The EMBO Journal vol.15 no.23 pp.6701–6715, 1996

A possible involvement of TIF1α and TIF1β in the epigenetic control of transcription by nuclear receptors

Bertrand Le Douarin, Anders Lade Nielsen, Jean-Marie Garnier, Hiroshi Ichinose, François Jeanmougin, Régine Losson[1] and Pierre Chambon

Institut de Génétique et de Biologie Moléculaire et Cellulaire, CNRS/INSERM/ULP, Collège de France, BP 163, 67404 Illkirch Cedex, France

[1]Corresponding author

Nuclear receptors (NRs) are ligand-inducible transcription factors that mediate complex effects on development, differentiation and homeostasis. They regulate the transcription of their target genes through binding to cognate DNA sequences as homodimers or heterodimers. The molecular mechanisms underlying transcriptional activation by NRs are still poorly understood, although intermediary factors (mediators) appear to be involved in mediating the transactivation functions of NRs. TIF1 has been identified previously as a protein that interacts specifically with the ligand binding domain of several nuclear receptors, both in yeast and *in vitro*. The characteristics of these interactions have led us to suggest that TIF1 might be a mediator of the NR ligand-inducible activation function AF-2. Using a two-hybrid screening in yeast, we have now identified two TIF1-binding proteins, mHP1α and mMOD1, that are mouse homologues of the *Drosophila* heterochromatinic protein 1. Using mHP1α as a bait in a second two-hybrid screening, we have isolated cDNAs encoding proteins that are also very likely to be involved in chromatin structure and function, as well as a protein structurally and functionally related to TIF1 (renamed TIF1α), which was named TIF1β. Here we discuss how the function of members of the TIF1 family in the control of transcription could be exerted at the level of the structure of the chromatin template.

Keywords: activation function AF-2/chromatin/HP1/SNF2β/transcriptional mediators

Introduction

Nuclear receptors (NRs) are transcriptional factors that control many aspects of development, differentiation and homeostasis upon binding of cognate hydrophobic ligands, such as steroid and thyroid hormones, vitamin D3 and retinoids. They act as homodimers or heterodimers by binding to *cis*-acting DNA response elements present in the regulatory regions of target genes (for reviews see Parker, 1993; Giguère, 1994; Glass, 1994; Tsai and O'Malley, 1994; Beato *et al.*, 1995; Kastner *et al.*, 1995; Keaveny and Stunnenberg, 1995; Mangelsdorf *et al.*, 1995a,b; Thummel, 1995; Gronemeyer and Laudet, 1995; Chambon, 1996). As many of the transcriptional regulatory proteins, NRs display a modular structure with five or six regions (denoted A–F, e.g. see Figure 3B and C) which exhibit different degrees of evolutionary conservation. The N-terminal A/B region contains an autonomous activation function AF-1, which can activate transcription constitutively in the absence of the ligand-binding domain (LBD) contained in region E. The highly conserved region C encompasses the DNA-binding domain (DBD) which is responsible for the specific recognition of cognate response elements (REs). In addition to the LBD, region E contains a dimerization surface and the ligand-dependent transcriptional activation function AF-2 (for references see the above reviews). AF-2 activity critically requires the integrity of an amphipathic α-helical motif, the AF-2 activating domain (AD) core, which is highly conserved and present in all known transcriptionally active members of the NR superfamily (Danielian *et al.*, 1992; Barettino *et al.*, 1994; Durand *et al.*, 1994; Chambon, 1996, and references therein). Both AF-1 and AF-2 can stimulate transcription in yeast cells (for references see Metzger *et al.*, 1988; Heery *et al.*, 1993, 1994) which do not contain NRs, indicating that important features of transcriptional activation have been conserved during evolution.

Transcriptional interference/squelching between the AFs of steroid receptors initially suggested the existence of transcriptional intermediary factors (TIFs, also designated mediators or coactivators) which may mediate the activity of AF-1s and AF-2s (Meyer *et al.*, 1989; Tasset *et al.*, 1990; Barettino *et al.*, 1994, and references therein). This concept was substantiated recently by the isolation and characterization of several proteins that interact with the AF-2-containing LBD of several NRs (steroid, thyroid, vitamin D3 and retinoid receptors) in the presence of agonistic ligands but not in the presence of antagonists. These proteins, which include RIP140 (Cavaillès *et al.*, 1995), TIF1 (Le Douarin *et al.*, 1995a), Trip1/SUG1 (Lee *et al.*, 1995; vom Baur *et al.*, 1996), SRC-1/p160 (Halachmi *et al.*, 1994; Onate *et al.*, 1995; Kamei *et al.*, 1996), CBP (Kamei *et al.*, 1996) and TIF2/Grip1 (Hong *et al.*, 1996; Voegel *et al.*, 1996), have been identified using expression libraries and either far-Western blotting or yeast two-hybrid screens (reviewed in Chambon, 1996). Interestingly, SRC-1/p160 and TIF2 exhibit sequence similarities that indicate the existence of a novel gene family of putative NR mediators (Onate *et al.*, 1995; Kamei *et al.*, 1996; Voegel *et al.*, 1996). The integrity of the AF-2 AD core appears to be mandatory for interactions between NR LBDs and the putative mediators. The discovery that major 3-D conformational changes (particularly those that bring the AF-2 core amphipathic α-helix into a new receptor environment) are triggered by ligand binding strongly suggests that the induction of the

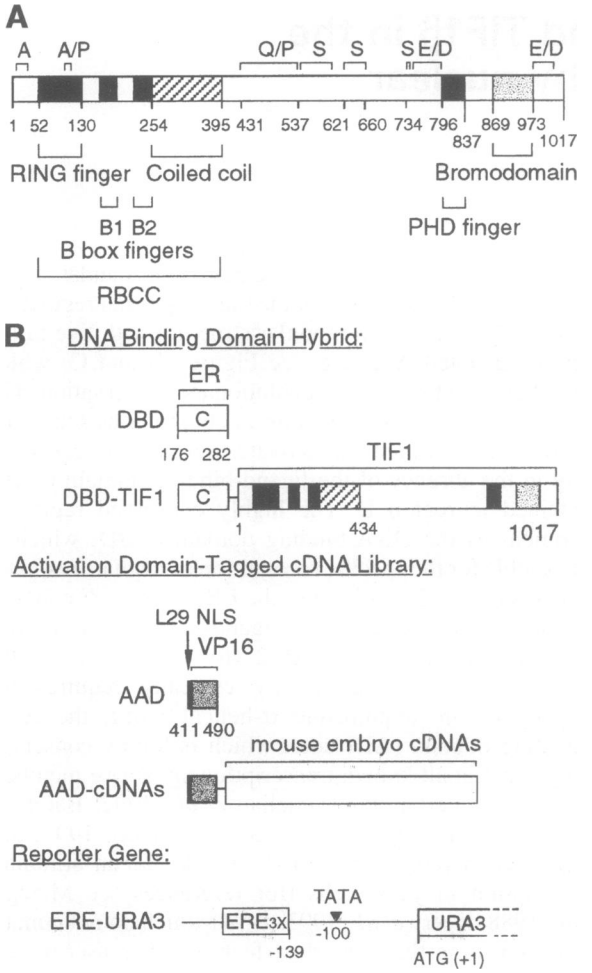


Fig. 1. Two-hybrid screening in yeast using TIF1 (TIF1α) as a bait. **(A)** Scheme of the domain organization of TIF1 (TIF1α). Numbers refer to amino acid positions (Le Douarin *et al.*, 1995a). **(B)** A schematic representation of the DBD of the ER is shown, unfused or fused to the complete coding sequence of TIF1. The VP16 AAD-tagged mouse embryo cDNA expression library is represented below. The VP16 AAD tag also includes codons specifying the nuclear localization signal (NLS) of the yeast ribosomal protein L29. Transcription of the integrated URA3 reporter gene, shown further below, is regulated by three oestrogen REs (ERE3×) in the yeast reporter strain PL3(α).

activation function AF-2 corresponds to the creation of the cognate surfaces required for efficient interaction with these putative mediators (Bourguet *et al.*, 1995; Renaud *et al.*, 1995; Wurtz *et al.*, 1996).

Conceptually, a TIF/mediator capable of mediating the transcriptional ligand-dependent activity of AF-2 may itself contain an autonomous activation function, relieve autosquelching and stimulate ligand-dependent AF-2 activity of the NRs with which it interacts. At present, these properties are exhibited by TIF2 (Voegel *et al.*, 1996) and SRC-1 (Onate *et al.*, 1995), whereas very little stimulation or even an inhibition of AF-2 activity was observed in the case of RIP140 (Cavaillès *et al.*, 1995) and TIF1 (Le Douarin *et al.*, 1995a), respectively.

TIF1 contains several conserved domains, including a RING finger, two B boxes, a coiled-coil domain, a PHD finger and a bromodomain, which are also present in a number of transcriptional regulatory proteins (Le Douarin *et al.*, 1995a, and references therein; see Figure 1A). The observations that TIF1 does not stimulate the AF-2 activity of NRs in transfected cultured cells, and apparently does not interact with any known component of the transcription machinery (Le Douarin *et al.*, 1995a), suggest that it could act as a mediator of an NR AF-2 activity related to chromatin remodelling (Le Douarin *et al.*, 1996). Indeed, it is well established that activation of transcription by NRs *in vivo* involves chromatin rearrangements (reviewed in Beato *et al.*, 1995).

To investigate further how TIF1 could mediate the AF-2 activity of NRs, we have performed a yeast two-hybrid screen using TIF1 as a bait and a mouse embryo cDNA expression library. Interestingly, this screen resulted in the isolation of two TIF1-interacting proteins, mHP1α and mMOD1, which are known to be associated with heterochromatin (Nicol and Jeppesen, 1994; Wreggett *et al.*, 1994) and represent mouse homologues of the heterochromatin-associated *Drosophila* HP1 protein (Singh *et al.*, 1991). Using mHP1α as a bait in a further two-hybrid screen, we identified a protein (TIFβ) exhibiting a domain organization similar to that of TIF1 (renamed TIF1α). Additional mHP1α-interacting proteins isolated in this screen included several proteins that either are known to be or could possibly be involved in chromatin structure and function. Here we discuss how members of the TIF1 gene family may participate in the control of the initiation of transcription in the light of the recent observation that TIF1s can interact with the transcriptional silencing domain of the *Drosophila* Krüppel-related KRAB proteins (P.Moosmann, O.Georgiev, B.Le Douarin and W.Schaffner, in preparation). We propose a tentative model in which TIF1s play a dual role in the control of transcription at the chromatin level, being involved in both repression (silencing), through the formation of 'inactive' heterochromatin-like 'condensed' structures, and activation, through reversion to 'active' euchromatin-like 'open' structures.

Results

TIF1 interacts with two mouse homologues of Drosophila heterochromatin protein 1 (dHP1) in yeast and in vitro

The yeast two-hybrid system (Fields and Sternglanz, 1994) was used to isolate cDNAs encoding proteins that interact with TIF1. A fusion between the LexA protein (amino acids 1–202) and the C-terminal half of TIF1 (amino acids 434–1017; see Figure 1A) was used as a bait to screen a library of mouse embryo cDNAs fused to the DNA sequence encoding the acidic activation domain (AAD) of the VP16 protein (Figure 1B; vom Baur *et al.*, 1996; see Materials and methods). Approximately 5×10^6 yeast transformants were screened; 68 were classified as positive when retested in another version of the two-hybrid system using the DBD of the oestrogen receptor (ER; amino acids 176–282) fused to full-length TIF1 (DBD–TIF1; Figure 1B) or an unfused ER DBD as a control in the reporter strain PL3(α), which contains a URA3 reporter gene driven by three oestrogen REs (ERE–URA3 in Figure 1B; Le Douarin *et al.*, 1995a,b). Sequence analyses revealed 11 classes of positive clones, designated TIF1-BPs (for TIF1-binding proteins). Most of these clones corresponded to previously uncharacterized genes and will be described

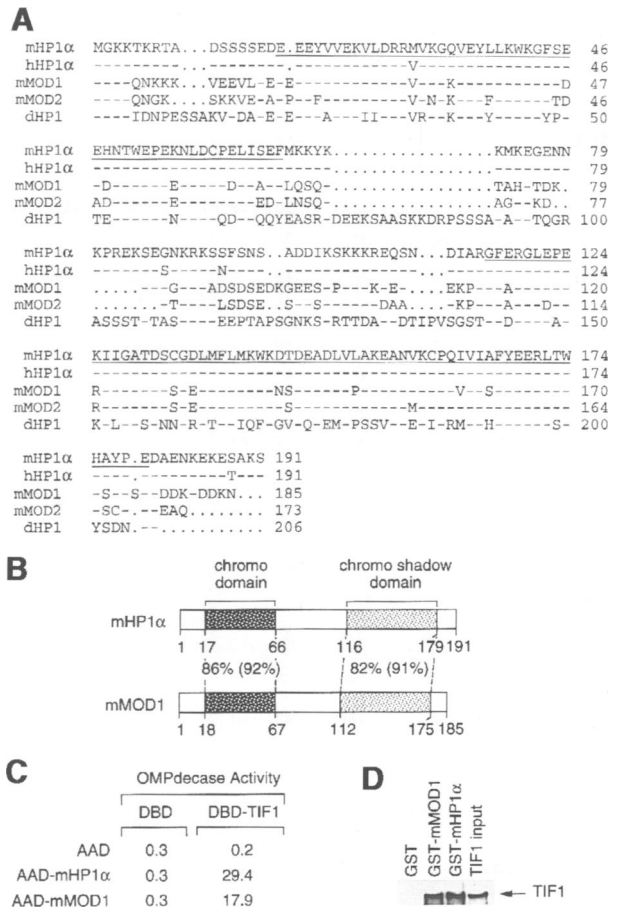


Fig. 2. TIF1 (TIF1α) interacts with two mouse homologues of dHP1. (**A**) Comparison of the predicted amino acid sequences of mouse HP1α, human HP1α (HP1^{Hsα}; Saunders *et al.*, 1993), mouse MOD1 (M31; Singh *et al.*, 1991), mouse MOD2 (M32; Singh *et al.*, 1991) and dHP1 (Clark and Elgin, 1992). Positions of amino acid identity with mHP1α are represented by dashes, while dots represent gaps used to align the sequences. The chromo and chromo shadow domains are underlined (see text). (**B**) Schematic alignment of mHP1α and mMOD1. The chromo and chromo shadow domains are represented, with the percentage of amino acid identity (similarity in parentheses) noted below each domain. (**C**) mHP1α and mMOD1 interact with TIF1 in yeast. Plasmids expressing mHP1α or mMOD1 fused to the VP16 AAD were introduced into the yeast reporter strain PL3(α), together with the unfused ER DBD or DBD–TIF1. Transformants were grown in liquid medium containing uracil. Extracts were prepared and assayed for OMPdecase activity, which is expressed in nmol substrate/min/mg protein. (**D**) mHP1α and mMOD1 interact with TIF1 *in vitro*. Purified His-TIF1 was incubated in a batch assay with 'control' GST (lane 1), GST–mMOD1 (lane 2) or GST–mHP1α (lane 3) bound to glutathione-S–Sepharose beads. Bound TIF1 was detected by Western blotting. Lane 4 shows 1/10 the amount of input His-TIF1, the position of which is indicated by an arrow.

elsewhere. We have focused here on two TIF1-BPs, TIF1-BP1 and TIF1-BP2 (see Materials and methods for a detailed description of the library isolates), both of which are mouse homologues of dHP1 (Clark and Elgin, 1992; Figure 2A). TIF1-BP1 is a novel mouse protein which is 53% identical to dHP1 and 98% identical to the human HP1 homologue HP1^{Hsα} (Saunders *et al.*, 1993; referred to as hHP1α in Figure 2A). TIF1-BP1 differs from hHP1α at only four amino acid positions (Figure 2A) and was therefore termed mHP1α. TIF1-BP2 is identical to the previously identified mouse modifier 1 protein (mMOD1; Singh *et al.*, 1991) that is 52% and 66% identical to dHP1 and mHP1α, respectively (Figure 2A). dHP1 and all three of its mammalian homologues identified so far, namely HP1α, MOD1 and MOD2 (Singh *et al.*, 1991; Figure 2A), belong to a subfamily of the chromatin organization modifier (chromo) superfamily (Aasland and Stewart, 1995, and references therein). These proteins not only have an N-terminal chromo domain similar to that found in the N-terminal part of some chromatin-binding proteins involved in gene silencing, such as the *Drosophila* Polycomb protein (Pc) (Figure 2B and Discussion), but also possess another chromo domain-like motif, the C-terminal chromo shadow domain (Aasland and Stewart, 1995; Figure 2B), which is sufficient for the binding of dHP1 to heterochromatin (Powers and Eissenberg, 1993; Platero *et al.*, 1995, 1996).

To characterize further the interactions between TIF1 and either mHP1α or mMOD1 in yeast, the entire coding sequences of mHP1α and mMOD1 were fused to the AAD of VP16 and the resulting hybrid proteins (AAD–mHP1α and AAD–mMOD1, respectively; Figure 2C) expressed in the reporter strain PL3(α) with either the unfused ER DBD (as a control) or DBD–TIF1 (Figure 1B). Activation of the URA3 reporter gene by these hybrid proteins was determined by measuring the activity of the URA3 gene product, orotidine 5′-monophosphate decarboxylase (OMPdecase; Figure 2C). When expressed with the DBD or the AAD controls, none of the hybrid proteins transactivated the URA3 reporter. In contrast, coexpression of DBD–TIF1 with either AAD–mHP1α or AAD–mMOD1 resulted in 60- to 100-fold increases in OMPdecase activity (Figure 2C), indicating that TIF1 interacts functionally with both mHP1α and mMOD1 in yeast cells.

To investigate whether these interactions correspond to direct interactions, binding assays were performed *in vitro* using purified recombinant proteins. Glutathione *S*-transferase (GST) fusion proteins containing the coding sequences of either mHP1α or mMOD1 (GST–mHP1α and GST–mMOD1, respectively; Figure 2D) were expressed in *Escherichia coli*, immobilized on glutathione–Sepharose beads and incubated with purified histidine-tagged TIF1 (His-TIF1; Le Douarin *et al.*, 1995a). The matrix-associated TIF1 was revealed by Western blotting. His-TIF1 was retained on beads coupled to either GST–mHP1α or GST–mMOD1, but not on 'control' GST beads (Figure 2D). Thus, TIF1 can interact with either mHP1α or mMOD1 both in yeast and *in vitro*.

NRs and the mouse HP1 homologues interact with two adjacent but distinct domains of TIF1

Various TIF1 mutants were fused to the ER DBD to map the region(s) required for interaction with mHP1α and mMOD1 with respect to the region shown previously to be sufficient for retinoid X receptor (RXR) α binding (residues 539–750; Le Douarin *et al.*, 1995a). A mutant lacking the N-terminal residues 1–433 of TIF1, which include the RING finger, the B boxes and the coiled-coil motif, and the C-terminal residues 751–1017, which contain the PHD finger and the bromodomain, retained its ability to interact in yeast with mHP1α, mMOD1 and RXRα [DBD–TIF1(434–750); Figure 3A; Le Douarin *et al.*, 1995a]. In contrast, a deletion mutant containing only residues 701–750 of TIF1 failed to interact with

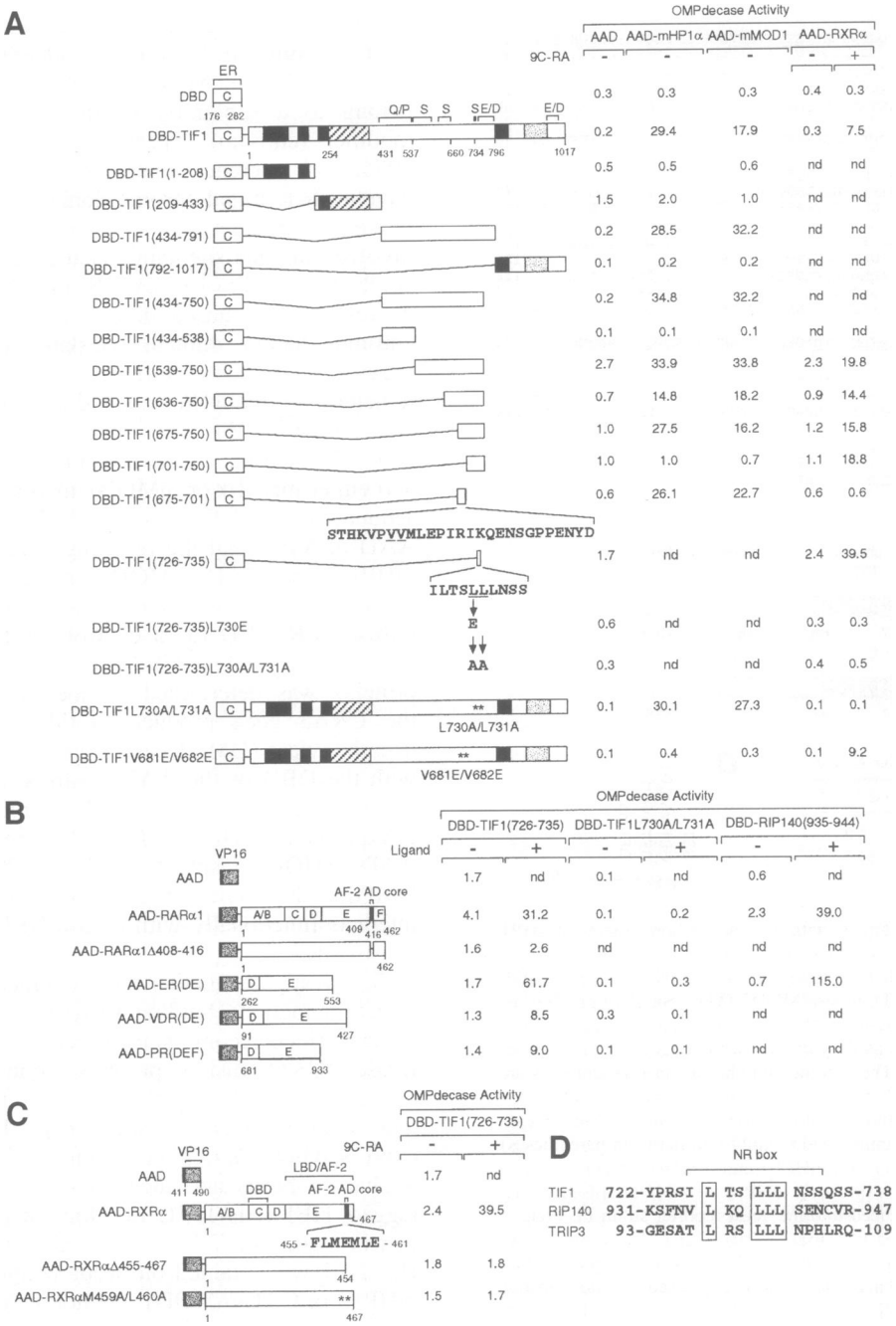


Fig. 3. NRs and the mouse HP1 homologues interact with two close but distinct domains of TIF1 (TIF1α). (**A**) Mapping of the mHP1α-, mMOD1- and RXRα-interacting domains within TIF1. Plasmids expressing TIF1 or individual regions of TIF1 fused to the ER DBD were introduced into PL3(α) together with VP16 AAD or AAD fusions, as indicated. Transformants were grown in the presence (+) or absence (–) of 1 μM 9 *cis*-retinoic acid (9C-RA). OMPdecase activities determined on each cell-free extract are expressed in nmol/min/mg protein. (**B**) Amino acids 726–735 of TIF1 and amino acids 935–944 of RIP140 (Cavaillès *et al.*, 1995) are sufficient for ligand-dependent interaction with NRs. DBD–TIF1(726–735), DBD–TIF1L730A/L731A or DBD–RIP140(935–944) were coexpressed in PL3(α) with AAD or AAD fusion receptors, as indicated to the left. Transformants were grown in the presence (+) or absence (–) of the cognate ligand (1 μM all-*trans* retinoic acid for RAR, 1 μM oestradiol for ER, 5 μM vitamin D3 for VDR, 10 μM R5020 for PR). OMPdecase activities are expressed as in (A). (**C**) The integrity of the RXRα AF-2 AD core is required for interaction with amino acids 726–735 of TIF1 (the NR box). The indicated mutants of RXRα were fused to the AAD of VP16 and assayed for interaction with the DBD–TIF1(726–735) fusion construct in the yeast reporter strain PL3(α) grown in the presence of 1 μM 9C-RA. OMPdecase activities are given as in (A). (**D**) Sequence alignment of the NR-interacting domain of TIF1 (residues 726–735) with RIP140 (Cavaillès *et al.*, 1995) and TRIP3 (Lee *et al.*, 1995) revealed a highly conserved region, referred to as the NR box. The conserved leucine residues are boxed. Note that the expression of all DBD and AAD fusion proteins indicated was confirmed by Western blotting using the F3 antibodies against the F region of ER and 2GV-4 against VP16, respectively (data not shown). In (A)–(C) the values (±10%) are the average of at least three independent experiments.

mHP1α and mMOD1, while still interacting with RXRα in a ligand-dependent manner (Figure 3A). Another deletion derivative containing residues 675–701 interacted with mHP1α or mMOD1 but not with RXRα (Figure 3A). Thus, two near but non-overlapping domains of TIF1 can interact with HP1 homologues and RXRα in yeast.

A minimal RXR-interacting domain was mapped between residues 726 and 735 of TIF1 (Figure 3A). Retinoic acid receptor (RAR) α and the LBD of either the oestrogen (ER), vitamin D3 (VDR) or progesterone (PR) receptor all interacted with the same region of TIF1 (Figure 3B). These interactions were ligand dependent (Figure 3B) and abolished by mutations within the core of the AF-2 AD of NRs (AF-2 AD core; Figure 3B and C). In contrast to wild-type RXRα, a C-terminally truncated mutant RXRαΔ455–467 lacking the AF-2 AD core, which is essential for AF-2 activity (Le Douarin *et al.*, 1995a), did not functionally interact with residues 726–735 of TIF1 (Figure 3C). Similarly, a point mutant RXRαM459A/L460A, in which the conserved hydrophobic residues M459 and L460 of the AF-2 AD core were replaced by alanine residues (AAD–RXRαM459A/L460A; Figure 3C), and a RARα mutant lacking the AF-2 AD core (AAD–RARΔ408–416; Figure 3B), both of which have lost AF-2 activity while still binding ligand (Durand *et al.*, 1994; Le Douarin *et al.*, 1995a), were unable to interact with residues 726–735 of TIF1. Thus, TIF1 contains a 10 amino acid-long sequence that is sufficient on its own to functionally interact with NRs in both a ligand- and AF-2 AD core-dependent manner, like full-length TIF1 (Le Douarin *et al.*, 1995a; vom Baur *et al.*, 1996). Similar amino acid sequences are present in RIP140 (Cavaillès *et al.*, 1995) and TRIP3 (Lee *et al.*, 1995), two other NR-interacting proteins (Figure 3D). In RIP140, it is localized in a region of the protein that interacts with ER (Cavaillès *et al.*, 1995). This RIP140 sequence, like that of TIF1, exhibited an NR-interacting activity when tested for interaction with RARα and the LBD of ER in the yeast reporter strain PL3 (Figure 3B). These homologous sequences were therefore termed NR boxes (Figure 3D). Mutations were introduced into the NR box of TIF1, replacing the conserved leucine residue L730 by a glutamic acid residue, or both the L730 and L731 leucine residues by alanine residues. These mutations yielded mutants [DBD–TIF1(726–735)L730E and DBD–TIF1(726–735) L730A/L731A, respectively] that failed to interact with RXRα in yeast (Figure 3A). To investigate whether TIF1 actually binds NRs through the NR box, the double mutation L730A/L731A was introduced in the context of full-length TIF1. No interaction was detected with any of the NRs tested, while binding to both mHP1α and mMOD1 was not affected (DBD–TIF1L730A/L731A; Figure 3A and B), indicating that TIF1 requires an intact NR box to interact with NRs but not with HP1 homologues.

Two other point mutations were introduced into the mHP1α/mMOD1-interacting domain of TIF1 (residues 675–701; hereafter designated the HP1 box), replacing the hydrophobic valine residues V681 and V682 by glutamic acid residues. In contrast to wild-type TIF1, the mutant protein (DBD–TIF1V681E/V682E; Figure 3A) was drastically impaired in its ability to interact with mHP1α and mMOD1 but not with RXRα (Figure 3A), indicating that valine at position 681 and/or valine at position 682 are critical for the interaction of TIF1 with HP1 homologues but not with RXRα. Therefore these two TIF1 interactions are genetically separable, which is consistent with the above conclusion that HP1 homologues and NRs interact with two near but distinct domains of

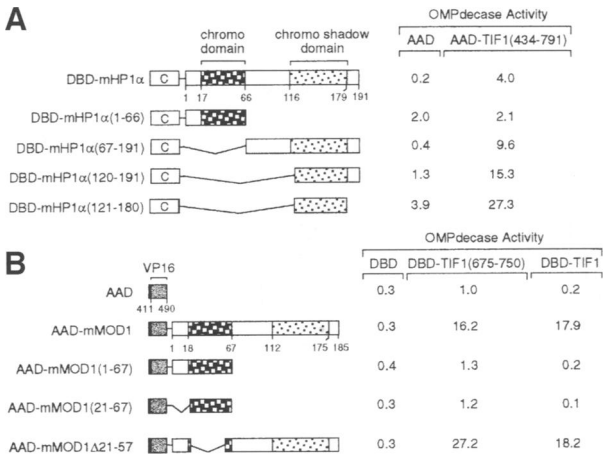


Fig. 4. The chromo shadow domains of mHP1α and mMOD1 are sufficient for interaction with TIF1 (TIF1α). (**A**) TIF1 interacts with the chromo shadow domain, but not the chromo domain, of mHP1α. Plasmids expressing mHP1α or deletion mutants of mHP1α fused to the DBD of ER were cotransformed into PL3(α) with AAD or AAD-TIF1(434–791). OMPdecase activities were expressed in nmol substrate/min/mg protein. (**B**) The chromo domain of mMOD1 is neither sufficient nor essential for interaction with TIF1. Various regions of mMOD1 were fused to the VP16 AAD and coexpressed with DBD, DBD–TIF1(675–750) or DBD–TIF1. OMPdecase activities were expressed as in (A). All proteins were expressed in yeast as assayed by Western blotting (data not shown).

TIF1. Note that no interaction was detected between NRs and HP1 homologues in yeast (data not shown).

The chromo shadow domain-containing moiety of mHP1α and mMOD1 is sufficient for interaction with TIF1

To identify the mHP1α and mMOD1 sequences required for interaction with TIF1, several deletion mutants were assayed for their functional interaction with TIF1 in yeast (Figure 4A and B). No increase in reporter gene activity was detected when a DBD fusion protein bearing mHP1α residues 1–66 was coexpressed with AAD–TIF1(434–791), indicating that the N-terminal region of mHP1α, which includes the chromo domain, is not sufficient for interaction with TIF1 (Figure 4A). In contrast, a TIF1-dependent activation was observed in the presence of N-terminally truncated fusion proteins lacking the chromo domain [DBD–mHP1α(67–191) and DBD–mHP1α(120–191); Figure 4A] or in the presence of a fusion protein bearing residues 121–180 of the chromo shadow domain [DBD–mHP1α(121–180); Figure 4A]. Similarly, mMOD1 derivatives lacking the C-terminal residues 67–185, which include the chromo shadow domain, failed to interact with TIF1 [see AAD–mMOD1(1–67) and AAD–mMOD1(21–67) coexpressed with DBD–TIF1(675–750) or DBD–TIF1; Figure 4B], whereas a mutant bearing an internal deletion of the chromo domain interacted with TIF1 (Figure 4B). Thus, the chromo domains of mHP1α and mMOD1 are neither sufficient nor essential for interaction with TIF1, whereas their C-terminal half, which contains the chromo shadow domain, can interact functionally with TIF1 in yeast.

Isolation of cDNAs for proteins that interact with mHP1α

A yeast two-hybrid screen was used to isolate cDNAs for proteins that interact with mHP1α. A fusion between the

Table I. Mouse embryo cDNA clones encoding mHP1α-binding proteins

Library isolate AAD fusion	Insert size[a]	GeneBank match		Relative OMPdecase activity in yeast expressing DBD–mHP1α
		Identity	Similarity[b]	
No	–	–	–	1.0
HP1-BP71	185	mMOD1	–	4.5
HP1-BP78	115	mHP1α	–	3.0
HP1-BP72	340	–	hSNF2β	24.0
HP1-BP84	513	–	bLAP	17.0
HP1-BP77	712	–	TIF1	11.0
HP1-BP38	852	–	hRAD54L	16.0
HP1-BP74	281	–	H1 and H1 variants	6.0

[a]Number of amino acids in frame with AAD.
[b]Similarity based on BLAST algorithm with Poisson probabilities $<10^{-8}$.

LexA protein and the coding sequence of mHP1α was used as a bait to screen the library of mouse embryo cDNAs fused to the AAD of VP16 (AAD–cDNAs; Figure 1B). Approximately 10^6 yeast transformants were screened, of which 11 were classified as positive when retested in the URA3-based two-hybrid system using the DBD of ER fused to mHP1α (DBD–mHP1α; Figure 4) or an unfused ER DBD as a control in the yeast reporter strain PL3(α). A complete or partial sequence analysis of these clones, designated HP1-BPs, revealed two previously identified genes and five new genes (Table I). The two known genes correspond to mMOD1 and mHP1α, respectively (Table I). Thus, in yeast cells, mHP1α can form homodimers or higher order homomultimer complexes, as well as heterodimers or higher order heteromultimer complexes, with mMOD1.

Of the five new cDNAs, two were found to encode proteins with a particularly high degree of sequence similarity to known proteins in other species (Table I). The 340 amino acids of HP1-BP72 are 99% identical to residues 295–634 of the human BRG1/SNF2β protein, which has been reported to cooperate with NRs in transcriptional activation (Khavari *et al.*, 1993; Chiba *et al.*, 1994) presumably through a chromatin-mediated mechanism (reviewed in Peterson, 1996; see Discussion). This protein was referred to as mSNF2β for mouse homologue of human SNF2β/BRG1. It apparently interacts specifically with mHP1α, as it was unable to interact with mMOD1 in yeast (data not shown). Note that a HeLa cell immunopurified SNF2β-containing SWI–SNF complex also interacted with mHP1α, but not with mMOD1, *in vitro* (data not shown). HP1-BP84 is 87% identical to the bovine leucine amino peptidase protein (bLAP; Table I), an enzyme presumably involved in the processing and turnover of intracellular proteins (Cuypers *et al.*, 1992); it therefore encodes a mouse homologue of this protein. Another novel protein, encoded by HP1-BP77 (Table I), showed significant homology to TIF1 and was therefore named TIF1β, while the original TIF1 was renamed TIF1α (see below).

In addition to these extensive similarities, two other HP1-BPs were isolated that contain regions exhibiting similarities to known proteins. The C-terminal portion of the HP1-BP38 sequence shares 57% identity and 74% similarity over 338 amino acids with the N-terminal portion of the human RAD54-like protein, a putative X-linked gene-encoded helicase (Stayton *et al.*, 1994; Figure 5A). The 70 C-terminal residues of HP1-BP74 showed significant similarity to the globular domains of histone H1 and its variants (Cerf *et al.*, 1993, and references

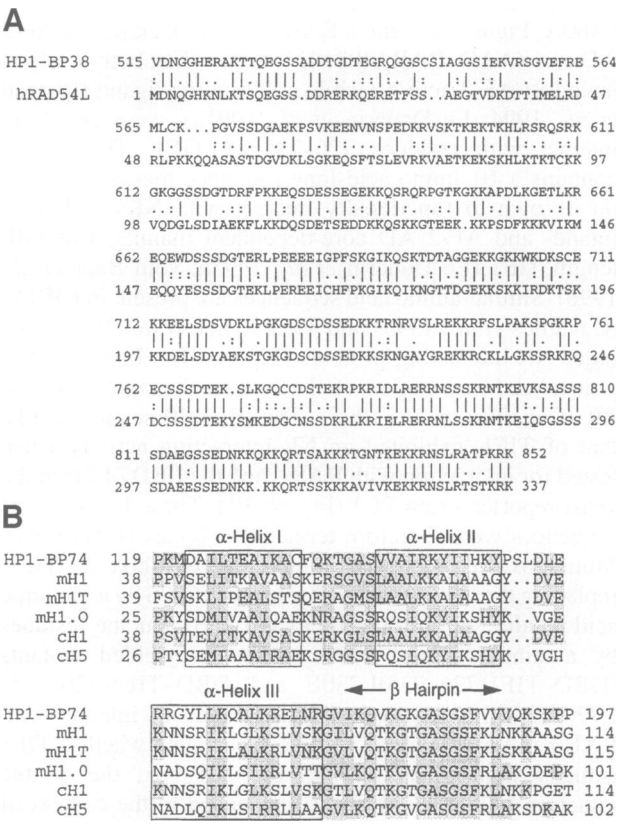


Fig. 5. Sequence similarities between two HP1-BPs and known proteins. (**A**) The 338 C-terminal residues of HP1-BP38 are shown aligned with the N-terminal residues of the human RAD54-like protein (Stayton *et al.*, 1994) using the GCG program BESTFIT. Vertical lines indicate identical amino acids; colons and points indicate conservative changes. (**B**) The predicted primary and secondary structures of the C-terminal part of HP1-BP74 are similar to those of the globular domains of histones H1 and H5 (see text). The amino acid sequence of HP1-BP74 is shown aligned with mouse histone H1 (mH1; accession No. M25365), mouse histone H1T (mH1T; accession No. Q07133), mouse histone H1.0 (mH1.0; accession No. P10922), chicken histone H1 (cH1; accession No. P09987) and chicken histone H5 (cH5; accession No. P02259) using the CLUSTAL W program (Thompson *et al.*, 1994b). Shaded boxes indicate residues that are identical or conserved between HP1-BP74 and the other proteins. A secondary structure prediction generated with the program PHD (Rost and Sander, 1994) is shown.

therein; 33% identity and 63% similarity with the globular domain of the mouse histone H1; Figure 5B). When secondary structural prediction methods were applied to this region of HP1-BP74, three α-helices and a β-hairpin were predicted (Figure 5B). This arrangement is similar to the structure of the globular domains of histones H1 (Cerf *et al.*, 1993) and H5 (Ramakrishnan *et al.*, 1993), determined by NMR spectroscopy and X-ray crystallography, respectively.

Note that all the HP1-BPs isolated interacted with the chromo shadow domain of mHP1α, and all of them, except HP1-BP72 and HP1-BP74, showed an interaction with DBD–mMOD1 in yeast (data not shown).

TIF1β: an HP1-binding protein structurally related to TIF1α

The amino acid sequence of TIF1β was deduced from the sequences of overlapping cDNA clones and a genomic clone encompassing the 5′ region of the cDNA (Figure 6). Over their entire length, TIF1α and TIF1β amino acid sequences exhibit 32% identity and 54% similarity. The homology is stronger in the N- and C-terminal regions spanning the conserved domains than in the central region (Figure 7A). The first region of homology between the two proteins, which is characteristic of the RBCC subfamily of RING finger proteins (Freemont, 1993; Le Douarin *et al.*, 1995a), contains a RING finger preceding one or two B box-type fingers and a putative coiled-coil domain (Figures 6 and 7A). This tripartite motif has been found in the N-terminal part of several putative transcription factors, ribonucleoproteins and proto-oncogene products, including PML, efp, RFP, RPT-1, SS-A/RO, XNF7 and PWA33 (Bellini *et al.*, 1993; for a review see Freemont, 1993; Inoue *et al.*, 1993). An amino acid comparison of the RBCC motifs of these proteins revealed only limited sequence identity, except between TIF1α and TIF1β which contain several highly conserved sequences, each of ~10 amino acids in length, within the RING finger, the B boxes and the coiled-coil motif (Figure 7B).

The second region of homology between TIF1α and TIF1β is located in their C-terminal parts and consists of a C4HC3 zinc finger or PHD finger followed by a bromodomain (Figures 6 and 7C). The bromodomain was identified originally as a conserved sequence motif of ~65 amino acids containing two predicted α-helices A and B (Haynes *et al.*, 1992; underlined in Figure 7C). Note that TIF1β contains a bromodomain motif which diverges from the consensus motif by lacking a number of conserved residues on the N-terminal side of the predicted α-helix A of the domain (Figure 7C). Interestingly, when secondary structural prediction methods are applied to an extended version of the original bromodomain, two additional α-helices, designated Z and C, respectively (Figure 7C), can be predicted at the N- and C-terminal sides of all bromodomains identified so far (F.Jeanmougin, J.M.Wurtz, B.Le Douarin, P.Chambon and R.Losson, manuscript in preparation). While the three predicted α-helices A, B and C span a number of residues conserved amongst all members of the bromodomain family, including TIF1α and TIF1β (Figure 7C), the α-helix Z sequence is well conserved only between closely related proteins. Thus, residues of helix Z of TIF1α, hSNF2α and p300 are highly similar to those of helix Z of TIF1β, hSNF2β and CBP, respectively (Figure 7C). In contrast, there is very little similarity between helices Z of two bromodomains present within a single protein (e.g. RING3/FSH-1 and RING3/FSH-2; Figure 7C) and between helices Z of bromodomains belonging to unrelated members of the family that, outside of the bromodomain, do not share any obvious similarity (e.g. TIF1, SNF2, CBP, RING3/FSH; Figure 7C). Thus, helix Z may provide specificity for an as yet uncharacterized activity carried out by the conserved or core part of the bromodomain (F.Jeanmougin, J.M.Wurtz, B.Le Douarin, P.Chambon and R.Losson, manuscript in preparation).

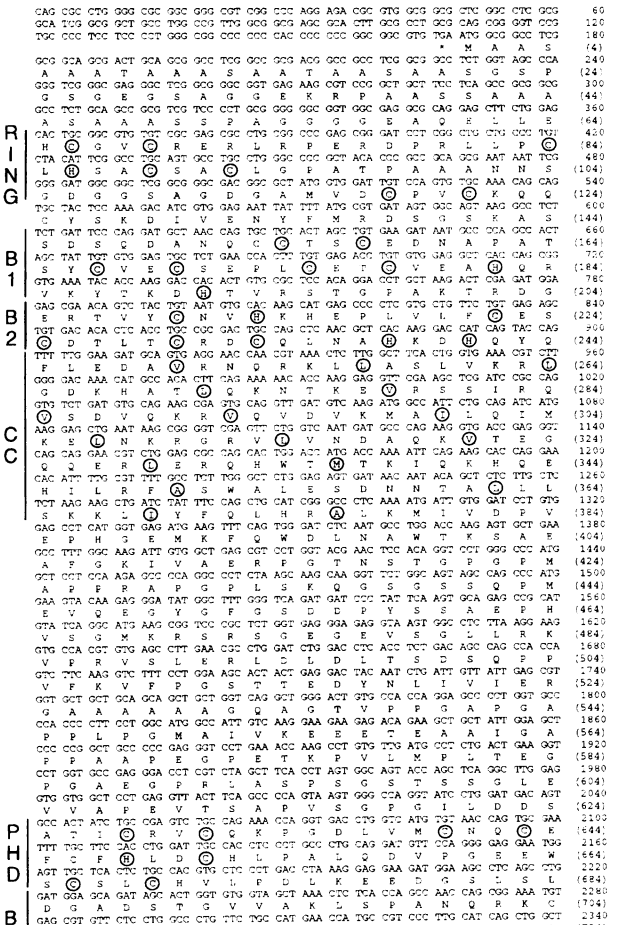
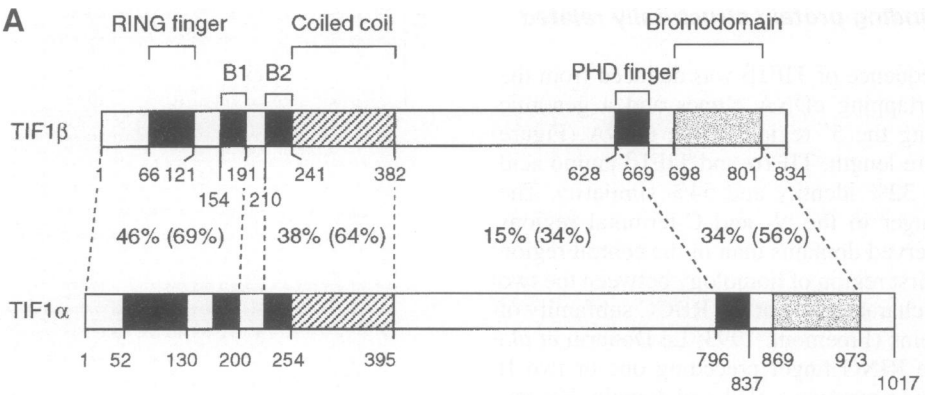


Fig. 6. Sequences of TIF1β. TIF1β cDNA and 5′-flanking genomic sequences, including an 834 amino acid open reading frame and flanking termination codons (underlined), are shown. The sequence was reconstituted from the original clone HP1-BP77 (nucleotides 535–2858; amino acid residues 123–834) and a genomic DNA clone encompassing the ATG initiation codon (nucleotides 1–618; amino acid residues 1–150). Cysteine and histidine residues belonging to the Cys/His-rich clusters (RING finger, B1 and B2 boxes, and the PHD finger) are highlighted with circles. The hydrophobic amino acids defining the heptad repeats of the putative coiled-coil structure (CC) are also circled, as are conserved residues of the bromodomain (BROMO).

TIF1α and TIF1β also exhibit a functional similarity because TIF1β, like TIF1α, can interact with mHP1α and mMOD1 in yeast cells and *in vitro* (Table I; data not shown). Note in this respect that residues 571–579 of TIF1β (Figure 6) present some similarity with residues 675–683 in the HP1 box of TIF1α (Figure 3A). In contrast, there is no motif clearly resembling the NR box of TIF1α in the TIF1β sequence.

Transcriptional repression by mHP1α and TIF1α in transfected mammalian cells

The *Drosophila* and mammalian Polycomb group (Pc-G) proteins have been shown previously to repress transcription in transfected mammalian cells when recruited to a promoter (Bunker and Kingston, 1994). Because it is currently assumed that HP1 and Pc-G proteins have analogous functions in the formation of a chromatin repressed state (for a review see Eissenberg *et al.*, 1995; Elgin, 1996), we investigated whether mHP1α could also exert a negative effect on the transcription of a reporter gene in transfected mammalian cells. The coding sequence of mHP1α was fused to the GAL4 DBD (amino acids 1–147). The transcriptional activity of the fusion protein was tested using a GAL4 reporter containing two GAL4-binding sites (17M2) in front of a β-globin (G) promoter–CAT fusion (17M2–ERE–G–CAT) in transiently trans-



B

RING Finger

```
TIF1β  63 LEHCGVCRERLRPERDPRLLPCLHSACSACLGPATPAAANNSGDGGSA......GDGAMVDCPVCKQQ 124
TIF1α  49 LDTCAVCHQNIQ.SRVPKLLPCLHSFCQRCLPAPQRYLMLTAPALGSA/24aa/TQVGVIRCPVCSQE 133
  PML  54 FLRCQQCQAEAK...CPKLLPCLHTLCSGCLEASGMQ.....................CPICQAP  94
  EFP  10 ELSCSVCLELFK...EPVTTPCGHNFCTSCLDETWVVQGPP.................YRCPQCRKV  56
  RFP  13 ETTCPVCLQYFA...EPMMLDCGHNICCACLARCWGTAETN.................VSCPQCRET  59
RPT-1  12 EVTCPICLELLK...EPVSADCNHSFCRACITLNYESNRNTDGK..............GNCPVCRVP  61
SSA/RO 13 EVTCPICLDPFV...EPVSIECGHSFCQECISQVGKGGGS..................VCPVCRQR  57
 XNF7 142 ELTCPLCVELFK...DPVMVACGHNFCRSCIDKAWEGQSS..................FACPECRES 187
PWA33 159 DLTCPLCRSLFK...EPVILECGHNFCKHCIDKSWESASA..................FSCPECKEV 204
```

B1 Box

```
TIF1β 154 CTSCEDNAPATSYCVECSEPLCETCVEAHQRVKYTKDH 191
TIF1α 163 CTSCEDNAEANGFCVECVEWLCKTCIRAHQRVKFTKDH 200
  PML 129 CTRCKES..ADFWCFECEQLLCAKCFEAHQ...WFLKH 161
  EFP 106 CDHCLTEI.AVKTCLVCMASFCQEHLRPHFDSPAFQDH 142
```

B2 Box

```
TIF1β 210 C..NVHKHEPL.VLFCESCDTLTCRDC.QLNA.H..KDH 241
TIF1α 223 C..PFHKKEQL.KLYCETCDKLTCRDC.QLLE.H..KEH 254
  PML 189 CSNPNHRTPTLTSIYCRGCSKPLCCSCALLDSSHSELKC 227
  EFP 156 C..TQHN..RLREFFCPEHGECICHIC..LVE.H..KTC 185
  RFP  96 C..EKHR.EPL.KLYCEEDQMPICVVCDRSRE.H..RGH 127
RPT-1  96 C..AQHG.EKL.RLFCRKDMMVICWLCERSQE.H..RGH 127
SSA/RO 92 C..AVHG.ERL.HLFCEKDGKALCWVCAQSRK.H..RDH 123
 XNF7 224 C..SEHD.ERL.KLYCKDDGTLSCVICRDSLK.H..ASH 255
PWA33 238 C..DEHD.ERL.KLFCKDDGTLACVICRDSLK.H..SNH 269
```

Coiled Coil

```
TIF1β 243 YQFLEDAVRNQRKLLASLVKRLGDKHATLQKNTKEVRSSIRQVSDVQKRVDVDVKMAILQIMKELNKRGRVLVNDAQKVT
TIF1α 256 YQFIEEAFQNQKVIIDTLITKLMEKTKYIKYTGNQTQNRIIEINQNQKQVEQDIKVAIFTLMVEINKKGKALLHQLESLA
  PML 227 CD.ISAEIQQRQEELDAMTQALQEQDSAFGAVHAQMHAAVGQLGRARAETEELIRERVRQVVAHVRAQERELLEAVDARY
  EFP 187 PTTLSQASADLEYKLRNKLTIMHSHINGATKALEDVRSKQQCVQDSMKRKMEQLRQEYMEMKAVIDAAETSSLRRLKEEE
  RFP 129 VLPLEEAVEGFKEQIQNQLDHLKRVKDLKKRRRAQGEQARAELLSLTQMERERKIVWEFEQLYHSLKEHEYRLLARLEELD
RPT-1 129 TALIEEVDQEYKEKLQGALWKLMKKAKICDEWQDDLQLQRVDWENQIQINVENVQRQFKGLRDLLDSKENEELQKLKKEK
SSA/RO 125 MVPLEEAAQEYQEKLQVALGELRRKQELAEKLEVEIAIKRADWKKTVETQKSRIHAEFVQQKNFVEEEQRQLQELEKDER
 XNF7 257 FLPILDAVGVYREELSAIVAPLEASLKVTEQLSSEQSDKIEQHNKNMSQYKEHITSEFEKLHKFLREREEKLLEQLKEQG
PWA33 271 FLPIQDAVGVYRDQLIALVSPLETTMKENQKLKCDQSQKISLHRENIVDCKKHIECEFEKLHQFLREKEAKMVEDLNAER

TIF1β      EGQQERLERQHWTMTKIQKHQEHILRFASWALESDNNTALLLSKKLTYFQLHRALKMIVD 382
TIF1α      KDHRMKLMQQQQEVAGLSKQLEHVMHFSKWAVSSGSSTALLYSKRLITYRLRHLLRARCD 395
  PML      QRDYEEMASRLGRLDAVLQRIRTGSALVQRMKCYASDQEVLDMHGFLRQALCRLRQEEPQ 365
  EFP      KRVYGKFDTIYQVLVKKKSEMQKLKAEVELIMDKGDEFEFLEKAAKLQGESTKPVYIPKI 326
  RFP      LAIYNSINGAITQFSCNISHLSSLIAQLEEKQQQPTRELLQDIGDTLSRAERIRIPEPWI 268
RPT-1      KEVMEKLEESENELEDQTELVRDLISDVEHHLELSTLEMLQGANCVLRRSQSLSLQQPQT 268
SSRA/RO    EQLRILGEKEAKLAQQSQALQELISELDRRCHSSALELLQEVIIVLERSESWNLKDLDIT 265
 XNF7      ENLLTEMENNLVKMQESQDAIKKTISLAKERMEDTDSISFLMDIKAFIDKCQEQQRAVIS 396
PWA33      EGLLKDMEANLVKMTDNCEFIEEAISTTQSRLNESDPIAFLTDIKSFIEKCCEEHRKGVP 410
```

fected COS-1 and HeLa cells. A 3- to 4-fold decrease in the transcriptional activity of the reporter was reproducibly observed in both cell types (Figure 8A; data not shown). Similar or stronger decreases were also observed with 17M–G–CAT reporters containing two or five GAL4-binding sites, and with the 17M2–ERE–G–CAT reporter transactivated by the chimeric transactivator ER(C)–VP16 (Figure 8C; data not shown). In contrast, no significant reduction in CAT activity was detected upon coexpression of a mHP1α unfused to the GAL4 DBD (Figure 8A). Thus, like the Pc-G proteins, mHP1α can function as a repressor of transcription when fused to a heterologous DBD.

Because TIF1α interacts with mHP1α, and mHP1α has a silencing activity, we also investigated whether a direct recruitment of TIF1α to a promoter could result in the repression of transcription. The cDNA encoding full-length TIF1α was cloned into the GAL4 fusion vector. The resulting plasmid (GAL4–TIF1α; Figure 8A) was cotransfected with the 17M2–ERE–G–CAT reporter into COS-1 cells. CAT activity was reduced to an extent similar to that achieved with GAL4–mHP1α (Figure 8A). This decrease, which was dependent on the dose of GAL4–TIF1α expression vector (Figure 8B), was also observed with a GAL4–TIF1αV681E/V682E fusion protein bearing mutations in the HP1 box, but not with TIF1α unfused to the GAL4 DBD (Figure 8A and B). Similar transcriptional repressions were also observed with the 17M–G–CAT reporter (data not shown) and the 17M2–ERE–G–CAT reporter transactivated by the ER(C)–VP16 activator (Figure 8C and D). Thus, TIF1α can mediate transcriptional repression when tethered to the DNA. However, this repression does not appear to depend on the TIF1α HP1 box which is required for interaction between TIF1α and either HP1α or MOD1 in yeast cells.

To define the region(s) in TIF1α that is responsible for transcriptional repression, a set of N-terminal deletions were cloned into the GAL4 fusion vector and assayed for their ability to repress transcription from a 17M2–ERE–G–CAT reporter in transfected COS-1 cells. Fusion proteins lacking the RBCC motif retained the repression activity, indicating that the RING finger, B boxes and coiled-coil are not required for repression (Figure 8E). Additional deletions enabled us to map the repression domain between residues 606 and 793 of TIF1α, in the region containing the HP1 and NR boxes (Figure 8E). Similarly, the domain

Fig. 7. TIF1β is structurally related to TIF1α. (**A**) Schematic comparison of TIF1α and TIF1β. Numbers attached to TIF1α and TIF1β indicate the amino acid position of the conserved domains, and amino acid identities (in %) (similarities in parentheses) are given. (**B**) Alignment and comparison of proteins belonging to the RBCC subfamily of RING finger proteins. The alignments were generated using the CLUSTAL W program (Thompson *et al.*, 1994b). Residues that are common to TIF1β and the other proteins are shaded. Stars indicate residues that are identical in all proteins. The hydrophobic amino acids defining the heptad repeats of the putative coiled-coil domains are boxed. Numbers refer to amino acid positions in the corresponding proteins. Database accession numbers: mouse TIF1α (S78219), mouse TIF1β (X99644), human PML (P29590), human EFP (D63902), human RFP (J03407), mouse RPT-1 (P15533), human SSA/RO (P19474), *Xenopus laevis* XNF7 (S64515) and *Pleurodeles waltii* PWA33 (L04190). (**C**) Comparative alignment of the TIF1β PHD and bromodomain with other PHD and bromodomain sequences. Conventions are as in (B). The consensus bromodomain sequence represents the most prevalent residue at each position. The bromodomain motif, as defined originally by Haynes *et al.* (1992), is underlined. Secondary structure predictions were made using the programs PHD (Rost and Sander, 1994) and SOPMA (Geourjon and Deleage, 1994), as implemented on the World Wide Web (WWW). Sequences corresponding to the predicted α-helices Z, A, B and C are boxed. RING3/FSH contains two bromodomains, as indicated by the suffixes -1 and -2. Note that TIF1β and HRX have imperfect bromodomains. Database accession numbers: human HRX (Q03164), human BR140 (M91585), human p300 (A54277), human CBP (S39162), human SFN2α/BRM (S45251), human SNF2β/BRG1 (S39059) and human RING3/FSH (P25440).

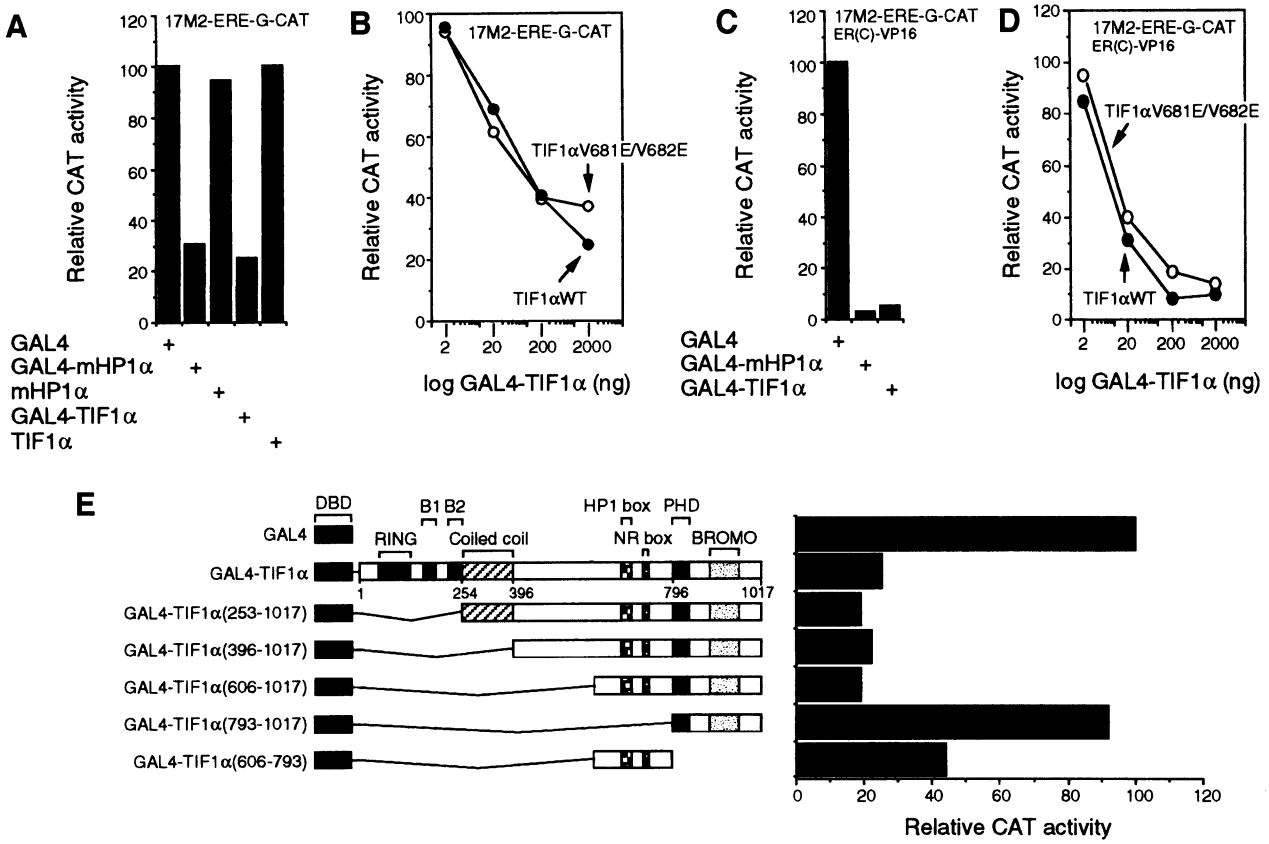


Fig. 8. mHP1α and TIF1α exert a transcriptional repressing (silencing) activity in transfected mammalian cells. (**A**) Transcriptional repression by TIF1α and mHP1α GAL4 fusion proteins. COS-1 cells were transiently cotransfected with 1 μg 17M2–ERE–G–CAT reporter, 1 μg pCH110 (expressing β-galactosidase) and 2 μg TIF1α or mHP1α expression vector as indicated. CAT activities are expressed relative to the CAT activity measured in the presence of the unfused GAL4 expression vector (taken as 100%). Values (±10%) represent the averages of two independent triplicated transfections after normalization for the internal control β-galactosidase activity of pCH110. (**B**) Dose-dependent repression by GAL4–TIF1α wild type (WT) or GAL4–TIF1αV681E/V682E bearing mutations in the HP1 box. 1 μg 17M2–ERE–G–CAT reporter and 1 μg pCH110 were cotransfected into HeLa cells, together with increasing amounts of GAL4–TIF1α (WT) or GAL4–TIF1αV681E/V682E expression vector. CAT activities were expressed as in (A). (**C**) GAL4–mHP1α or GAL4–TIF1α repress transcription activated by VP16. 17M2–ERE–G–CAT (1 μg) and pCH110 (1 μg) were cotransfected into HeLa cells with ER(C)–VP16 (100 ng), together with 1 μg TIF1α or mHP1α expression vector as indicated. CAT activities (±10%) resulting from activation by ER(C)–VP16 are expressed relative to that measured in the presence of the unfused GAL4 expression vector (see above). (**D**) GAL4–TIF1α represses transcription activated by VP16 in a dose-dependent manner. 17M2–ERE–G–CAT (1 μg) was cotransfected into HeLa cells with ER(C)–VP16 (100 ng), together with increasing amounts of GAL4–TIF1α (WT) or GAL4–TIF1αV681E/V682E expression vector. CAT activities resulting from activation by VP16 are expressed as in (C). (**E**) Residues 606–793 of TIF1α can repress transcription when fused to GAL4. 17M2–ERE–G–CAT (1 μg) was transfected into COS-1 cells with GAL4 unfused or fused to various regions of TIF1α (1 μg) as indicated. CAT activities were expressed as in (A).

responsible for repression of the transcription enhanced by the ER(C)–VP16 activator appears to be located essentially within the same region (data not shown). Note that the C-terminal residues 793–1017 of the protein including the PHD finger and the bromodomain may also contribute to the repression potential of TIF1α, as the fusion protein GAL4–TIF1α(606–793) was a less potent repressor than the fusion protein GAL4–TIF1α(606–1017) (Figure 8E).

Discussion

The domains of interaction of TIF1α with HP1 homologues and NRs are adjoining but not overlapping

TIF1α was originally identified as a protein that interacts directly with the LBDs of several NRs in a ligand- and AF-2 AD-dependent manner both *in vivo* and *in vitro* (Le Douarin *et al.*, 1995a; vom Baur *et al.*, 1996). Here, we have isolated and characterized two additional TIF1α-interacting proteins, mHP1α and mMOD1. Both of them are mouse homologues of dHP1, a non-histone chromosomal protein that exerts dose-dependent effects on heterochromatin-mediated gene silencing (for reviews see Eissenberg *et al.*, 1995; Elgin, 1996). This protein shares a conserved N-terminal domain, the chromo domain, with other eukaryotic proteins such as *Drosophila* Pc, a repressor of homeotic gene expression (Paro and Hogness, 1991; Koonin *et al.*, 1995). The dHP1 and Pc chromo domains have been shown to target chimeric proteins to distinct chromosomal sites (Platero *et al.*, 1995, 1996). The Pc chromo domain is both necessary and sufficient for nuclear localization and binding to euchromatin sites in polytene spreads (Messmer *et al.*, 1992), whereas the HP1 chromo domain is sufficient, although dispensable, for binding to heterochromatin (Powers and Eissenberg, 1993; Platero *et al.*, 1995). HP1, but not Pc, contains an additional chromatin-targeting activity which, like the chromo domain, is sufficient for heterochromatin binding (Platero *et al.*, 1995). This activity has been associated with a chromo domain-like motif, the chromo shadow

domain, located in the C-terminal moiety of HP1 (Aasland and Stewart, 1995). This domain is present in the known vertebrate HP1 homologues HP1α, MOD1 and MOD2, and has also been identified in the *Schizosaccharomyces pombe* fission yeast SWI6 protein, which is involved in the repression of silent mating-type loci (Lorentz *et al.*, 1994; Aasland and Stewart, 1995). We have demonstrated here that the chromo shadow domain is both necessary and sufficient for the interaction of mHP1α and mMOD1 with TIF1α.

The region of TIF1α that interacts with mHP1α and mMOD1 (TIF1α residues 675–701; the HP1 box) is close to but distinct from the region that interacts with the LBDs of NRs (residues 726–735; the NR box). Mutations in the HP1 box that impair the interaction of TIF1α with mHP1α and mMOD1 have no effect on TIF1α binding to NRs. Reciprocally, mutations in the NR box that abolish an interaction with NRs do no affect binding to mHP1α and mMOD1, thus demonstrating that these two interactions are genetically separable.

TIF1α and TIF1β share several highly conserved domains and interacting proteins

Among the proteins that, like TIF1α, interact with mHP1α, TIF1β is particularly interesting because the two TIF1 proteins exhibit the same domain organization. Like TIF1α and some other members of the RING finger family, TIF1β contains an N-terminal RBCC motif which includes a RING finger with two B boxes and a coiled-coil motif (reviewed in Freemont, 1993; Le Douarin *et al.*, 1995a). Although the actual functional significance of this tripartite motif is unknown, it is currently assumed that it is involved in protein–protein interactions (see also below). Interestingly, three out of the nine presently known RBCC proteins, i.e. PML, RFP and TIF1α (Figure 7B), have been identified in the context of fusion oncoproteins, in which the RBCC motif is fused to truncated products of other genes (RARα, ret and B-raf, respectively; Le Douarin *et al.*, 1995a, and references therein).

Another domain of unknown function, the bromodomain, is present in the C-terminal moiety of TIF1α and TIF1β, but not in that of other RBCC-containing proteins. The bromodomain, which is conserved from yeast to humans (Haynes *et al.*, 1992), has been identified in a number of proteins involved in transcriptional regulation (Tamkun *et al.*, 1992; Lygerou *et al.*, 1994); in certain cases it was found in association with catalytic domains. For instance, in addition to the bromodomain, SWI2–SNF2 has a DNA-dependent ATPase domain (Laurent *et al.*, 1993), GCN5 contains a histone acetyl-transferase A domain (Brownell *et al.*, 1996) and $TAF_{II}250$ has two distinct kinase domains (Dickstein *et al.*, 1996). Because all of these proteins reside in large multiprotein complexes, the SWI–SNF, ADA and TFIID transcription complexes, respectively (reviewed in Guarente, 1995), it has been proposed that the bromodomain may mediate protein–protein interactions influencing the assembly and/or activity of these complexes (Haynes *et al.*, 1992). The bromodomain, as defined initially, contained two predicted α-helices. We show here and elsewhere (F.Jeanmougin, J.M.Wurtz, B.Le Douarin, P.Chambon and R.Losson, manuscript in preparation) that all bromodomains identified so far in fact correspond to ~110 amino acid residues with four predicted α-helices. Interestingly, the amino acid sequence of helix Z, the first of these predicted helices, is conserved only between bromodomains of related members, whereas the three other α-helices (A, B and C) are conserved among all bromodomains in the family (Figure 7C). We suggest that the conserved residues of α-helices A, B and C may be important for the structural integrity and certain function(s) of the bromodomain, whereas α-helix Z residues may confer some specificity.

On the N-terminal side of the bromodomain, TIF1α and TIF1β contain a conserved zinc finger motif, the C4HC3 motif or PHD finger (Aasland *et al.*, 1995). In vertebrates, this motif is also present in four bromodomain-containing proteins: CBP and p300 (two transcriptional coactivators; Lundblad *et al.*, 1995, and references therein), BR140 (a nuclear protein of unknown function; Thompson *et al.*, 1994a) and HRX/ALL-1 (a human homologue of the *Drosophila* protein Trithorax which is involved in chromosomal translocations found in acute leukaemias; Gu *et al.*, 1992) (Figure 7C). The occurrence of the PHD finger in a number of chromatin-related proteins, including the Trithorax and Polycomb-like proteins which have antagonistic effects on chromatin structure and function in *Drosophila*, has suggested that it may be involved in interactions between chromatinic proteins (Aasland *et al.*, 1995, and references therein).

Importantly, TIF1α and TIF1β are not only structurally related, but they also exhibit functional similarities. They both interact with mammalian homologues of dHP1. Whether TIF1β, which does not possess the consensus NR box present in TIF1α, RIP140 and TRIP3 (Figure 3D), interacts with NR LBDs remains to be seen. Most interestingly, both TIF1α and TIF1β also interact with the 97 amino acid KRAB domain (P.Moosmann, O.Georgiev, B.Le Douarin and W.Schaffner, in preparation; our unpublished results), an evolutionarily conserved transcriptional silencing domain present in a large number of vertebrate putative DNA-binding proteins containing multiple zinc-binding motifs, which are related to those of the *Drosophila* Krüppel protein (Bellefroid *et al.*, 1991; Margolin *et al.*, 1994; Witzgall *et al.*, 1994, and references therein). The region of TIF1α that interacts with the KRAB domain of the human protein KOX1 has been located within a segment of 187 amino acids (amino acids 247–433) which contains the coiled-coil motif but not the HP1 box or the NR box (P.Moosmann, O.Georgiev, B.Le Douarin and W.Schaffner, manuscript in preparation). On the basis of their structural and functional similarities, we propose that TIF1α and TIF1β may be the first members of a new gene family.

mHP1α and mMOD1 interact with proteins involved in chromatin structure and function

We have isolated here a number of mouse cDNAs encoding proteins that interact specifically with mHP1α in yeast. Included in this protein group are mHP1α itself and mMOD1, suggesting that mHP1α may function by forming dimer or higher order multimer complexes with itself or mMOD1, consistent with the previous proposal that *Drosophila* HP1 could multimerize through direct or indirect interactions (Platero *et al.*, 1995, 1996; Elgin, 1996).

Among the cDNAs encoding mHP1α-interacting

proteins, we have isolated a partial cDNA for mSNF2β, one of the two mouse homologues of the *Drosophila* Brahma protein and of the yeast transcriptional coactivator SWI2–SNF2, which belongs to the SWI–SNF multiprotein complex and is presumably involved in chromatin remodelling to counteract the repressive effects of histones and other proteins involved in gene silencing (Peterson and Tamkun, 1995; Peterson, 1996, and references therein). How the SWI–SNF complex might remodel chromatin is still unclear. However, it has been reported recently that the yeast SWI–SNF complex can bind four-way junction (4 WJ) DNA with high affinity (Quinn *et al.*, 1996). Interestingly, 4 WJ DNA may be structurally similar to the DNA crossovers present at the entry and exit points of DNA on the nucleosome (Lilley, 1992; Varga-Weisz *et al.*, 1994), thus indicating that the binding of SWI–SNF complexes to nucleosomal DNA crossovers may be important in chromatin structure remodelling (Quinn *et al.*, 1996, and references therein). Our data, which indicate that mSNF2β-containing complexes interact with mHP1, therefore suggest that these complexes may be involved in the remodelling of heterochromatin-like structures. Interestingly, no such interactions were observed with mMOD1, indicating that mHP1α and mMOD1, although being structurally highly related, might be somewhat functionally distinct.

Two additional novel mHP1α-interacting proteins were isolated in our study which, on the basis of their amino acid sequences, may play a role in chromatin structure and/or function. HP1-BP38 is a potential helicase that exhibits a similarity with the yeast RAD54 protein which may function in opening 'inaccessible' chromatin structures (Sugawara *et al.*, 1995). Interestingly, the HP1-BP74 protein contains a region that is highly similar to the globular domain of the linker histones H1 and H5 and their variants, which has been shown to bind preferentially to 4 WJ DNA, similarly to the SWI–SNF complex (Varga-Weisz *et al.*, 1994; Goytisolo *et al.*, 1996).

What role might members of the TIF1 gene family play in the control of transcription?

TIF1α and TIF1β may belong to a new class of intermediary factors (mediators) that play a dual role in the control of transcription at the chromatin level, being involved in both repression (silencing) through the formation of 'inactive' heterochromatin-like 'condensed' structures, and activation, through reversion to 'active' euchromatin-like 'open' structures. In transcriptional silencing, TIF1α and TIF1β, which interact with the KRAB domain of KOX1 but not with mutated KRAB domains that do not repress transcription in transfected cultured cells (P.Moosmann, O.Georgiev, B.Le Douarin and W.Schaffner, manuscript in preparation), may mediate the silencing activity of this domain (Margolin *et al.*, 1994; Witzgall *et al.*, 1994; Deusche *et al.*, 1995). This possibility is supported by the observation that TIF1α (this study) and TIF1β (P.Moosmann, O.Georgiev, B.Le Douarin and W.Schaffner, manuscript in preparation) can exert a silencing effect in transfected cells when recruited to the promoter region of a reporter gene. By analogy with the known function of *Drosophila* HP1, which, presumably through self-association and the formation of a silencing complex, promotes heterochromatin-induced gene repression (Platero *et al.*, 1995; for reviews see Weiler and Wakimoto, 1995; Elgin, 1996), it is tempting to speculate that the binding of HP1 and mMOD1 to TIF1α and TIF1β may exert a silencing effect via the formation of transcriptionally 'inactive' heterochromatin-like structures. In this respect, note that HP1 can interact with the HP1-BP74 protein, which contains a domain homologous to the globular domain of histone H1 and its variants (e.g. H5), known to preferentially bind to DNA crossovers present at the entry and exit points of DNA on nucleosomes (see above). Therefore it is possible that H1-type histones and HP1-BP74-like proteins serve as bridging factors linking HP1α to nucleosomal DNA. We therefore suggest a hypothetical mechanism through which the numerous KRAB domain-containing proteins, which can presumably bind to DNA through (some of) their multiple zinc finger motifs (Bellefroid *et al.*, 1995, and references therein), recruit TIF1s, HP1 and HP1-related proteins to assemble silencing complexes, possibly at specific DNA sites. The formation of heterochromatin-like 'condensed' structures would then be cooperatively propagated both by self-association of HP1/MOD1 as proposed by Platero *et al.* (1995) in the *Drosophila* case, and by interactions of HP1 with HP1-BP74-like proteins bound to the nucleosomal DNA crossovers. This model assumes that the KRAB domain exerts its silencing activity through chromatin effects, and not through repression of the evolutionarily conserved transcriptional machinery (Pengue and Lania, 1996), in agreement with its lack of silencing effect in yeast and *Drosophila* cells (P.Moosmann, O.Georgiev, B.Le Douarin and W.Schaffner, manuscript in preparation). However, although compatible with most of our results, this tentative model does not fit with the lack of an effect of mutations within the TIF1α HP1 box on TIF1α silencing activity in transfected cultured cells. Whether this discrepancy reflects additional interactions between TIF1α and HP1 in animal cells not revealed by the yeast two-hybrid assay, the existence of two silencing domains in TIF1α and/or the use of transient transfection assays in cultured cells, in which it is unlikely that bona fide chromatin structures are assembled, deserves further investigation.

Previously we have proposed that TIF1α may be required to mediate transcriptional activation by NR AF-2 (Le Douarin *et al.*, 1995a; vom Baur *et al.*, 1996). However, coexpression of TIF1α and various NRs in transiently transfected cultured cells did not result in any stimulation of transcription of cognate reporter genes (Le Douarin *et al.*, 1995a). Together with the lack of *in vitro* interaction of TIF1α with known components of the general transcription machinery (TBP and TFIIB) and of the TFIID complex (Le Douarin *et al.*, 1995a), this indicated that TIF1α may mediate AF-2 activity on the chromatin template rather than on components of the transcription machinery (Le Douarin *et al.*, 1996). Our data suggest that the binding of liganded NR to TIF1α may promote the conversion of transcriptionally 'inactive' heterochromatin-like structures to 'active' euchromatin-like 'open' structures by triggering the release of HP1 and MOD1. The SNF2β complex as well as the RAD54-related putative helicase protein HP1-BP38, both of which have been shown here to interact with HP1 (see above), may facilitate this process. Note that the yeast SWI–SNF

complex has been reported to bind to 4 WJ DNA, and that this binding may be crucial for the generation of transcriptionally active chromatin templates (see above and Quinn *et al.*, 1996). Thus, the binding of liganded NRs to TIF1α might induce a chromatin remodelling which, in turn, would allow NRs and other transactivators to bind to their cognate REs. The subsequent binding of other TIFs [e.g. SRC-1 (Onate *et al.*, 1995) and TIF2 (Voegel *et al.*, 1996)] to NR LBDs would then mediate additional activation effects of AF-2 on components of the transcription machinery either directly or indirectly through coactivators such as CBP (Kamei *et al.*, 1996, and references therein).

Our tentative model predicts that TIF1 is a bifunctional protein involved at the level of the chromatin template in both repression and activation of transcription, and that it might be associated with chromatin to participate in the epigenetic regulation of transcription that is critical in the controlled expression of developmental programmes in higher eukaryotes (for a review and references see Eissenberg *et al.*, 1995; Elgin, 1996). In support of this model, preliminary confocal electron microscopic immunocytological studies indicate that TIF1α is associated with both euchromatin and heterochromatin in mammalian cells (Y.Lutz, J.L.Vonesch and A.Gansmuller, unpublished data), whereas HP1α (Nicol and Jeppesen, 1994) and MOD1 (Wreggett *et al.*, 1994) have been found only in heterochromatin. Furthermore, TIF1α, like HP1α, appears to be tightly associated with chromatin prepared from nuclei of P19 embryocarcinoma cells (E.Remboutsika, unpublished results). However, other models could also account for our results. For instance, TIF1α could be involved in possible ligand-dependent repression processes. Further biochemical and genetic approaches (e.g. TIF1 knockouts) are clearly required to investigate the validity of our model, which is essentially based on molecular interaction studies performed in non-physiological contexts.

Materials and methods

Plasmids

Receptor cDNAs used in this study correspond to mouse RXRα and human RARα1, ER, VDR and PR (EMBL Data Bank; Le Douarin *et al.*, 1995a; vom Baur *et al.*, 1996). DBD and AAD fusion proteins were expressed from the yeast multicopy plasmids pBL1 and pASV3, respectively (Le Douarin *et al.*, 1995b). pBL1 contains the HIS3 marker and directs the synthesis of epitope (F region of human ER)-tagged ER DBD fusion proteins. pASV3 contains the LEU2 marker and a cassette expressing a nuclear localized VP16 AAD, preceding a polylinker with cloning sites for the cDNA and stop codons in all reading frames. All inserts cloned into pBL1 and pASV3 were obtained by PCR and verified by sequencing. The LexA fusion proteins LexA–TIF1(434–1017) and LexA–mHP1α were expressed from a derivative of pBTM116 (Vojtek *et al.*, 1993). For *in vitro* binding assays, the indicated cDNAs were fused to GST in the pGEX-2T plasmid (Pharmacia). The His-TIF1 construct has been described previously (Le Douarin *et al.*, 1995a). For transfection studies in mammalian cells, the indicated cDNAs were cloned into pSG5 (Green *et al.*, 1988). The GAL4(1–147) chimeras were constructed by PCR amplification of the indicated regions of TIF1α and mHP1α, followed by subcloning into pG4MpolyII (Tora *et al.*, 1989). The chimeric protein ER(C)–VP16, which encodes amino acids 176–280 of ER and amino acids 413–490 of VP16, has been described previously (Tora *et al.*, 1989), as well as the reporter gene 17M2–ERE–G–CAT. Details concerning each construction are available upon request.

cDNA library screening

A VP16-tagged cDNA library derived from 9.5 to 12.5 d.p.c. mouse embryonic poly(A)$^+$ RNAs was constructed in the leucine-selectable plasmid pASV3 (vom Baur *et al.*, 1996) and introduced by LiAc transformation into the *Saccharomyces cerevisiae* L40 strain [*MATα trp1-901 leu2-3, 112 his3-Δ200 ade2 LYS2::(LexAop)4-HIS3 URA3::(LexAop)8-LacZ*] expressing the fusion protein LexA–TIF1(434–1017) from a derivative of the tryptophan-selectable expression vector pBTM116 (Vojtek *et al.*, 1993). Approximately 5×10^6 yeast transformants were selected on Trp$^-$ Leu$^-$ plates, and replated at a multiplicity of ~10 onto His$^-$ Trp$^-$ Leu$^-$ plates containing 10 mM 3-aminotriazole (3-AT). After 5 days, 223 His$^+$ clones exhibiting *LacZ* expression on X-Gal indicator filters were isolated. Library plasmids were rescued in *E.coli* strain JM110 (leuB$^-$) and introduced into the yeast reporter strain PL3(α) (*MATα ura3-Δ1 his3-Δ200 leu2-Δ1 trp1::3ERE-URA3*; Pierrat *et al.*, 1992) expressing full-length TIF1 fused to the ER DBD (DBD–TIF1; Figure 1) or an unfused ER DBD as a control. In all, 68 clones were considered positive for the interaction with TIF1. DNA sequencing revealed 11 different proteins designated TIF1-BPs. Two of these cDNAs, TIF1-BP1 and TIF1-BP2, encoded VP16 fusions containing the entire coding sequence of mHP1α (amino acids 1–191) and residues 1–170 of mMOD1, respectively. Full-length mMOD1 cDNA was cloned by RT–PCR from mouse embryo RNAs.

To isolate cDNAs encoding proteins that interact with mHP1α, the mouse embryo cDNA library was screened against LexA–mHP1α in yeast strain L40. A total of 10^6 yeast transformants were screened directly for histidine prototrophy on His$^-$ Trp$^-$ Leu$^-$ plates without 3-AT. A total of 11 were classified as positive for the interaction with mHP1α when retested in the URA3-based two-hybrid system (Figure 1) using the DBD of ER fused to mHP1α or an unfused ER DBD in the PL3 reporter strain. The BLAST program (Altschul *et al.*, 1990) was used to search for sequence homology in the GeneBank and EMBL databases.

Transactivation assays

Yeast PL3 transformants were grown exponentially for about five generations in selective medium containing uracil in the presence or absence of ligand. Yeast extracts were prepared and assayed for OMPdecase activity as described previously (Pierrat *et al.*, 1992). The transient transfection of mammalian cells and CAT assays were as described previously (Durand *et al.*, 1994).

Antibodies

Anti-TIF1α monoclonal antibody 5T-1E8 was raised against amino acids 396–682 of TIF1α (Le Douarin *et al.*, 1995a). Monoclonal antibodies 2GV-4 and F3 are directed against VP16 (White *et al.*, 1992) and the F region of human ER (Ali *et al.*, 1993), respectively. Monoclonal antibody 2GV-3 is directed against the DBD of yeast GAL4 (amino acids 1–147).

In vitro binding assays

1 μg purified baculovirus-expressed 6× His-tagged TIF1 (His-TIF1; Le Douarin *et al.*, 1995a) was incubated with 12 μg bacterially expressed GST fusion proteins loaded on glutathione-S–Sepharose beads for 1 h at 4°C in a final volume of 100 μl binding buffer (BB: 50 mM Tris–HCl, pH 8.0, 100 mM NaCl, 0.3 mM dithiothreitol, 10 mM $MgCl_2$, 10% glycerol, 0.1% NP-40). The beads were washed four times with 1 ml BB buffer, resuspended in an SDS-containing buffer, boiled for 10 min and proteins analysed by SDS–PAGE. The enhanced chemiluminescence detection system was used for immunodetection as recommended by the supplier (Amersham). Variation of the NaCl concentration in buffer BB indicated that the TIF1α–mHP1α and TIF1α–mMOD1 interactions were stable between 100 and 500 mM NaCl.

Accession numbers

The EMBL accession numbers for the sequences reported in this paper, i.e. mHP1, HP1-BP74, HP1-BP38 and TIF1, are XX99641, XX99642, XX99643 and XX99644, respectively.

Acknowledgements

We are grateful to S.M.Hollenberg for the generous gifts of yeast strain L40 and plasmid pBTM116. We thank P.Moosmann, O.Georgiev and W.Schaffner for communication of their results before publication and gifts of plasmids. We also thank S.Vicaire for DNA sequencing, F.Ruffenach and A.Staub for oligonucleotide synthesis, Y.Lutz for antibody preparation and our colleagues of the yeast and retinoid groups for helpful discussions and for communicating unpublished results. This work was supported by the Groupement de Recherches et d'Etudes sur les Génomes (GREG47/95), the Centre National de la Recherche Scientifique, the Institut National de la Santé et de la Recherche Médicale,

the Centre Hospitalier Universitaire Régional, the Association pour la Recherche sur le Cancer and the Collège de France. A.N. is the recipient of a fellowship from EMBO.

References

Aasland,R. and Stewart,A.F. (1995) The chromo shadow domain, a second chromo domain in heterochromatin-binding protein 1, HP1. *Nucleic Acids Res.*, **23**, 3163–3173.

Aasland,R., Gibson,T.J. and Stewart,A.F. (1995) The PHD finger: implications for chromatin-mediated transcriptional regulation. *Trends Biochem. Sci.*, **20**, 56–59.

Ali,S., Lutz,Y., Bellocq,J.P., Chenard-Neu,M.P., Rouyer,N. and Metzger,D. (1993) Production and characterization of monoclonal antibodies recognising defined regions of the human oestrogen receptor. *Hybridoma*, **12**, 391–405.

Altschul,S.F., Gish,W., Miller,W., Myers,E.W. and Lipman,D.J. (1990) Basic local alignment search tool. *J. Mol. Biol.*, **215**, 403–410.

Barettino,D., Vivanco-Ruiz,M.M. and Stunnenberg,H.G. (1994) Characterization of the ligand-dependent transactivation domain of thyroid hormone receptor. *EMBO J.*, **13**, 3039–3049.

Beato,M., Herrlich,P. and Schütz,G. (1995) Steroid hormone receptors: many actors in search of a plot. *Cell*, **83**, 851–857.

Bellefroid,E.J., Poncelet,D.A., Lecocq,P.J., Revelant,O. and Martial,J.A. (1991) The evolutionarily conserved Krüppel-associated box domain defines a subfamily of eukaryotic multifingered proteins. *Proc. Natl Acad. Sci. USA*, **88**, 3608–3612.

Bellefroid,E.J. *et al.* (1995) Emergence of the ZNF91 Krüppel-associated box-containing zinc finger gene family in the last common ancestor of anthropoidea. *Proc. Natl Acad. Sci. USA*, **92**, 10757–10761.

Bellini,M., Lacroix,J.C. and Gall,J.G. (1993) A putative zinc-binding protein on lampbrush chromosome loops. *EMBO J.*, **12**, 107–114.

Bourguet,W., Ruff,M., Chambon,P., Gronemeyer,H.G. and Moras,D. (1995) Crystal structure of the ligand binding domain of the human nuclear receptor RXRα. *Nature*, **375**, 377–382.

Brownell,J.E., Zhou,J., Ranalli,T., Kobayashi,R., Edmondson,D.G., Roth,S.Y. and Allis,C.D. (1996) *Tetrahymena* histone acetyltransferase A: a homology to yeast Gcn5p linking histone acetylation to gene activation. *Cell*, **84**, 843–851.

Bunker,C.A. and Kingston,R.E. (1994) Transcriptional repression by *Drosophila* and mammalian Polycomb group proteins in transfected mammalian cells. *Mol. Cell. Biol.*, **14**, 1721–1732.

Cavaillès,V., Dauvois,S., L'Horset,F., Lopez,G., Hoare,S., Kushner,P.J. and Parker,M.G. (1995) Nuclear factor RIP140 modulates transcriptional activation by the estrogen receptor. *EMBO J.*, **14**, 3741–3751.

Cerf,C., Lippens,G., Muyldermans,S., Segers,A., Ramakrishnan,V., Wodak,S.J., Hallenga,K. and Wyns,L. (1993) Homo- and heteronuclear two-dimensional NMR studies of the globular domain of histone H1: sequential assignment and secondary strutures. *Biochemistry*, **32**, 11345–11351.

Chambon,P. (1996) A decade of molecular biology of retinoic acid receptors. *FASEB J.*, **10**, 940–954.

Chiba,H., Muramatsu,M., Nomoto,A. and Kato,H. (1994) Two human homologues of *Saccharomyces cerevisiae* SWI2/SNF2 and *Drosophila* brahma are transcriptional coactivators cooperating with the estrogen receptor and the retinoic acid receptor. *Nucleic Acids Res.*, **22**, 1815–1820.

Clark,R.F. and Elgin,S.C.R. (1992) Heterochromatin protein 1, a known suppressor of position-effect variegation, is highly conserved in *Drosophila*. *Nucleic Acids Res.*, **20**, 6067–6074.

Cuypers,H.T., van Loon-Klaassen,L.A., Egberts,W.T., de Jong,W.W. and Bloemendal,H. (1992) The primary structure of leucine aminopeptidase from bovine eye lens. *J. Biol. Chem.*, **257**, 7077–7085.

Danielian,P.S., White,R., Lees,J.A. and Parker,M.G. (1992) Identification of a conserved region required for hormone dependent transcriptional activation by steroid hormone receptors. *EMBO J.*, **11**, 1025–1033.

Deutsche,U., Meyer,W. and Thiesen,H.J. (1995) Tetracycline-reversible silencing of eukaryotic promoters. *Mol. Cell. Biol.*, **15**, 1907–1914.

Dickstein,R., Ruppert,S. and Tjian,R. (1996) $TAF_{II}250$ is a bipartite protein kinase that phosphorylates the basal transcription factor RAP74. *Cell*, **84**, 781–790.

Durand,B., Saunders,M., Gaudon,C., Roy,B., Losson,R. and Chambon,P. (1994) Activation function 2 (AF-2) of RAR and RXR: presence of a conserved autonomous constitutive activating domain and influence of the nature of the response element on AF-2 activity. *EMBO J.*, **13**, 5370–5382.

Eissenberg,J.C., Elgin,S.C.R. and Paro,R. (1995) Epigenetic regulation in *Drosophila*: a conspiracy of silence. In Elgin;S.C.R. (ed.), *Chromatin Structure and Gene Expression*. Frontiers in Molecular Biology. Oxford University Press, Oxford, UK, pp. 147–171.

Elgin,S.R.C. (1996) Heterochromatin and gene regulation in *Drosophila*. *Curr. Opin. Genet. Dev.*, **6**, 193–202.

Fields,S. and Sternglanz,R. (1994) The two-hybrid system: an assay for protein–protein interactions. *Trends Genet.*, **10**, 286–292.

Freemont,P.S. (1993) The RING finger. A novel protein sequence motif related to the zinc finger. *Ann. N.Y. Acad. Sci.*, **684**, 174–192.

Geourjon,C. and Delage,G. (1994) SOPM: a self-optimized method for protein secondary structure prediction. *Protein Engng*, **7**, 157–164.

Giguère,V. (1994) Retinoic acid receptors and cellular retinoid binding proteins: complex interplay in retinoid signaling. *Endocr. Rev.*, **15**, 61–79.

Glass,C.K. (1994) Differential recognition of target genes by nuclear receptor monomers, dimers, and heterodimers. *Endocr. Rev.*, **15**, 391–407.

Goytisolo,F.M., Gerchman,S.-E., Yu,X., Rees,C., Graziano,V., Ramakrishnan,V. and Thomas,J.O. (1996) Identification of two DNA-binding sites on the globular domain of histone H5. *EMBO J.*, **15**, 3421–3429.

Green,S., Issemann,I. and Scheer,E. (1988) A versatile *in vivo* and *in vitro* eukaryotic expression vector for protein engineering. *Nucleic Acids Res.*, **16**, 369.

Gronemeyer,H. and Laudet,V. (1995) *Transcription Factors 3: Nuclear Receptors. Protein Profile Volume 2.* Academic Press.

Gu,Y., Nakamura,T., Alder,H., Prasad,R., Canaani,O., Cimino,G., Croce,C.M. and Canaani,E. (1992) The t(4; 11) chromosome translocation of human acute leukemias fuses the ALL-1 gene, related to *Drosophila* trithorax, to the AF-4 gene. *Cell*, **71**, 701–708.

Guarente,L. (1995) Transcriptional coactivators in yeast and beyond. *Trends Biochem. Sci.*, **20**, 517–521.

Halachmi,S., Marden,E., Martin,G., MacKay,H., Abbondanza,C. and Brown,M. (1994) Oestrogen receptor-associated proteins: possible mediators of hormone-induced transcription. *Science*, **264**, 1455–1458.

Haynes,S.R., Dollard,C., Winston,F., Beck,S., Trowsdale,J. and Dawid,I.B. (1992) The bromodomain: a conserved sequence found in human, *Drosophila* and yeast proteins. *Nucleic Acids Res.*, **20**, 2603.

Heery,D.M., Zacharewski,T., Pierrat,B., Gronemeyer,H., Chambon,P. and Losson,R. (1993) Efficient transactivation by retinoic acid receptors in yeast requires retinoid X receptors. *Proc. Natl Acad. Sci. USA*, **90**, 4281–4285.

Heery,D.M., Pierrat,B., Gronemeyer,H., Chambon,P. and Losson,R. (1994) Homo- and heterodimers of the retinoid X receptor (RXR) activate transcription in yeast. *Nucleic Acids Res.*, **22**, 726–731.

Hong,H., Kohli,K., Trivedi,A., Johnson,D.L. and Stallcup,M.R. (1996) GRIP1, a novel mouse protein that serves as a transcriptional coactivator in yeast for the hormone binding domains of steroid receptors. *Proc. Natl Acad. Sci. USA*, **93**, 4948–4952.

Inoue,S. *et al.* (1993) Genomic binding-site cloning reveals an estrogen-responsive gene that encodes a RING finger protein. *Proc. Natl Acad. Sci. USA*, **90**, 11117–11121.

Kamei,Y. *et al.* (1996) A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors. *Cell*, **85**, 403–414.

Kastner,P., Mark,M. and Chambon,P. (1995) Nonsteroid nuclear receptors: what are genetic studies telling us about their role in real life? *Cell*, **83**, 859–869.

Keaveny,M. and Stunnenberg,H.G. (1995) Retinoic acid receptors. In Bauerle,P.A. (ed.), *Inducible Gene Expression*. Birkhäuser, Boston, MA, Vol. 2, pp. 187–242.

Khavari,P.A., Peterson,C.L., Tamkun,J.W., Mendel,D.B. and Crabtree, G.R. (1993) BRG1 contains a conserved domain of the SWI2/SNF2 family necessary for normal mitotic growth and transcription. *Nature*, **366**, 170–174.

Koonin,E.V., Zhou,S. and Lucchesi,J.C. (1995) The chromo superfamily: new members, duplication of the chromo domain and possible role in delivering transcription regulators to chromatin. *Nucleic Acids Res.*, **23**, 4229–4233.

Laurent,B.C., Treich,I. and Carlson,M. (1993) The yeast SNF2/SWI2 protein has DNA-stimulated ATPase activity required for transcriptional activation. *Genes Dev.*, **7**, 583–591.

Le Douarin,B. *et al.* (1995a) The N-terminal part of TIF1, a putative mediator of the ligand-dependent activation function (AF-2) of nuclear receptors, is fused to B-raf in the oncogenic protein T18. *EMBO J.*, **14**, 2020–2033.

Le Douarin,B., Pierrat,B., vom Baur,E., Chambon,P. and Losson,R. (1995b) A new version of the two-hybrid assay for detection of protein–protein interactions. *Nucleic Acids Res.*, **23**, 876–878.

Le Douarin,B., vom Baur,E., Zechel,C., Heery,D., Heine,M., Vivat,V., Gronemeyer,H., Losson,R. and Chambon,P. (1996) Ligand-dependent interaction of nuclear receptors with potential transcriptional intermediary factors (mediators). *Philos. Trans. R. Soc. Lond.*, **B351**, 569–578.

Lee,J.W., Ryan,F., Swaffield,J.C., Johnston,S.A. and Moore,D.A. (1995) Interaction of thyroid-hormone receptor with a conserved transcriptional mediator. *Nature*, **374**, 91–94.

Lilley,D.M. (1992) DNA–protein interactions. HMG has DNA wrapped up. *Nature*, **357**, 282–283.

Lorentz,A., Ostermann,K., Fleck,O. and Schmidt,H. (1994) Switching gene *swi6*, involved in repression of silent mating-type loci in fission yeast, encodes a homologue of chromatin-associated proteins from *Drosophila* and mammals. *Gene*, **143**, 139–143.

Lundblad,J.R., Kwok,R.P., Laurance,M.E., Harter,M.L. and Goodman, R.H. (1995) Adenoviral E1A-associated protein p300 as a functional homologue of the transcriptional co-activator CBP. *Nature*, **374**, 85–88.

Lygerou,Z., Conesa,C., Lesage,P., Swanson,R.N., Ruet,A., Carlson,M., Sentenac,A. and Seraphin,B. (1994) The yeast BDF1 gene encodes a transcription factor involved in the expression of a broad class of genes including snRNAs. *Nucleic Acids Res.*, **22**, 5332–5340.

Mangelsdorf,D.J. and Evans,R.M. (1995b) The RXR heterodimers and orphan receptors. *Cell*, **83**, 841–850.

Mangelsdorf,D.J. *et al.* (1995a) The nuclear receptor superfamily: the second decade. *Cell*, **83**, 835–839.

Margolin,J.F., Friedman,J.R., Meyer,W.K.-H., Vissing,H., Thiesen,H.-J. and Rauscher,F.J.,III (1994) Krüppel-associated boxes are potent transcriptional repression domains. *Proc. Natl Acad. Sci. USA*, **91**, 4509–4513.

Messmer,S., Franke,A. and Paro,R. (1992) Analysis of the functional role of the Polycomb chromo domain in *Drosophila melanogaster*. *Genes Dev.*, **6**, 1241–1254.

Metzger,D., White,J.H. and Chambon,P. (1988) The human oestrogen receptor functions in yeast. *Nature*, **334**, 31–36.

Meyer,M.E., Gronemeyer,H., Turcotte,B., Bocquel,M.T., Tasset,D. and Chambon,P. (1989) Steroid hormone receptors compete for factors that mediate their enhancer function. *Cell*, **57**, 433–442.

Nicol,L. and Jeppesen,P. (1994) Human autoimmune sera recognize a conserved 26 kD protein associated with mammalian heterochromatin that is homologous to heterochromatin protein 1 of *Drosophila*. *Chromosome Res.*, **2**, 245–253.

Onate,S.A., Tsai,S.Y., Tsai,M.J. and O'Malley,B. (1995) Sequence and characterization of a coactivator for the steroid hormone receptor superfamily. *Science*, **270**, 1354–1357.

Parker,M.G. (1993) Steroid and related receptors. *Curr. Opin. Cell Biol.*, **5**, 499–504.

Paro,R. and Hogness,D.S. (1991) The Polycomb protein shares a homologous domain with a heterochromatin-associated protein of *Drosophila*. *Proc. Natl Acad. Sci. USA*, **88**, 263–267.

Pengue,G. and Lania,L. (1996) Krüppel-associated box-mediated repression of RNA polymerase II promoters is influenced by the arrangement of basal promoter elements. *Proc. Natl Acad. Sci. USA*, **93**, 1015–1020.

Peterson,C.L. (1996) Multiples switches to turn on chromatin? *Curr. Opin. Genet. Dev.*, **6**, 171–175.

Peterson,C.L. and Tamkun,W. (1995) The SWI–SNF complex: a chromatin remodeling machine? *Trends Biochem. Sci.*, **20**, 143–146.

Pierrat,B., Heery,D.M., Lemoine,Y. and Losson,R. (1992) Functional analysis of the human oestrogen receptor using a phenotypic transactivation assay in yeast. *Gene*, **119**, 237–245.

Platero,J.S., Hartnett,T. and Eissenberg,J.C. (1995) Functional analysis of the chromo domain of HP1. *EMBO J.*, **14**, 3977–3986.

Platero,J.S., Sharp,E.J., Adler,P.N. and Eissenberg,J.C. (1996) *In vivo* assay for protein–protein interactions using *Drosophila* chromosomes. *Chromosoma*, **104**, 393–404.

Powers,J.A. and Eissenberg,J.C. (1993) Overlapping domains of the heterochromatin-associated protein HP1 mediate nuclear localization and heterochromatin binding. *J. Cell Biol.*, **120**, 291–299.

Quinn,J., Fyrberg,A.M., Ganster,R.W., Schmidt,M.C. and Peterson,C.L. (1996) DNA-binding properties of the yeast SWI/SNF complex. *Nature*, **379**, 844–847.

Ramakrishnan,V., Finch,J.T., Graziano,V., Lee,P.L. and Sweet,R.M. (1993) Crystal structure of globular domain of histone H5 and its implications for nucleosome binding. *Nature*, **362**, 219–223.

Renaud,J.P., Rochel,N., Ruff,M., Vivat,V., Chambon,P., Gronemeyer,H. and Moras,D. (1995) Crystal structure of the RARγ ligand-binding domain bound to all-*trans* retinoic acid. *Nature*, **378**, 681–689.

Rost,B. and Sander,C. (1994) Conservation and prediction of solvent accessibility in protein families. *Proteins*, **20**, 216–226.

Saunders,W.S. *et al.* (1993) Molecular cloning of a human homologue of *Drosophila* heterochromatin protein HP1 using anti-centromere autoantibodies with anti-chromo specificity. *J. Cell Sci.*, **104**, 573–582.

Singh,P.B., Miller,J.R., Pearce,J., Kothary,R., Burton,R.D., Paro,R., James,T.C. and Gaunt,S.J. (1991) A sequence motif found in a *Drosophila* heterochromatin protein is conserved in animals and plants. *Nucleic Acids Res.*, **19**, 789–794.

Stayton,C.L. *et al.* (1994) Cloning and characterization of a new human Xq13 gene encoding a putative helicase. *Hum. Mol. Genet.*, **3**, 1957–1964.

Sugawara,N., Ivanov,E.L., Fishman-Lobell,J., Ray,B.L., Wu,X. and Haber,J.E. (1995) DNA structure-dependent requirements for yeast RAD genes in gene conversion. *Nature*, **373**, 84–86.

Tamkun,J.W., Deuring,R., Scott,M.P., Kissinger,M., Pattatucci,A.M., Kaufman,T.C. and Kennison,J.A. (1992) Brahma: a regulator of *Drosophila* homeotic genes structurally related to the yeast transcriptional activator SNF2/SWI2. *Cell*, **68**, 561–572.

Tasset,D., Tora,L., Fromental,C., Scheer,E. and Chambon,P. (1990) Distinct classes of transcriptional activating domains function by different mechanisms. *Cell*, **62**, 1177–1187.

Thompson,K.A., Wang,B., Argraves,W.S., Giancotti,F.G., Schranck,D.P. and Ruoslahti,E. (1994a) BR140, a novel zinc-finger protein with homology to the TAF250 subunit of TFIID. *Biochem. Biophys. Res. Commun.*, **198**, 1143–1152.

Thompson,J.D., Higgins,D.G. and Gibson,T.J. (1994b) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.*, **22**, 4673–4680.

Thummel,C.S. (1995) From embryogenesis to metamorphosis: the regulation and function of *Drosophila* nuclear receptor superfamily members. *Cell*, **83**, 871–877.

Tora,L., White,J., Brou,C., Tasset,D., Webster,N., Scheer,E. and Chambon,P. (1989) The human oestrogen receptor has two independent nonacidic transcriptional activation functions. *Cell*, **59**, 447–487.

Tsai,M.J. and O'Malley,B.W. (1994) Molecular mechanisms of action of steroid/thyroid receptor superfamily members. *Annu. Rev. Biochem.*, **63**, 451–486.

Varga-Weisz,P., Zlatanova,J., Leuba,S.H., Schroth,G.P. and van Holde,K. (1994) Binding of histone H1 and H5 and their globular domains to four-way junction DNA. *Proc. Natl Acad. Sci. USA*, **91**, 3525–3529.

Voegel,J.J., Heine,M.J.S., Zechel,C., Chambon,P. and Gronemeyer,H. (1996) A 160 kDa transcriptional mediator for the ligand-dependent activation function AF-2 of nuclear receptors. *EMBO J.*, **15**, 3667–3675.

Vojtek,A.B., Hollenberg,S.M. and Cooper,J.A. (1993) Mammalian Ras interacts directly with the serine/threonine kinase Raf. *Cell*, **74**, 205–214.

vom Baur,E. *et al.* (1996) Differential ligand-dependent interactions between the AF-2 activating domain of nuclear receptors and the putative transcriptional intermediary factors mSUG1 and TIF1. *EMBO J.*, **15**, 110–124.

Weiler,K.S. and Wakimoto,B.T. (1995) Heterochromatin and gene expression in *Drosophila*. *Annu. Rev. Genet.*, **29**, 577–605.

White,R.J., Brou,C., Wu,J., Lutz,Y., Moncollin,V. and Chambon,P. (1992) The acidic transcriptional activator GAL–VP16 acts on preformed template-committed complexes. *EMBO J.*, **11**, 2229–2240.

Witzgall,R., O'Leary,E., Leaf,A., Önaldi,D. and Bonventre,J.V. (1994) The Krüppel-associated box-A (KRAB-A) domain of zinc finger proteins mediates transcriptional repression. *Proc. Natl Acad. Sci. USA*, **91**, 4514–4518.

Wreggett,K.A., Hill,F., James,P.S., Hutchings,A., Butcher,G.W. and Singh,P.B. (1994) A mammalian homologue of *Drosophila* heterochromatin protein 1 (HP1) is a component of constitutive heterochromatin. *Cytogenet. Cell. Genet.*, **66**, 99–103.

Wurtz,J.M., Bourguet,W., Renaud,J.P., Vivat,V., Chambon,P., Moras,D. and Gronemeyer,H. (1996) A canonical structure for the ligand binding domain of nuclear receptors. *Nature Struct. Biol.*, **3**, 87–94.

Received on June 14, 1996; revised on July 23, 1996