments confirmed that all point-mutant proteins were expressed at similar levels compared with wild-type GAL(399–499) and that these levels were not affected by TGF- β or VP-H3 (data not shown), thereby demonstrating the specificity of the Y497D and Y491D effect. Finally, several other point mutations of the proline rich-domain, including mutation of serine 495 to aspartate or alanine, as well as the triple TRD mutation (see earlier), were all regulated by TGF- β and interacted with VP-H3 equally well to GAL(399–499) (data not shown). Thus, several independent lines of evidence link TGF- β regulation of CTF-1 transcriptional activity with histone H3 interaction in vivo.

Figure 6. Interaction of histone H3 with glutamine-rich TADs in the superactivation assay. NIH-3T3 cells were transiently transfected with G5BCAT, CMVβgal, and expression vectors for chimeric activators containing the CTF-1 proline-rich or the Sp1 or Oct2 glutamine-rich activation domains [GAL(399–499), GAL-Sp1, or GAL-Oct2, respectively] and vectors expressing either VP-H3 or the VP16 TAD alone (VP), as indicated. CAT activities were determined 18 hr after transfection, and results are expressed relative to the normalized CAT activity of cells transfected with GAL(399–499) and VP, which was set to 100.

Discussion

Transcription factors are thought to regulate gene expression by interacting with components of the basal transcriptional machinery but also by somehow modifying the structure of chromatin. Here, we have analyzed the regulation of CTF-1 transcriptional activity by TGF-β, and we have found that the TRD of CTF-1 mediates a novel type of interaction with histone H3. Our findings suggest that TGF-β may regulate transcription by modulating the interaction of chromatin components with transcriptional regulatory proteins.

Growth factor regulation of CTF/NF-1 requires a TRD

In this study, we have shown that TGF-β induces the transcriptional activity of CTF-1, a member of the CTF/NF-1 family of transcription factors. TGF-β induction was specific for the proline-rich TAD of CTF-1, as a panel of other TADs failed to confer TGF-β inducibility. We identified a small element at the extreme carboxy-terminal portion of the TAD of CTF-1, the TRD, that is necessary for TGF-β induction. The TRD is well conserved within some but not all members of the CTF/NF-1 family. Consistently, the alternatively spliced CTF/NF-1 isoform CTF-2 that lacks this element did not mediate TGF-β regulation. Previous studies had implicated binding sites for CTF/NF-1 in the induction of the collagen α2(I) promoter by TGF-β (Rossi et al. 1988). Our results now demonstrate that TGF-β regulates CTF/NF-1 transcriptional activity, and they further imply selective

CTF/NF-1 variants in the TGF-β induction of this promoter. This indicates that other growth factors known to regulate α2(I) collagen gene expression may regulate CTF-1 transcriptional activity in a similar manner. We have found recently that tumor necrosis factor-α (TNF-α), which represses α2(I) collagen expression at the level of transcriptional initiation (Kähäri et al. 1990), also specifically represses the transcriptional activity of CTF-1 through the TRD (A. Alevizopoulos and N. Mermod, in prep.). Our findings therefore provide a mechanism for the transcriptional regulation of the collagen α2(I) promoter by growth factors and further indicate that CTF-1 may be a major regulator of α2(I) collagen gene expression in vivo.

TRD-dependent interaction of CTF-1 with histone H3

Although the primary sequence of the TRD contains three potential phosphate acceptor sites, our genetic analysis demonstrated that TGF-β induction is not mediated through direct phosphorylation of these residues. We therefore hypothesized that other proteins may interact with the proline-rich TAD of CTF-1 and may regulate or contribute to its transcriptional activity. Our genetic screening in yeast identified histone H3 as a CTF-1-interacting protein. We subsequently found that histone H3 interacts with mammalian CTF/NF-1 proteins and that the proline-rich TAD of CTF-1 specifically mediates this interaction in vivo. Furthermore, our data provide evidence for the interaction of the CTF-1 TAD with histone H3 on promoters stably integrated within cellular chromatin. The TADs of several acidic transcription factors and that of a synthetic proline-rich activator failed to interact with histone H3, thereby demonstrating the specificity of the proposed interaction. Finally, the occurrence of the CTF-1 interaction with histone H3 was ascertained in vitro. Thus, our results demonstrate a novel function for a transcriptional activator, whereby it interacts with a chromatin component with its TAD.

The TGF-β-responsive region of CTF-1 was required for both transcriptional activation and efficient interaction with histone H3, suggesting that these might be coupled functions of the CTF-1 proline-rich domain in vivo. Consistently, the interaction of CTF-1 with histone H3 was upregulated by TGF-β in the superactivation assay, as was the CTF-1 TRD transcriptional activity. Moreover, two point mutations in the CTF-1 TRD (Y491D and Y497D) compromised both TGF-β induction and the efficiency of interaction with histone H3. Finally, the naturally occurring CTF/NF-1 variant CTF-2, which lacks a TRD, activated transcription very poorly and interacted weakly, if at all, with histone H3 in vivo. In contrast, a distinct portion of CTF-1 shown previously to interact with TBP in vitro (Xiao et al. 1994) displayed no transcriptional activity in fibroblastic cells and did not contribute to TGF-β regulation. Thus, although our findings do not exclude a role for other types of regulatory interactions, they strongly implicate the CTF-1–histone H3 interaction in transcriptional regulation. The TRD

A TGF- β -responsive transcription factor interacts with H3

Figure 7. VP-H3 interacts with the TGF- β -regulated transcriptionally active region of CTF-1 in vivo. (A) The TRD is required for strong interaction with histone H3. NIH-3T3 cells were transfected with G5BCAT, CMV β gal, and expression vectors for the indicated GAL-CTF fusions, as well as with vectors coding for VP-H3 or VP alone. Cells were induced with TGF- β or carrier, as indicated, and CAT activity was determined 40 hr later. Results are expressed relative to the normalized CAT activity obtained from cells expressing GAL(399-499) in the presence of VP, which was set to 100. (B) A mutation in the TRD correspondingly inhibits TGF- β inducibility and interaction with VP-H3. Cells were transfected with expression vectors coding for GAL-CTF1, GAL-CTF2 [referred to as GAL(399-430)], GAL-AP2, and GAL-Sp1 fusions, as described in A and in the legends to Figs. 2 and 6. The tyrosine at the position 497 of CTF-1 sequence was either left unchanged [GAL(399-499) W.T.] or mutated to phenylalanine [GAL(399-499) Y497F] or aspartic acid [GAL(399-499) Y497D], as indicated. CAT activities were determined 20 hr after transfection, and the results are expressed relative to the normalized CAT activity obtained using cells expressing GAL(399-499) and VP, which was set to 100.

bears no significant homology to other known proteins, but the features that might be relevant for its function are several bulky hydrophobic residues (such as leucine, isoleucine, and tryptophan) interspersed with prolines, glutamines, and aspartic acid. Interestingly, similar elements were identified in other TADs (Gill et al. 1994), including that of the histone H3-interacting transcription factor Sp1, which has led to the proposal that they might be relevant for transcriptional activation (Maniatis and Tjian 1994).

Functional implications of the CTF-1 interaction with histone H3

In contrast to histone H3, histone H4 did not interact directly with CTF-1 in vitro. However, histone H3-H4 oligomers were specifically retained by immobilized GST-CTF1. Moreover, footprinting studies implied that

CTF-1, but not its TAD-truncated derivative, affected the structure of the core nucleosome reconstituted vitB1 promoter. These results obtained using purified proteins are consistent with the direct interaction of the CTF-1 TAD with chromatin in vitro. However, the precise nature of the CTF-1-mediated chromatin alteration is unknown. The DNase I digestion pattern observed with CTF-1-bound reconstituted DNA was conspicuously similar to that obtained previously with rotationally phased nucleosomes on the vitB1 promoter (Schild et al. 1993). Thus, a potential function of CTF-1 might be to alter nucleosome positioning of the vitB1 promoter. Accordingly, nucleosome positioning would maximize the cooperation between CTF-1, ER, and other factors on the this promoter, allowing for efficient estrogen-dependent activation of transcription (Schild et al. 1993). Although further experiments will be required to evaluate this hypothesis, it is supported by the previous demonstration

that chromatin reconstitution of the vitB1 promoter favors *in vitro* transcription in the presence, but not in the absence, of the high-affinity CTF/NF-I binding site at position -110 [Corthsy et al. 1990]. Thus, the proposed alteration of the vitB1 nucleosomal structure by CTF-1 correlates well with the known chromatin-dependent transcriptional stimulation of this promoter.

The MMTV long terminal repeat (LTR) provides a second example of a chromatin-regulated promoter that requires cooperation of CTF/NF-I and steroid receptors for optimal activity. In the resting state, MMTV promoter DNA is packaged into six translationally positioned nucleosomes, one of which excludes CTF/NF-I binding. Following hormonal induction, an activated steroid receptor dimer binds hormone response elements within one of the nucleosomes (referred to as nucleosome B). Nucleosome B is then thought to be rearranged, histone H1 is displaced, and CTF/NF-I and other factors are recruited to the DNA, followed by transcription initiation [Archer et al. 1992; Truss et al. 1995; for review, see Wolffe 1994]. How CTF-1 contributes to maximal transcriptional activation of the MMTV promoter is unclear at present. However, following DNA binding, CTF-1 might interact with histone H3 in nucleosome B and participate in its remodeling. Alternatively, CTF-1 may interact with the adjacent nucleosome that covers the transcription initiation site, thereby facilitating transcriptional initiation. Another issue, not addressed here, is that CTF-1 may recruit the rather unspecific chromatin-remodeling SWI/SNF complex [Winston and Carlson 1992] and direct it specifically to nearby nucleosomes.

The role of histone H3 in transcriptional activation

We have provided evidence that TGF- β upregulates the interaction of histone H3 with the proline-rich domain of CTF-1, suggesting that nucleosome components such as histone H3 may serve as targets of growth factor regulation. Previous studies have linked chromatin structure alterations with growth factor-regulated gene expression. For instance, it was demonstrated that histone H3 becomes phosphorylated in response to growth factor stimulation of mammalian cells and that this phosphorylation correlates with increased expression of immediate-early genes [Mahadevan et al. 1991]. It is therefore conceivable that TGF- β may upregulate the interaction of CTF-1 with histone H3 by directly modulating phosphorylation of chromatin constituents.

Our results indicated that the amino-terminal tail of histone H3 is dispensable for interaction with CTF-1 *in vivo* and that the interaction of CTF-1 with histone H3 occurs even when the latter is within nucleosomes. Thus, the globular domain of nucleosomal histone H3 must be accessible to transcription factors, and mutations in this domain might be expected to affect transcriptional activation. Recent studies from other laboratories support this prediction. Refinement of the octamer core three-dimensional structure [Arents et al. 1991] shows that several regions of the globular domain of histone H3 are stereochemically exposed and may

thus mediate regulatory interactions (for review, see Pruss et al. 1995). Moreover, several point mutations of yeast genes referred to as switch-independent or SIN mutations restore inducible gene expression in mutant yeast cells [Herskowitz et al. 1992; Winston and Carlson 1992; Prelich and Winston 1993]. Some of the SIN mutations map in the exposed part of the globular domain of histone H3 [Prelich and Winston 1993; discussed in Paranjape et al. 1994]. Our results thus may provide a molecular basis for the functional interpretation of the SIN phenotype, as some of these mutants may directly alter the interaction of nucleosomes with transcription factors, thus affecting disruption or reconfiguration of target nucleosomes on promoters.

Relevance of the CTF-1–histone H3 interaction in the control of DNA replication

In the cases described above, we have considered the relevance of CTF-1–H3 interaction with respect to nucleosomal fate during transcriptional activation. However, all activities that rely on DNA–protein interactions are potentially regulated by chromatin. For instance, nucleosomes have to be removed during the initiation of DNA replication, on viral replication origins (for review, see DePamphilis 1993). In the case of the small DNA virus SV40, initiation of DNA replication can be activated by the concerted action of specific host-provided transcription factors, such as Sp1 [DePamphilis 1993]. Previous studies showed that CTF/NF-I can stimulate the initiation of SV40 DNA replication *in vivo*, as does Sp1, and that *in vitro* CTF/NF-I counteracts chromatin-mediated repression of DNA replication [Cheng and Kelly 1989]. Although the exact mechanism of CTF/NF-I-mediated activation of SV40 DNA replication is unknown, these findings suggested that CTF/NF-I may somehow modify the chromatin structure over the SV40 replication origin, thereby indirectly activating replication. Our findings now provide a rationale for the stimulation of SV40 DNA replication by cellular *trans*-activators, such as CTF-1 and Sp1. According to our results, CTF-1 and Sp1 may bind histone H3 on target nucleosomes close to the initiation site of SV40 DNA replication. As a result of this high-affinity interaction, rotational phasing, structure, or positioning of these nucleosomes may be altered, allowing for the recruitment of the replication machinery to the origin. Interestingly, the very strong transcriptional activator GAL–VP16 does not activate SV40 replication *in vivo* [DePamphilis 1993], and we find that it does not interact with histone H3. Thus, the capacity to activate SV40 replication correlates with the ability of transcription factors to bind histone H3.

In conclusion, the interaction of *trans*-acting factors such as CTF-1 and Sp1 with chromatin is potentially relevant for a number of processes in eukaryotic nuclei, including transcription, replication, and perhaps also other activities. Analysis of such interactions may help unravel the complex interplay between chromatin and sequence-specific regulatory proteins.

Materials and methods

Plasmid constructions

The mammalian expression vectors for GAL4 fusion proteins were constructed in the pSG424 vector encoding the DNA-binding domain of GAL4 (amino acids 1–147; Sadowski and Ptashne 1989) by inserting appropriate portions of the human CTF-1 cDNA (Mermod et al. 1989) and are termed GAL x - y , where x and y indicate CTF-1 insert end-point amino-acid positions. GAL(399–472) and GAL(399–486) result from the insertion of the small *HindIII*–*AflIII* and *HindIII*–*BamHI* fragments of GAL(399–499), respectively, in pSG424 cleaved with *HindIII* and *EcoRI*, after filling-in of the *AflIII*, *BamHI*, and *EcoRI* sites with the Klenow enzyme. The small *HindIII*–*AflIII* and *AflIII*–*XbaI* fragments of GAL(438–499) were then ligated to the large *HindIII*–*SmaI* and *SmaI*–*XbaI* pSG424 vector fragments, to produce GAL(438–472) and GAL(472–499), respectively, with the *AflIII* sites filled-in by Klenow. The *AflIII* and Klenow-repaired *BamHI* small fragment of GAL(472–499) was ligated with the *AflIII* and Klenow-repaired *BamHI* large pSG424 vector fragment, resulting in the GAL(486–499) expression vector. To construct GAL(479–499) and GAL(479–499) 3xM, double-stranded DNA fragments bearing unique *EcoRI* and *SmaI* sites were synthesized by PCR using appropriate oligos and GAL(399–499) as template, digested with *EcoRI* and *SmaI*, and cloned between the *EcoRI* and Klenow-repaired *XbaI* sites of pSG424. In GAL(479–499) 3xM, four point mutations have been introduced at positions 1539, 1550, and 1552 and 1557 of the wild-type human CTF-1 cDNA sequence (Mermod et al. 1989), changing tyrosine 491 to phenylalanine (TAT $\rightarrow$ TTT), serine 495 to alanine (TCC $\rightarrow$ GCA), and tyrosine 497 to phenylalanine (TAT $\rightarrow$ TTT), respectively. The intermediate *HindIII*–*BamHI* fragment of GAL(399–499) was ligated to the large *HindIII*–*BamHI* GAL(479–499) 3xM vector fragment, generating GAL(399–499) 3xM. The GAL(399–499) Y491D, Y491F, Y497D, and Y497F point mutants were constructed by introducing appropriate single point mutations at codons 491 and 497 of CTF-1 cDNA, using oligo-directed PCR mutagenesis, after subcloning from the corresponding GAL(479–499) intermediates, as above. GAL(399–499), GAL(1–399), GAL(399–438), GAL(438–499), and the CTF-2 fusion GAL(399–430) vectors have been described (Martinez et al. 1991).

The small *PvuII*–*XbaI* fragments of pGAL4(1–93)–AP2(31–76), pGAL4(1–93)–Oct2(99–161), pGAL4(1–93)–Sp1(132–243), and pGAL4(1–93)–P₃₄ (Seipel et al. 1992), were ligated to the large (Klenow-repaired) *BamHI*–*XbaI* pSG424 fragment, generating the GAL–AP2, GAL–Oct2, GAL–Sp1, and GAL–P₃₄ expression vectors, respectively. A synthetic double-stranded oligonucleotide coding for amino acids 437–445 of the VP16 TAD and bearing cohesive *EcoRI* and *XbaI* extensions was inserted between the corresponding sites of pSG424 to generate the GAL–VP16(437–445) expression vector.

The CEN-based plasmid, pGAL1–CTF1–CTF/NFI–*lacZ*, contains the human CTF-1 cDNA under the control of the galactose-inducible yeast *GAL1* promoter and a CTF/NFI-dependent *lacZ* reporter gene. pGAL1–CTF1–CTF/NFI–*lacZ* was constructed by subcloning the CTF/NFI–*lacZ* reporter gene as a *XhoI*–*Apal* fragment from pLG–CTF/NFI into a CEN-based derivative of p2HG–CTF1 (M. Tsai-Pflugfelder, S.M. Gasser, and W. Wahli, in prep.).

The SD10–VP–H3(10–136) clone was obtained by screening the SD.10 yeast expression library (see below). It expresses the VP16 transcriptional activation domain (Dalton and Treisman 1992) fused to the coding sequence of human histone H3.3 from amino acid 10 to 136. Such fusion constructs are termed VP–H3 x - y where x and y indicate the border amino-terminal and car-

boxy-terminal amino acid coordinates of the histone sequence. The mammalian expression vector pMLV–VP–H3(10–136) was constructed by inserting the VP–H3 coding sequence of SD10–VP–H3(10–136) in pMLV β plink (Dalton and Treisman 1992), after cleavage with *BamHI* and *XhoI*. pMLV–VP–H3(10–98) and pMLV–VP–H3(10–82) were generated by the excision of the small fragments of pMLV–VP–H3(10–136) cleaved with *XhoI* and *StuI* (partial) or with *XhoI* and *BglII*, respectively, Klenow-repair of the staggered ends and ligation. pMLV–VP–H3(10–136) was excised of the VP16-encoding *BamHI* and *EcoRI* fragment or of the histone H3-encoding *XhoI* and *EcoRI* fragment and Klenow-repaired, generating pMLV–H3(10–136) and pMLV–VP, respectively. pMLV–VP–H3(30–136) and pMLV–VP–H3(1–136) were generated by PCR amplification of pBLUE + H3.3 (Hraba-Renevey and Kress 1989) and subcloning in pMLV–VP–H3(10–136), after excision of the H3(10–136)-coding sequence. PCR-amplified full-length sequences encoding mouse H4 (Seiler-Tuyns and Birnstiel 1981), H2A, H2B (Gruber et al. 1990), and H1 (Cheng et al. 1989) were used to generate pMLV–VP–H4, pMLV–VP–H2A, pMLV–VP–H2B, and pMLV–VP–H1, respectively, as for the construction of pMLV–VP–H3(1–136).

pMLV–VP–TFIIB, pMLV–VP–TBP, and pMLV–VP–PC4 contain the entire coding sequence of TFIIB (from p6H–TFIIB; Hoffmann and Roeder 1991), TBP (Hoffmann et al. 1990), and PC4 (from pGEX–2TK–PC4; Ge and Roeder 1994), introduced between the *EcoRI* and *XhoI* sites of pMLV–VP–H3(10–136), after removal of the histone H3 sequences.

In vitro transcription and translation vectors were constructed by cleaving the corresponding pMLV–VP–histone vectors with *NcoI* and *XhoI* and inserting the resulting VP–histone fusion-coding fragment in pT7–CTF1 (Mermod et al. 1989), after excision of the CTF-1 sequences with *NcoI* and *Sall*, generating pT7–VP–H3, pT7–VP–H4, pT7–VP–H2A, pT7–VP–H2B, and pT7–VP–H1.

The pGEX–CTF1 vector for the bacterial expression of the fusion of GST with full-length CTF1 was generated by inserting the CTF-1-coding *EcoRI* and Klenow-repaired *SpeI* fragment of pCTF-1 (Santoro et al. 1988) in the *EcoRI* and Klenow-repaired *BamHI*-cleaved pGEX–3X vector (Pharmacia). The *NcoI* and *SacI* CTF-1 fragment of pGEX–CTF1 was replaced by the corresponding sequence of the CTF1–mut6 mutant (Armentero et al. 1994), in pGEX–CTF1–mut6. The introduction of an oligonucleotide with translational stops in all frames (Pharmacia) in the *PpuMI* site of pGEX–CTF1, in pGEX–CTF1 Δ , was used to express a fusion of the amino-terminal 235 amino acids of CTF-1, thereby truncated of the transcriptional activation domain. pGEX–AP2 results from the insertion of a partial *NcoI* and Klenow-repaired *XhoI* fragment of pPADH–AP2 (Williams et al. 1988), inserted in pGEX–CTF1 with the CTF-1 sequence removed with *NcoI* and *SmaI*.

Correct fusion and PCR amplification of all recombinant clones was confirmed by dideoxy DNA sequencing.

Two-hybrid screening in yeast

The screening procedure for CTF-1-interacting proteins in a two-hybrid system using a VP16-tagged HeLa cell cDNA yeast expression library was a slight variation of that described by Dalton and Treisman (1992). A protease-deficient yeast strain (MATa *GAL*⁺ *pep4-3* *ura3* *his3* *bar1* *suc2 Δ 9*) was transformed with pGAL1–CTF1–CTF/NFI–*lacZ* and maintained under selection for the *HIS3* marker. Yeast cells were subsequently transformed with the pSD.10 library (Dalton and Treisman 1992) and grown on HIS⁺ and URA⁺ selective media containing 2% glucose. After 3–4 days at 30°C, transformants were replica-

plated onto Amersham Hybond filters. The filters were then transferred to fresh HIS⁻ and URA⁻ selective media containing 2% galactose and incubated at 30°C for 24 hr to induce CTF-1 and the VP16-cDNA protein expression. The colony color β -galactosidase activity assay was performed as described (Dalton and Treisman 1992), allowing a color development time ranging from 30 min to 6 hr at 30°C. Positive (blue) colonies were picked from the original plates (HIS⁻ and URA⁻ selective media containing 2% glucose) and colony purified.

To test whether positive colonies required the presence of CTF-1 or the VP16-cDNA fusion protein, two assays were performed. First, β -galactosidase activity was determined on glucose-grown, noninduced cells. Then, the VP16-cDNA-containing plasmids were isolated from the remaining galactose-dependent positive clones and retransformed in yeast cells containing pGAL1-CTF1-CTF/NFI-lacZ vector or the pGAL1-CTF/NFI-lacZ derivative that does not express CTF-1, to score for VP16-cDNA and for CTF-1-dependent β -galactosidase activity.

Cell culture, transfections, and bandshift analysis

NIH-3T3 cells were grown at 37°C in a humidified incubator containing 5% CO₂ in DMEM medium (GIBCO) supplemented with 10% fetal bovine serum (FBS) and antibiotics. For transient transfection, 4.5×10^6 exponentially growing cells were resuspended in 350 μ l of serum-free medium, mixed with 70 μ g of total plasmid DNA (in a volume of 30–50 μ l) in a 0.4-cm electroporation cuvette, and pulsed once at 960 μ F and 250 V at room temperature, according to the instructions of the electroporator manufacturer (Bio-Rad). Cells were allowed to recover for 15 min at room temperature, transferred to 37°C, and grown overnight in 5 ml of DMEM with 10% FBS medium. Cells were then harvested and lysed in 120 μ l of 1 $\times$ reporter lysis buffer (Promega). CAT activities were determined using standard procedures and normalized according to the activity resulting from an internal control plasmid expressing β -galactosidase (CMV β gal; Clontech). For the experiments using TGF- β , the contents of one pulsed cuvette were split in two and cells were brought in DMEM plus 0.5% FBS, plated, and allowed to adhere to the dish for 3–5 hr. Cultures were then induced with ethanol vehicle or with 5 ng/ml of human TGF- β 1 (Nacalai Tesque, Japan) for a period of 18–40 hr. The values shown in the figures are means of three experiments, except for Figure 1C ($n=2$) and Figure 7A ($n=5-9$), with <30% s.d. The plasmid DNA mixture used for transient transfections typically contained 20 μ g of the GIBCAT and G5BCAT GAL4-responsive reporters (Lillie and Green 1989), 25 μ g of pCAT- Δ 87–3xAd and pCAT- Δ 55 reporters (Santoro et al. 1988), 2 μ g of CMV β gal, 15 μ g of GAL-CTF expression vector, and 2 μ g of MLV-VP-H3(10–136) expression vectors (these quantities were used for all other GAL4 and VP-fusion derivatives). The total amount of plasmid DNA was adjusted to 70 μ g with sonicated, salmon sperm carrier DNA (Sigma).

For stable transfection, 4.5×10^6 cells were electroporated with 20 μ g of total plasmid DNA (5 μ g of G0BCAT, G1BCAT, or G5BCAT reporter and 15 μ g of carrier) and plated in 10% FBS medium as above. Cells were selected with 500 μ g/ml of G418 (GIBCO) added to the growth medium 24 hr after transfection over a period of 2 weeks with regular medium changes. Several hundred colonies of stably transfected cells were pooled and expanded to polyclonal cell lines.

For bandshift analysis of GAL4 fusion proteins, transiently transfected NIH-3T3 cells were lysed in extraction buffer (20 mM Tris at pH 7.5, 20% glycerol, 500 mM KCl, 1 mM DTT, 1 mM PMSF) as described by Martinez et al. (1991). Whole-cell lysates were normalized for protein concentration and incubated with

an end-labeled double-stranded DNA probe, containing the 17-bp GAL4 binding site (Carey et al. 1989). Protein-DNA complexes were separated from free probe on native polyacrylamide gels and revealed by autoradiography.

In vitro translation of proteins and GST pull-down assays

In vitro transcription and translation of VP16-histone fusion proteins were performed by in vitro transcription of the plasmids of the pT7 series with T7 RNA polymerase, followed by translation of the RNA in rabbit reticulocyte lysate (Promega) according to the manufacturer's instructions. Before use, in vitro-translated proteins were treated with micrococcal nuclease, and ethidium bromide was added to a concentration of 200 ng/ml, as described by Li and Botchan (1993).

To produce GST-CTF fusion proteins in bacteria, *Escherichia coli* strain W3110 (a kind gift from D. Haas) was transformed with appropriate GST expression vectors. GST fusion proteins were expressed in bacterial cells and purified on glutathione-sepharose 4B beads (Pharmacia), as described by the manufacturer. Nonspecifically bound bacterial proteins and nucleic acids were removed by washing the beads twice with a buffer containing 40 mM HEPES (pH 7.9), 10 mM EDTA, 100 mM KCl, 1% Triton X-100, 5 mM DTT, 0.2 mM PMSF, twice with R buffer [20 mM HEPES (pH 7.9), 20% glycerol, 2 mM MgCl₂, 0.2 mM EDTA, 0.1% NP-40, 0.2 mM PMSF, 1 mM DTT, 200 ng/ml of ethidium bromide] containing 600 mM KCl, and twice with R buffer containing 100 mM KCl and 0.5% nonfat dry milk. Equal volumes of bead slurry, coated with equivalent amount of GST-fusion proteins, were then mixed with in vitro-translated histone fusions and incubated at 4°C for 1 hr with rotation. Unbound proteins were removed by washing four times in R buffer with 100 mM KCl and 0.5% nonfat dry milk, followed by two washes with R buffer containing 100 mM KCl. Beads were finally mixed with 20 μ l of SDS-PAGE sample buffer and heated at 95°C for 3 min. Eluted proteins were resolved on 10% SDS-PAGE; the gel was dried and exposed for autoradiography.

Nucleosomal core reconstitution and DNase I footprinting

The DNA probe used in nucleosome reconstitution was prepared by the PCR amplification of a *Xenopus* vitB1 promoter fragment ranging from the –301 to the +136 position (Seiler-Thuyns et al. 1986), using the 5'-GAAATACCTG AATACTGTGTATAACA and the ³²PO₄-labeled 5'-TCTTAGTTGGC-TATGGTCTATG oligonucleotides. Purification of chicken erythrocyte core histones, reconstitution of nucleosomal octamers on the DNA probe by salt dialysis, and analysis of the reconstitutes by gel mobility-shift analysis and DNase I footprinting were as described (Schild et al. 1993), except that the dialysis was performed in the absence of urea, which resulted in nonpositioned nucleosomal cores on the DNA. Histone/DNA mass ratios of 0.9 and 1.5 resulted predominantly in one or two nucleosomal octamers per 437-bp probe, under these reconstitution conditions. Full-length CTF-1 and the proline-rich domain-truncated derivative CTF-1 Δ (amino acids 1–220) were overexpressed in HeLa cells using recombinant vaccinia virus vectors and purified to homogeneity as described previously (Dusserre and Mermod 1992).

Acknowledgments

We would like to thank Drs. B. Amati, D. Haas, S. Hraba-Renvey, P. Mitchell, R. Roeder, W. Schaffner, D. Schümperli, A. Seiler-Thuyns, and A. Skoultschi for the gift of plasmids and

strains. We are grateful to Dr. R. Treisman for the gift of the HeLa cDNA library and for plasmids and to Dr. C. Schild for purified histones and helpful discussions. We acknowledge Drs. A. Wolffe, G. Krey, P. Devchand, and E. Farmer for critical reading of the manuscript. N.M. was supported by a career development START award and by a grant from the Swiss National Science Foundation and the Etat de Vaud.

The publication costs of this article were defrayed in part by payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact.

References

- Archer, T.K., P. Lefebvre, R.G. Wolford, and G.L. Hager. 1992. Transcription factor loading on the MMTV promoter: A bimodal mechanism for promoter activation. *Science* **255**: 1573–1576.
- Arents, G., R.W. Burlingame, B.W. Wang, W.E. Love, and E.N. Moudrianakis. 1991. The nucleosomal core histone octamer at 3.1 Å resolution: A tripartite protein assembly and left-handed superhelix. *Proc. Natl. Acad. Sci.* **88**: 10148–10152.
- Armentero, M.-T., M. Horwitz, and N. Mermod. 1994. Targeting of DNA polymerase to the Adenovirus origin of DNA replication by interaction with Nuclear Factor I. *Proc. Natl. Acad. Sci.* **91**: 11537–11541.
- Border, W.A., S. Okuda, L.R. Languino, M.B. Sporn, and E. Ruoslahti. 1990. Suppression of experimental glomerulonephritis by antiserum against transforming growth factor β 1. *Nature* **346**: 371–374.
- Cardinaux, J.-R., S. Chapel, and W. Wahli. 1994. Complex organization of CTF/NF-I, C/EBP, and HNF3 binding sites within the promoter of the liver-specific vitellogenin gene. *J. Biol. Chem.* **269**: 32947–32956.
- Carey, M., H. Kakidani, J. Leatherwood, F. Mostashari, and M. Ptashne. 1989. An aminoterminal fragment of GAL4 binds DNA as a dimer. *J. Mol. Biol.* **209**: 423–432.
- Cheng, G., A. Nandi, S. Clerk, and A.I. Skoultschi. 1989. Different 3'-end processing produces two independently regulated mRNAs from a single H1 histone gene. *Proc. Natl. Acad. Sci.* **86**: 7002–7006.
- Cheng, L. and T.J. Kelly. 1989. Transcriptional activator Nuclear Factor I stimulates the replication of SV40 minichromosomes in vivo and in vitro. *Cell* **59**: 541–551.
- Chiang, C.-M. and R.G. Roeder. 1995. Cloning of an intrinsic human TFIID subunit that interacts with multiple transcriptional activators. *Science* **267**: 531–536.
- Corthésy, B., P. Léonard, and W. Wahli. 1990. Transcriptional potentiation of the vitellogenin B1 promoter by a combination of both nucleosome assembly and transcription factors: An in vitro dissection. *Mol. Cell. Biol.* **10**: 3926–3933.
- Dalton, S. and R. Treisman. 1992. Characterization of SAP-1, a protein recruited by Serum Response Factor to the serum response element. *Cell* **68**: 597–612.
- DePamphilis, M. 1993. How transcription factors regulate the origin of DNA replication in eukaryotic cells. *Trends Cell Biol.* **3**: 161–167.
- Dusserre, Y. and N. Mermod. 1992. Purified cofactors and histone H1 mediate transcriptional regulation by CTF/NF-I. *Mol. Cell. Biol.* **12**: 5228–5237.
- Fisher-Adams, G. and M. Grunstein. 1995. Yeast histone H4 and H3 N-termini have different effects on the chromatin structure of the *GAL1* promoter. *EMBO J.* **14**: 1468–1477.
- Ge, H. and R.G. Roeder. 1994. Purification, cloning and characterization of a human coactivator, PC4, that mediates transcriptional activation of class II genes. *Cell* **78**: 513–523.
- Gill, G., E. Pascal, Z.H. Tseng, and R. Tjian. 1994. A glutamine-rich hydrophobic patch in transcription factor Sp1 contacts the dTAF_{II}110 component of the Drosophila TFIID complex and mediates transcriptional activation. *Proc. Natl. Acad. Sci.* **91**: 192–196.
- Gruber, A., A. Streit, M. Reist, P. Benninger, R. Böhni, and D. Schümperli. 1990. Structure of a mouse histone-encoding cluster. *Gene* **95**: 303–304.
- Herskowitz, I., B. Andrews, W. Kruger, J. Ogas, A. Sil, C. Coburn, and C. Peterson. 1992. Integration of multiple regulatory inputs in the control of *HO* expression in yeast. In *Transcriptional regulation* (ed. S.L. McKnight and K.R. Yamamoto), pp. 949–974. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
- Hoffmann, A. and R.G. Roeder. 1991. Purification of his-tagged proteins in non-denaturing conditions suggests a convenient method for protein interaction studies. *Nucleic Acids Res.* **19**: 6337–6338.
- Hoffmann, A., E. Sinn, T. Yamamoto, J. Wang, A. Roy, M. Horiuchi, and R.G. Roeder. 1990. Highly conserved core domain and unique N terminus with presumptive regulatory motifs in a human TATA factor (TFIID). *Nature* **346**: 387–390.
- Hraba-Renevey, S. and M. Kress. 1989. Expression of a mouse replacement histone H3.3 gene with a highly conserved 3' noncoding region during SV40- and polyoma-induced G0 to S-phase transition. *Nucleic Acids Res.* **17**: 2449–2461.
- Ignatz, R.A., T. Endo, and J. Massagué. 1987. Regulation of fibronectin and type I collagen mRNA levels by Transforming Growth Factor- β . *J. Biol. Chem.* **262**: 6443–6446.
- Inagaki, Y., S. Truter, and F. Ramirez. 1994. Transforming Growth Factor- β stimulates α 2(I) collagen gene expression through a cis-acting element that contains an Sp1-binding site. *J. Biol. Chem.* **269**: 14828–14834.
- Jones, K., J.T. Kadonaga, P.J. Rosenfeld, T. Kelly, and R. Tjian. 1987. A cellular DNA-binding protein that activates eukaryotic transcription and DNA replication. *Cell* **48**: 79–89.
- Kähäri, V.-M., Y.Q. Chen, M.W. Su, F. Ramirez, and J. Uitto. 1990. Tumour Necrosis Factor- α and Interferon- γ suppress the activation of human type I collagen gene expression by Transforming Growth Factor- β 1. *J. Clin. Invest.* **86**: 1489–1495.
- Kim, T.K. and R.G. Roeder. 1994. Proline-rich activator CTF1 targets the TFIIB assembly step during transcriptional activation. *Proc. Natl. Acad. Sci.* **91**: 4170–4174.
- Kingsley, D.M. 1994. The TGF- β superfamily: New members, new receptors, and new genetic tests of function in different organisms. *Genes & Dev.* **8**: 133–146.
- Lewin, B. 1994. Chromatin and gene expression: Constant questions, but changing answers. *Cell* **79**: 397–406.
- Li, R. and M.R. Botchan. 1993. The acidic transcriptional activation domains of VP16 and p53 bind the cellular replication protein A and stimulate in vitro BPV-1 DNA replication. *Cell* **73**: 1207–1221.
- Lillie, J.W. and M.R. Green. 1989. Transcription activation by the adenovirus E1A protein. *Nature* **338**: 39–44.
- Mahadevan, L.C., A.C. Willis, and M.J. Barratt. 1991. Rapid histone H3 phosphorylation in response to growth factors, phorbol esters, okadaic acid, and protein synthesis inhibitors. *Cell* **65**: 775–783.
- Maniatis, T. and R. Tjian. 1994. Transcriptional activation: A complex puzzle with few easy pieces. *Cell* **77**: 5–8.
- Martinez, E., Y. Dusserre, W. Wahli, and N. Mermod. 1991. Synergistic transcriptional activation by CTF/NF-I and the Estrogen Receptor involves stabilized interactions with a limiting target factor. *Mol. Cell. Biol.* **11**: 2937–2945.

- Massagué, J. 1990. The Transforming Growth Factor- β family. *Annu. Rev. Cell Biol.* **6**: 597–641.
- Mauviel, A., J.-C. Lapière, C. Halcin, C.H. Evans, and J. Uitto. 1994. Differential cytokine regulation of type I and type VII collagen gene expression in cultured human dermal fibroblasts. *J. Biol. Chem.* **269**: 25–28.
- Mermod, N., E.A. O'Neill, T.J. Kelly, and R. Tijan. 1989. The proline-rich transcriptional activator of CTF/NF-I is distinct from the replication and DNA binding domain. *Cell* **58**: 741–753.
- Morse, R.H. 1993. Nucleosome disruption by transcription factor binding in yeast. *Science* **262**: 1563–1566.
- Ohana, B., P.A. Moore, S.M. Ruben, C.D. Southgate, M.R. Green, and G.A. Rosen. 1993. The type 1 Human Immunodeficiency Virus Tat binding protein is a transcriptional activator belonging to an additional family of evolutionarily conserved genes. *Proc. Natl. Acad. Sci.* **90**: 138–142.
- Paranjape, S.M., R.T. Kamakaka, and J.T. Kadonaga. 1994. Role of chromatin structure in the regulation of transcription by RNA polymerase II. *Annu. Rev. Biochem.* **63**: 265–297.
- Prelich, G. and F. Winston. 1993. Mutations that suppress the deletion of an upstream activating sequence in yeast: Involvement of a protein kinase and histone H3 in repressing transcription in vivo. *Genetics* **135**: 665–676.
- Pruss, D., J.J. Hayes, and A.P. Wolffe. 1995. Nucleosomal anatomy: Where are the histones? *BioEssays* **17**: 161–170.
- Riccio, A., P.V. Pedone, L.R. Rund, T. Olesen, H.S. Olsen, and P.A. Andreasen. 1992. Transforming growth factor β 1-responsive element: Closely associated binding sites for USF and CCAAT-binding transcription factor—Nuclear factor I in the type 1 plasminogen activator inhibitor gene. *Mol. Cell. Biol.* **12**: 1846–1855.
- Rossi, P., G. Karsenty, A.B. Roberts, N.S. Roche, M.B. Sporn, and B. deCrombrughe. 1988. A nuclear factor I binding site mediates the transcriptional activation of a type I collagen promoter by transforming growth factor- β . *Cell* **52**: 405–414.
- Sadowski, I. and M. Ptashne. 1989. A vector expressing GAL4 (1-147) fusions in mammalian cells. *Nucleic Acids Res.* **17**: 7539.
- Santoro, C., N. Mermod, P. Andrews, and R. Tijan. 1988. A family of human CCAAT-box-binding proteins active in transcription and DNA replication: Cloning and expression of multiple cDNAs. *Nature* **334**: 218–224.
- Schild, C., F.-X. Claret, W. Wahli, and A.P. Wolffe. 1993. A nucleosome-dependent static loop potentiates estrogen-regulated transcription from the *Xenopus* vitellogenin B1 promoter in vitro. *EMBO J.* **12**: 423–433.
- Seiler-Tuyns, A. and M.L. Birnstiel. 1981. Structure and expression in L cells of a cloned H4 histone gene of the mouse. *J. Mol. Biol.* **151**: 607–625.
- Seiler-Tuyns, A., P. Walker, E. Martinez, A.M. Merillat, F. Givel, and W. Wahli. 1986. Identification of estrogen-responsive DNA sequences by transient expression experiments in a human breast cancer cell line. *Nucleic Acids Res.* **14**: 8755–8770.
- Seipel, K., O. Georgiev, and W. Schaffner. 1992. Different activation domains stimulate transcription from remote ("enhancer") and proximal ("promoter") positions. *EMBO J.* **11**: 4961–4968.
- Taylor, C.A., J.L. Workman, T.J. Schuetz, and R.E. Kingston. 1991. Facilitated binding of GAL4 and heat shock factor to nucleosomal templates: Differential function of DNA-binding domains. *Genes & Dev.* **5**: 1285–1298.
- Truss, M., J. Bartsch, A. Schelbert, R.J.G. Haché, and M. Beato. 1995. Hormone induces binding of receptors and transcription factors to a rearranged nucleosome on the MMTV promoter in vivo. *EMBO J.* **14**: 1737–1751.
- Vertese-Dadey, M., P. Walter, H. Chen, L.-J. Juan, and J.L. Workman. 1994. Role of the histone amino termini in facilitated binding of a transcription factor, GAL4-AH, to nucleosome cores. *Mol. Cell. Biol.* **14**: 970–981.
- Wells, D., D. Hoffman, and L. Kedes. 1987. Unusual structure, evolutionary conservation of non-coding sequences and numerous pseudogenes characterize the human H3.3 histone multigene family. *Nucleic Acids Res.* **15**: 2871–2889.
- Williams, T., A. Admon, B. Lüscher, and R. Tijan. 1988. Cloning and expression of AP-2, a cell-type-specific transcription factor that activates inducible enhancer elements. *Genes & Dev.* **2**: 1557–1569.
- Winston, F. and M. Carlson. 1992. Yeast SNF/SWI transcriptional activators and the SPT/SIN chromatin connection. *Trends Genet.* **8**: 387–391.
- Wolffe, A.P. 1994. Nucleosome positioning and modification: Chromatin structures that potentiate transcription. *Trends Biochem. Sci.* **19**: 240–244.
- Workman, J.L. and A.R. Buchman. 1993. Multiple functions of nucleosomes and regulatory factors in transcription. *Trends Biochem. Sci.* **18**: 90–95.
- Wrana, J.L., L. Attisano, J. Carcamo, A. Zentella, J. Doody, M. Laiho, X.-F. Wang, and J. Massagué. 1992. TGF β signals through a heteromeric protein kinase receptor complex. *Cell* **71**: 1003–1014.
- Wrana, J.L., L. Attisano, R. Wieser, F. Ventura, and J. Massagué. 1994. Mechanism of activation of the TGF β receptor. *Nature* **370**: 341–347.
- Xiao, H., J.T. Lis, H. Xiao, J. Greenblatt, and J.D. Friesen. 1994. The upstream activator CTF/NF1 and RNA polymerase II share a common element involved in transcriptional activation. *Nucleic Acids Res.* **22**: 1966–1973.

A proline-rich TGF-beta-responsive transcriptional activator interacts with histone H3.

A Alevizopoulos, Y Dusserre, M Tsai-Pflugfelder, et al.

Genes Dev. 1995, **9**:

Access the most recent version at doi:[10.1101/gad.9.24.3051](https://doi.org/10.1101/gad.9.24.3051)

References

This article cites 59 articles, 22 of which can be accessed free at:
<http://genesdev.cshlp.org/content/9/24/3051.full.html#ref-list-1>

License

Email Alerting Service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or [click here](#).