p300 stimulates transcription instigated by ligand-bound thyroid hormone receptor at a step subsequent to chromatin disruption

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We investigate the role of the transcriptional coactivator p300 in gene activation by thyroid hormone receptor (TR) on addition of ligand. The ligand-bound TR targets chromatin disruption independently of gene activation. Exogenous p300 facilitates transcription from a disrupted chromatin template, but does not itself disrupt chromatin in the presence or absence of ligand-bound receptor. Nevertheless, the acetyltransferase activity of p300 is required to facilitate transcription from a disrupted chromatin template. Expression of E1A prevents aspects of chromatin remodeling and transcriptional activation dependent on TR and p300. E1A selectively inhibits the acetylation of non-histone substrates. E1A does not prevent the assembly of a DNase I-hypersensitive site induced by TR, but does inhibit topological alterations and the loss of canonical nucleosome arrays dependent on the addition of ligand. Mutants of E1A incompetent for interaction with p300 partially inhibit chromatin disruption but still allow nuclear receptors to activate transcription. We conclude that p300 has no essential role in chromatin disruption, but makes use of acetyltransferase activity to stimulate transcription at a subsequent step.

Keywords: chromatin disruption/E1A/non-histone acetylation/nuclear receptors/p300

Introduction

The precise mechanisms by which coactivators function to regulate transcription remain unclear. Coactivators have a structural role in serving as focal points for multiple protein–protein interactions including association with many transcriptional activation domains (Verrijzer and Tjian, 1996; Shikama *et al.*, 1997; Xu *et al.*, 1999). These interactions might help recruit components of the basal transcriptional machinery including RNA polymerase to a particular promoter (Barlev *et al.*, 1995; Nakajima *et al.*, 1997a,b). Coactivators can also function as enzymes that modify both other transcriptional regulators and the chromatin environment within which transcription occurs (Brownell and Allis, 1996; Gregory and Horz, 1998). The exact significance of these diverse roles is a topic of substantial research interest.

Among the best studied systems for the analysis of coactivator function is the GCN5p–ADA2p–ADA3p com-

plex (Berger *et al.*, 1992; Candau and Berger, 1996; Candau *et al.*, 1997; Grant *et al.*, 1997). Components of this complex physically contact transcription activation domains and components of the basal transcriptional machinery (Barlev *et al.*, 1995). The bromodomain of GCN5p also makes specific contacts with the N-terminal tail of histone H4 (Ornaghi *et al.*, 1999). GCN5p is an acetyltransferase that modifies histones (Brownell *et al.*, 1996). Histone acetylation occurs in the vicinity of promoters to which it is targeted (Kuo *et al.*, 1998) and acetyltransferase activity is required to regulate transcription (Kuo *et al.*, 1998; Wang *et al.*, 1998). The histone acetyltransferase activity of GCN5p contributes to alterations in chromatin structure *in vivo* (Gregory *et al.*, 1998), and mutations in the core histones that mimic the consequences of acetylation relieve the requirement for GCN5p for gene activation *in vivo* (Zhang *et al.*, 1998). This is impressive progress, yet the exact biochemical mechanism by which the GCN5p coactivator stimulates transcription remains unclear. For example, histone modification and acetyltransferase activity might be required either to disrupt chromatin or to maintain a fully disrupted active state. Histone acetylation might yet be a secondary consequence of recruitment and modification of the basal transcriptional machinery by GCN5p.

Histone acetylation provided an early link between transcription and chromatin modification (Allfrey *et al.*, 1964). Genetic, biochemical and chromatin immunoprecipitation experiments in yeast all reinforce this association (reviewed by Edmondson *et al.*, 1998; Gregory and Horz, 1998; Mizzen *et al.*, 1998). Structural studies indicate that histone acetylation destabilizes both local and higher-order chromatin folding, thereby facilitating transcription (Lee *et al.*, 1993; Vettesse-Dadey *et al.*, 1996; Ura *et al.*, 1997; Krajewski and Becker, 1998; Nightingale *et al.*, 1998; Tse *et al.*, 1998). It is also probable that the acetylation of specific lysines in the core histones provides novel recognition surfaces to promote the association of positive regulators of the transcription process (Dutnall *et al.*, 1998). What is clear is that histone acetylation is not a universal transcriptional activation mechanism (Van Lint *et al.*, 1996a), and that while some genes can be selectively activated by increases in histone acetylation (Van Lint *et al.*, 1996b), others are not (Bresnick *et al.*, 1990). An explanation for this variation may lie either in specific aspects of nucleoprotein architecture to which histone acetylation might contribute (van Holde, 1993), or in the recognition that acetyltransferases modify many transcriptional regulators aside from the histones (Gu and Roeder, 1997; Imhof *et al.*, 1997; Boyes *et al.*, 1998) with largely unknown functional consequences.

In contrast to the experiments in yeast that establish a role for the coactivator GCN5p in chromatin remodeling (Gregory and Horz, 1998, Gregory *et al.*, 1998), the

capacity of targeted transcriptional coactivators to remodel chromatin in animal systems has not been investigated. Many transcriptional activators, including nuclear hormone receptors, interact with the structurally related coactivators p300 and CREB-binding protein (CBP) (Chrivia *et al.*, 1993; Chakravarti *et al.*, 1996; Hanstein *et al.*, 1996; Kamei *et al.*, 1996; Smith *et al.*, 1996; Chen *et al.*, 1997; Nakajima *et al.*, 1997a,b; Puri *et al.*, 1997; Ramirez *et al.*, 1997; Shikama *et al.*, 1997; Li *et al.*, 1998). The basal transcription factors TATA-binding protein (TBP) and TFIIB also make contact with CBP and p300 (Kwok *et al.*, 1994; Yuan *et al.*, 1996), as does RNA polymerase (Nakajima *et al.*, 1997a,b). These diverse interactions might facilitate recruitment of the basal transcriptional machinery to promoters. p300 and CBP are HATs (Ogryzko *et al.*, 1996) and this enzymatic activity stimulates transcription in model systems (Li *et al.*, 1998; Martinez-Balbas *et al.*, 1998). One model for transcriptional control by coactivators suggests that their acetyltransferase activity is important for rendering chromatin accessible to the basal transcriptional machinery (Wolffe and Pruss, 1996; Jenster *et al.*, 1997; Mizzen *et al.*, 1998). However, the influence of p300 and CBP on chromatin disruption has not yet been examined.

Chromatin disruption has long been associated with transcription of chromosomal DNA (reviewed by van Holde, 1988). Disruption has been defined through the use of diverse assays. These include alterations in cleavage of DNA using nucleases such as DNase I (Zaret and Yamamoto, 1984; Emerson *et al.*, 1985; Gregory *et al.*, 1998), micrococcal nuclease (Almer and Horz, 1986; Carr and Richard-Foy, 1990; Cavalli and Thoma, 1993; Tsukiyama *et al.*, 1994; Cavalli *et al.*, 1996) and restriction endonucleases (Emerson and Felsenfeld, 1984; Fascher *et al.*, 1993; Lee and Archer, 1994; Truss *et al.*, 1995; Logie and Peterson, 1997; Varga-Weisz *et al.*, 1997). Alterations in the topology of closed circular DNA molecules have also been used to monitor changes in nucleosome integrity (Norton *et al.*, 1989; Kwon *et al.*, 1994; Wechsler *et al.*, 1997). The assay for topological change of minichromosomes monitors chromatin reconfiguration or disruption. It is based on the fact that each nucleosome constrains a single negative superhelical turn (Germond *et al.*, 1975; Simpson *et al.*, 1985). Removal of nucleosomes (Randall and Kelly, 1992), reduction in DNA wrapping (Norton *et al.*, 1989) and alterations in higher-order structure dependent on DNA crossing over at the entry and exit of the nucleosome (Worcel *et al.*, 1981) can, in principle, all be monitored by the reduction in negative superhelicity. These chromatin disruption assays have allowed several investigators to establish unambiguously that chromatin disruption can occur in the absence of transcription itself, so that it potentially functions to prepare the promoter for true activation that would occur as a subsequent step (Fascher *et al.*, 1993; Becker, 1994; Owen-Hughes *et al.*, 1996; Svaren and Horz, 1997; Wong *et al.*, 1997a).

The regulation of gene expression by nuclear hormone receptors has proven particularly useful in determining the relationships between coactivators, corepressors and chromatin (Parker, 1998; Torchia *et al.*, 1998; Stunnenberg *et al.*, 1999; Xu *et al.*, 1999). Nuclear receptors have the capacity to bind to their recognition

elements efficiently in chromatin (Wong *et al.*, 1995; Ciana *et al.*, 1998; Minucci *et al.*, 1998). In the absence of ligand, the thyroid hormone receptor (TR) and retinoic acid receptor (RAR) recruit a corepressor complex that contains histone deacetylase (Alland *et al.*, 1997; Heinzel *et al.*, 1997; Nagy *et al.*, 1997). This enzymatic activity is essential for transcriptional silencing in the *Xenopus* oocyte (Wong *et al.*, 1998). On the addition of hormone the corepressor complex is released (Chen and Evans, 1995; Horlein *et al.*, 1995; Collingwood *et al.*, 1998) and a complex of histone acetyltransferases is recruited to the receptor (Chakravarti *et al.*, 1996; Kamei *et al.*, 1996; Chen *et al.*, 1997). The TR can target chromatin disruption as assayed by micrococcal nuclease cleavage and topological change in the absence of transcription (Wong *et al.*, 1997a). This leads to a model for gene regulation in which the unliganded nuclear receptor initially targets the assembly of a repressive chromatin structure in response to ligand, the receptor recruits coactivators with histone acetyltransferase activity to first destabilize the repressive structure and subsequently activate transcription (Jenster *et al.*, 1997; Pazin and Kadonaga, 1997; Wolffe, 1997).

In this work, we have examined the role of p300 and acetylation in chromatin dynamics and transcriptional activation in response to the addition of hormone to chromatin-bound TR. Surprisingly, we find that p300 itself neither disrupts chromatin, nor activates transcription from a non-disrupted template. However, p300 facilitates transcription from a previously disrupted chromatin template. This activation of transcription requires acetyltransferase activity. E1A is known to influence transcriptional activation by nuclear receptors (Berkensham *et al.*, 1992; Meyer *et al.*, 1996; Kurokawa *et al.*, 1998; Wahlstrom *et al.*, 1999). We make use of E1A to explore further the role of p300 and acetylation in transcriptional control by the TR in chromatin. We establish that E1A acts selectively to impair chromatin remodeling and that there are additional targets involved in this process apart from p300.

Results

p300 does not stimulate transcription from a chromatin-bound TR unless chromatin is disrupted in the presence of ligand

We microinjected the *Xenopus* thyroid hormone receptor β A (TR β A) promoter into *Xenopus* oocyte nuclei in single-stranded form. Replication-coupled chromatin assembly drives the repression of basal transcription under these conditions (Almouzni and Wolffe, 1993). Expression of exogenous TR further stabilizes this repression of transcription in the absence of hormone (Wong *et al.*, 1995, 1998). The expression of exogenous p300 in the *Xenopus* oocyte (Li *et al.*, 1998) does not lead to a significant relief of the repression of transcription established on the TR β A promoter containing template, which occurs in the presence of unliganded TR-retinoid X receptor (RXR) (Figure 1A, compare lanes 1 and 2; Wong *et al.*, 1995, 1998). The addition of ligand activates transcription from the TR β A promoter and the expression of exogenous p300 further facilitates transcription (Figure 1A and B, compare lanes 1–4). Under these conditions, all of the minichromosomes

assembled in the *Xenopus* oocyte nucleus are bound by the TR-RXR (Wong *et al.*, 1995, 1997a, 1998). This is shown by *in vivo* footprinting of the receptor on the TR β A promoter, by complete receptor-dependent repression of basal transcription and by the receptor altering the topology of all the minichromosomes in the presence of ligand (Figure 1C and D). Since we have a robust connection between chromatin structure and function in this system, we next asked whether the p300 facilitation of transcription (Figure 1A and B) would lead to a further change in minichromosome topology.

The expression of exogenous p300 has no effect on the topology of minichromosomes in the presence of TR-

RXR (Figure 1C, compare lanes 1 and 2). Addition of ligand in the presence of TR-RXR leads to chromatin reconfiguration or disruption as revealed by the decrease in negative superhelical turns (equivalent to loss of nucleosomes) (Figure 1C, compare lanes 1 and 3). Remarkably, expression of exogenous p300, which facilitates transcription in the presence of ligand-bound TR-RXR (Figure 1A and B), does not further disrupt chromatin (Figure 1C, compare lanes 1 and 4). In fact, exogenous p300 appears to stabilize the minichromosome because the topological change is reduced relative to that directed by ligand-bound TR-RXR alone (Figure 1C, compare lanes 3 and 4). Quantitation of topological change using a different con-

centration of chloroquine (Figure 1D) indicates that ligand-bound TR-RXR on the TR β A promoter targets the loss of eight negative superhelical turns, each a putative reconfigured or disrupted nucleosome, while in the presence of exogenous p300 only five negative superhelical turns are lost. The loss of negative superhelical turns is dependent on the abundance of TR-RXR and the total of four thyroid hormone response elements (TREs) present in the TR β A regulatory DNA, which span from -800 to +264 (Wong *et al.*, 1997a; F.Urnov, manuscript in preparation). Each TR-RXR and TRE can target the loss of two or three negative superhelical turns in the presence of ligand (Wong *et al.*, 1997a). As a control, we examined whether the major topological change was dependent on transcription itself. Addition of α -amanitin at concentrations sufficient to inhibit transcription (Figure 1E) did not prevent significant chromatin disruption targeted by hormone-bound TR-RXR (Figure 1F). The addition of α -amanitin did lead to minor topological changes comparable to those observed with p300 (Figure 1F, compare lanes 3–6). This would be consistent with a small level of nucleosomal stabilization in the absence of transcription. Finally, it is conceivable that TR bound to DNA in the absence of hormone, as in Figure 1A–D, prevents p300 effects. To test this possibility, we compared transcription and DNA topology plus or minus p300 in the presence or absence of ligand-bound TR-RXR (Figure 1G–I). We find that p300 alone has no influence on transcription or topology (Figure 1G–I, compare lanes 1 and 3). However, p300 augments transcription in the presence of TR-RXR and hormone (Figure 1G, compare lanes 2 and 4). As seen previously (Figure 1C and D), p300 also leads to recovery of topology in the presence of TR-RXR and ligand (Figure 1H and I, compare lanes 2 and 4). We conclude that TR bound in the absence of hormone does not interfere with the activity of p300.

Taken together, these results suggest that chromatin disruption is largely independent of transcription and that exogenous p300 facilitates transcription but does not do

so by further disrupting chromatin. We do not yet understand why the expression of exogenous p300 would reduce topological change or potentially stabilize nucleosomes. The expression of p300 may capacitate transcription by facilitating the assembly of regulatory nucleoprotein complexes other than nucleosomes, which nevertheless wrap DNA in a comparable way (e.g. Du *et al.*, 1993; Katsani *et al.*, 1999). It has been proposed that the TFIID complex itself may resemble a histone octamer (Hoffman *et al.*, 1997). It is also possible that the overexpression of p300 competes for endogenous chromatin disrupting factors, thereby inhibiting topological change; however, we do not see such marked effects on topology following expression of SRC-1 or p300/CBP-associating factor (PCAF) (see Figure 3 and Discussion).

Earlier work has established that in mammalian cells the association of transcriptional coactivators with nuclear receptors is usually hormone dependent (Chakravarti *et al.*, 1996; Kamei *et al.*, 1996; Henttu *et al.*, 1997; Collingwood *et al.*, 1998); however, under certain circumstances coactivators can interact with nuclear receptors through hormone-independent pathways (White *et al.*, 1997; Blanco *et al.*, 1998; Hammer *et al.*, 1999; Tremblay *et al.*, 1999; reviewed by Freedman, 1999). We examined the association of p300 with the TR-RXR in oocytes in the presence or absence of ligand. Surprisingly, p300 interacts with TR-RXR independently of ligand (Figure 2A, compare lanes 7 and 8; see also Figure 3A, lanes 6 and 8). The N-terminal domain of p300 was sufficient for association with TR-RXR (Figure 2A, compare lanes 9 and 10) as described previously (Chakravarti *et al.*, 1996; Kamei *et al.*, 1996), and negative controls using *Xenopus* heat-shock transcription factor, a known positive regulator of transcription in *Xenopus* oocytes (Landsberger and Wolffe, 1995), indicated that the association was specific (Figure 2A, lane 11). In the absence of the expressed p300 protein, the M2-antibody immunoprecipitates very few radioactive proteins (Figure 2A, lane 12). This further indicates that interaction with p300 is necessary for

Fig. 1. Effects of p300 on TR-RXR-dependent transcription and DNA topology of the TR β A minichromosome in the *Xenopus* oocytes. (A) p300 augments transcriptional activation by ligand-bound TR-RXR from the TR β A promoter. *Xenopus* oocytes were first injected with different mRNAs for protein synthesis. Two hours after the cytoplasmic injection, single-stranded TR β A promoter was injected into the nucleus in 9.2 nl (0.1 μ g/ μ l). The oocytes were then incubated in the presence or absence of 50 nM T₃ hormone at 18°C for 16 h. The microinjected oocytes (15–20) for each experimental group were collected for assay of transcription activity by primer extension. Lane 1, TR and RXR mRNA (2 fmol) without hormone induction; lane 2, TR, RXR mRNA (2 fmol) and p300 mRNA (2 fmol) without T₃ induction; lane 3, mRNA injection as in lane 1 but with T₃ induction; lane 4, mRNA injection as in lane 2 but with T₃ induction. Primer extensions of endogenous histone H4 mRNA serves as a loading control (Materials and methods). The Southern blots of chloroquine gels serve as control for the microinjection of constant amounts of template DNA (C and D). (B) Quantification of the experiment in (A) by PhosphorImager analysis. The endogenous H4 signal is used as a loading control. The transcription signals are plotted as fold induction relative to control [lane 1 in (A)]. A.U. indicates arbitrary units of transcriptional activity. (C) p300 decreases the DNA topological change caused by ligand-bound TR-RXR. DNA from the same batch of oocytes as in (A) was purified and analyzed for its topology on a 1.2% agarose gel in 1 $\times$ TPE buffer with 90 μ g/ml chloroquine. The detection of DNA was done by conventional Southern blotting. Non-injected DNA (In) and topoisomerase I relaxed DNA (Re) were used as markers for the Southern analysis. Lanes 1–4 as lanes 1–4 in (A). The arrow indicates the direction of increase in negative superhelicity (i.e. nucleosomes). NC shows the migration of nicked circular DNA. (D) The experimental procedure is as in (C) except that the chloroquine concentration is 30 μ g/ml. NC shows the migration of nicked circular DNA. Densitometric scans of lanes 1–4 are shown for ease of comparison of topoisomers. (E) α -amanitin blocks transcription from TR β A promoter. The oocytes were first injected with different mRNA, then with single-stranded TR β A promoter (1 ng). α -amanitin (6 ng) was coinjected with the DNA. The microinjected oocytes (15–20) for each experimental group were collected for the transcription activity by primer extension. Lane 1, TR and RXR mRNA (2 fmol) without T₃ induction; lane 2, as in lane 1 except with coinjected α -amanitin; lane 3, TR and RXR mRNA (2 fmol) with T₃ induction; lane 4, as in lane 3 except with α -amanitin; lane 5, TR, RXR mRNA (2 fmol) and p300 mRNA (2 fmol) with T₃ induction; lane 6, as in lane 5 except with coinjected α -amanitin. (F) DNA topological change is independent of transcription. DNA from the same batch of oocytes as in (E) was purified and analyzed for its topology on 1.2% agarose with 60 μ g/ml chloroquine. The detection of DNA was done by conventional Southern blot analysis. Lanes 1–6 as lanes 1–6 in (E). (G) Effects of p300 alone on transcription in the absence of TR-RXR as in (A) except lane 1, no hormone addition; lane 2, TR, RXR mRNA (2 fmol) with hormone induction; lane 3, p300 mRNA (2 fmol) with hormone; lane 4, TR, RXR mRNA (2 fmol) and p300 mRNA (2 fmol) with hormone induction. (H) Topological analysis of minichromosomes transcribed in (G). Experimental procedures were as described in (C). Topoisomers were resolved in 90 μ g/ml chloroquine. (I) As in (H), except topoisomers were resolved in 30 μ g/ml chloroquine. Densitometric scans of lanes 1–4 are shown to facilitate comparison of topoisomers.

Fig. 4. p300 stimulation of transcriptional activity on the TRβA promoter is acetyltransferase activity (AT) domain-dependent. (A) The AT mutant of p300 (hm) is able to interact with TR-RXR equivalently to the wild-type p300 (WT). The oocytes were injected with different mRNA as indicated and incubated with T₃ in the presence or absence of [³⁵S]methionine at 18°C for 18 h for exogenous protein synthesis. The homogenized oocytes were then subjected to SDS-PAGE for monitoring protein synthesis (lanes 1–3) and immunoprecipitation by antibody against TR (lanes 4–6) or antibody against p300 (lanes 7–8). (B) The AT mutant of p300 (hm) can not enhance transcriptional activation from TRβA promoter. Oocytes were first injected with different mRNAs, then with single-stranded TRβA promoter (1 ng) and incubated in the presence or absence of 50 nM T₃ hormone at 18°C for 16 h. The microinjected oocytes (15–20) for each experimental group were collected for assay of transcription activity by primer extension. Lane 1, TR and RXR mRNA (1 fmol) without hormone induction; lane 2, TR and RXR mRNA (1 fmol) with T₃ induction; lane 3, TR, RXR mRNA (1 fmol) and the acetyltransferase mutant of p300 mRNA (2 fmol) with T₃ induction. (C) The AT mutant of p300 can not reduce DNA topological change. DNA from the same batch of oocytes as in (B) was purified and analyzed for its topology on 1.2% agarose gel in 1× TPE buffer with 30 μg/ml chloroquine. The detection of DNA was done by conventional Southern blotting. Lanes 1–3 as lanes 1–3 in (B). The arrow indicates the direction of increasing negative superhelicity (i.e. nucleosomes). NC shows the migration of nicked circular DNA. (D) The experimental procedure is as in (C) except that the chloroquine concentration is 90 μg/ml. Non-injected DNA (In) and topoisomerase I relaxed DNA (Re) were used as markers for the Southern analysis. NC shows the migration of nicked circular DNA.

Fig. 5. E1A inhibits transcriptional activation and topological changes induced by ligand-bound TR-RXR, p300 and PCAF. (A) E1A inhibits the transcriptional activation by p300 and PCAF. The oocytes were first injected with different mRNA as indicated. Two hours later, single-stranded TRβA promoter was injected into the nucleus in 9.2 nl (0.1 μg/μl). The oocytes were then incubated with 50 nM T₃ hormone at 18°C for 16 h. The microinjected oocytes (15–20) for each experimental group were collected for the transcription activity by primer extension. Lane 1, oocytes without exogenous mRNA; lane 2, TR and RXR mRNA (1 fmol); lane 3, TR, RXR mRNA (1 fmol) and p300 mRNA (2 fmol); lane 4, TR, RXR mRNA (1 fmol), p300 mRNA (2 fmol) and E1A mRNA (2 fmol); lane 5, TR, RXR mRNA (1 fmol) and PCAF mRNA (2 fmol); lane 6, TR, RXR mRNA (1 fmol), PCAF mRNA (2 fmol) and E1A mRNA (2 fmol). (B) E1A prevents the topological change. DNA from the same batch of oocytes as in (A) was purified and analyzed for its topology on 1.2% agarose with 90 μg/ml chloroquine. The detection of DNA was carried out by conventional Southern blotting. Non-injected DNA (In) and topoisomerase I relaxed DNA (Re) were used as markers for the Southern analysis. Lanes 1–4 as lanes 1–4 in (A). NC shows the migration of nicked circular DNA. (C) The experimental procedure is as in (B) except that the chloroquine concentration is 30 μg/ml. Densitometric scans of the lanes indicated are shown.

immunoprecipitation. We note that in our assay, less TR is precipitated by anti-p300 serum in the presence of ligand compared with the absence of ligand (Figure 2A, compare lanes 7 and 8, 9 and 10). This might be the consequence of a competition between exogenous p300 and other, endogenous, coactivators for interaction interfaces on the surface of liganded TR. The fraction of intracellular receptor bound to p300 would thus be lowered. As a control, we also examined the association of SRC-1 (Onate *et al.*, 1995; Kalkhoven *et al.*, 1998)

with TR-RXR and found it to be dependent on ligand (Figure 2B, lanes 1–3; see Lanz *et al.*, 1999). The interaction of SRC-1 with TR-RXR was dependent on the intact N-terminal domain of the coactivator (Figure 2B, lanes 6–9) demonstrating specificity (Korzus *et al.*, 1998). Note that endogenous levels of p300 are extremely low (Li *et al.*, 1998) and are not detectable in this assay.

These results indicate that within the oocyte nucleus, exogenous p300 is bound to the TR independently of ligand. This association is insufficient to activate transcrip-

Fig. 6. E1A selectively inhibits acetylation of non-histone substrate by p300. (A) Recombinant TFIIIEβ was used as an acetylation substrate for p300 at a concentration of 3 μM. Molar ratios of E1A to histone of 1:5 or 2:1 were used and the kinetics of modification of TFIIIEβ by p300 assayed. After a 30 min incubation, a 20 μl aliquot of the reaction mixture was loaded onto an SDS-polyacrylamide gel, stained with Coomassie Blue, destained, treated with Amplify (Amersham) for 15 min, dried and the gel exposed for 24 h. The left panel shows the quantitation, whereas the right panel shows both a stained gel and the autoradiograph. (B) Various other substrates have been used for acetylation assays. Two micrograms of GST-MyoD (lanes 1 and 2); TR-RXR heterodimers (lanes 3 and 4) at molar ratios of E1A to substrate of 2:1; TFIIIF (lanes 5 and 6); TFIIIEβ (lanes 7 and 8) at molar ratios of E1A to substrate of 1:1; and core histones (lanes 9 and 10) at molar ratios of E1A to substrate of 1:5 were incubated with p300 (40 fmol) in the presence (lanes 2, 4 and 6) or absence (lanes 1, 3 and 5) of GST-E1A in a total volume of 20 μl with an acetyl-CoA concentration of 20 μM for 30 min at 37°C. Reactions were loaded on a 12% SDS-polyacrylamide gel, stained with Coomassie Blue, destained, treated with Amplify (Amersham) for 15 min, dried and exposed for 24 h. (C) Quantitation of acetylation reactions shown in (B).

tion significantly from a chromatin template (Figure 1A, compare lanes 1 and 2, and Figure 1G, compare lanes 1 and 3). On addition of ligand, chromatin is disrupted independently of the presence of exogenous p300 (Figure 1C and D). Once chromatin has been disrupted, exogenous p300 can facilitate transcription but without further removal of nucleosomes (Figure 1). In this model, chromatin disruption would be a necessary first step before p300 could stimulate transcription.

The interaction of p300 with TR-RXR in the absence of ligand is surprising; however, this association also occurs in two hybrid experiments (T.Collingwood, unpublished observations). It is possible that the overexpression of p300 and TR-RXR in oocytes forces a non-physiological interaction between these proteins, or titrates away transcriptional corepressors that would normally contribute to ligand-dependent control of association (Blanco *et al.*, 1998). Blanco *et al.* (1998) observed that the release of corepressor facilitates the binding of PCAF to RAR-RXR. We have also observed the constitutive association of PCAF with TR-RXR when overexpressed in *Xenopus* oocytes, consistent with such a corepressor titration effect

(data not shown). Any potential titration of corepressors does not, however, influence the regulated recruitment of SRC-1 (Figure 2B).

We next asked whether the capacity to augment transcription without additional chromatin disruption was a general function of transcriptional coactivators, both those that show ligand-dependent recruitment, like SRC-1 (Figure 2B) and those that do not, such as p300 (Figure 2A) and PCAF (data not shown). Neither SRC-1 nor PCAF influence basal transcription in the absence of TR-RXR (Figure 3A and B); however, in the presence of TR-RXR and ligand, PCAF (Figure 3A and B, lane 7) provides a significant increase in transcription (2- to 3-fold; also see Figure 5), whereas SRC-1 only augments transcription by 50% (Figure 3A and B, lane 6). Neither PCAF nor SRC-1 overexpression leads to any significant topological charge beyond that induced by receptor alone (Figure 3C). We deliberately used conditions in which a limiting level of TR-RXR increases expression only 4-fold in the presence of ligand (Figure 3A and B, compare lanes 4 and 5) such that the change in DNA topology (Figure 3C) is less than under the conditions of TR-RXR saturation used in

Fig. 7. E1A effects on TSA-activated transcription and chromatin remodeling of the TRβA minichromosome. *Xenopus* oocytes were either incubated or not in 10 ng/ml TSA as indicated. Where indicated, groups of 15–20 oocytes were injected with 2 fmol of E1A mRNA. Two hours after this cytoplasmic injection, single-stranded TRβA promoter was injected into the nucleus in 9.2 nl (0.1 μg/μl). The oocytes were then incubated for 16 h at 18°C. (A) Transcriptional activity as assayed by primer extension. The positions of the TRβA transcripts and the histone H4 loading control are indicated. (B) Nucleosomal array organization as assayed by micrococcal nuclease digestion of the minichromosomes whose transcriptional activity was digested with 4–120 U of micrococcal nuclease at 25°C for 2 min. The DNA was then purified, separated on a 2% agarose gel, transferred to Hybond+ membrane and hybridized with random primed probe from the entire TRβA plasmid. Lanes 1–3 as lane 1 in (A); lanes 4–6 as lane 2 in (A); lanes 7–9 as lane 3 in (A); and lanes 10–12 as lane 4 in (A). The numbers indicate mono-, di-, tri- and tetranucleosomal size DNA fragments. (C) TSA and E1A effects on topology. DNA from the same batch of oocytes as in (A) was purified and analyzed for its topology on a 1.2% agarose gel in 1× TPE buffer with 90 μg/ml chloroquine. The detection of DNA was done by conventional Southern blotting. Non-injected DNA (In) and topoisomerase I relaxed DNA (Re) were used as markers for the Southern analysis. Lanes 1–4 as lanes 1–4 in (A). The arrow indicates the direction of increase in negative superhelicity (i.e. nucleosomes). NC shows the migration of nicked circular DNA. (D) The experimental procedure is as in (C) except that the chloroquine concentration is 30 μg/ml. The densitometric scans of lanes 1–4 are for ease of comparison of the topoisomers.

Figure 1A–D. We wished to be able to detect sensitively any augmentation of chromatin disruption by the coactivators. The densitometric scans demonstrate that expression of the SRC-1 and PCAF coactivators does not further alter topology towards a more disrupted state. The stimulation of transcription by PCAF in the presence of ligand-bound TR-RXR (Figure 3A and B, lanes 5 and 7) is comparable to that obtained with p300 (Figure 1A and B, lanes 3 and 4). However, unlike p300, PCAF does not appear to help

restore the original topology of the minichromosome (see also Figure 5).

The acetyltransferase activity of p300 is required for transcriptional stimulation

As exogenous p300 is not required to disrupt chromatin, we next examined whether acetyltransferase activity would be required to stimulate transcription. We expressed a mutant form of p300 in which the acetyltransferase activity had been eliminated by a deletion in the active site (Li *et al.*, 1998; Martinez-Balbas *et al.*, 1998). This mutant form of p300 interacts with TR-RXR (Figure 4A, lanes 5 and 7), but does not stimulate transcription in the presence of ligand (Figure 4B), nor does the association of the mutant p300 with the receptor prevent chromatin disruption (Figure 4C). Put another way, the mutant p300 that binds efficiently to TR-RXR does not exert a dominant-negative function on chromatin disruption. This is consistent with our hypothesis that p300 (endogenous and exogenous) is not required for chromatin disruption. Interestingly, the mutant p300 deficient in acetyltransferase activity does not cause any topological recovery in disrupted chromatin (Figure 4C, compare lanes 2 and 3). We conclude that the acetyltransferase activity of p300 is not essential for chromatin disruption, but is essential for transcriptional stimulation of a disrupted chromatin template.

E1A inhibits transcription instigated by ligand-bound TR-RXR and inhibits topological change dependent on ligand-bound TR-RXR and p300

p300 was originally defined through interactions with the adenovirus oncoprotein E1A (Eckner *et al.*, 1994). Subsequent experiments have indicated that E1A interferes with p300 function at several levels (Eckner *et al.*, 1994, 1996a,b; Arany *et al.*, 1995; Lundblad *et al.*, 1995; Chakravarti *et al.*, 1996; Gerritsen *et al.*, 1997; Aarnisalo *et al.*, 1998; Blobel *et al.*, 1998). This inhibition of p300 function can be a consequence of E1A interference with the association of p300 with transcriptional activation domains or other coactivators such as PCAF (Yang *et al.*, 1996; Kurokawa *et al.*, 1998). E1A also influences the histone acetyltransferase activity of p300 (Ait-Si-Ali *et al.*, 1998; Chakravarti *et al.*, 1999; Hamamori *et al.*, 1999). Importantly, the site of E1A interaction with p300 is distinct from the N-terminal domain of p300 that interacts with TR-RXR; therefore, E1A does not influence the association of TR-RXR with p300 (data not shown; see also Kurokawa *et al.*, 1998; Wahlstrom *et al.*, 1999). We find that expression of exogenous E1A blocks transcriptional activation by TR-RXR in the presence of ligand and exogenous p300 (Figure 5A, lanes 1–4). The small amount of basal transcription is not activated by E1A and there is a reduction in transcription below basal levels in the presence of p300, ligand-bound receptor and E1A (Figure 5A, compare lanes 1 and 4; data not shown). This contrasts with the substantial activation of the RARβ2 promoter dependent on the RAR, TFIID and an E1A-mediated bridge between the two transcription factors (Berkensam *et al.*, 1992). E1A has also recently been reported to facilitate transcriptional activation by the TR in mammalian cells during transfection experiments in the presence and absence of ligand using direct repeat and

palindromic hormone response sites in model templates (Wahlstrom *et al.*, 1999). As we discuss later, E1A could have diverse effects on transcription dependent on the excess of the protein relative to other components of the transcriptional machinery (see Figure 6). We next examined the influence of E1A on the topological changes instigated by ligand-bound TR-RXR and exogenous p300 under these particular conditions. We find that E1A inhibits the topological change induced by ligand-bound receptor and p300 (Figure 5B and C, compare lanes 1–4). These results suggest that E1A inhibits chromatin disruption. We also tested the effect of E1A on transcriptional control by PCAF (Reid *et al.*, 1998). Expression of exogenous PCAF

facilitates transcriptional activation by ligand-bound TR-RXR (Figure 5A, compare lanes 2 and 5). Addition of E1A interferes with this activation (Figure 5A, compare lanes 5 and 6). As seen previously (Figure 3), PCAF does not augment the topological change induced by ligand-bound TR-RXR (Figure 5B and C, compare lanes 1 and 2 with 5 and 6), nor does PCAF lead to partial topological recovery in contrast to p300 (Figure 5B and C, compare lanes 2, 3 and 5). E1A blocks any topological changes induced by TR-RXR in the presence or absence of PCAF.

The acetyltransferase activity of p300 is important for both transcriptional stimulation and the partial topological recovery of disrupted chromatin (Figure 4). However, our

evidence demonstrates that the acetyltransferase activity of p300 is not required for chromatin disruption. Therefore, either the acetyltransferase function is required to maintain (but not establish) a disrupted chromatin structure through the modification of histones, or a non-histone substrate is being modified with functional consequences. Recent observations have produced divergent results concerning the influence of E1A on the acetyltransferase function of p300. In a mammalian cell line, E1A stimulates histone acetylation (Ait-Si-Ali *et al.*, 1998), whereas *in vitro* E1A inhibits histone acetylation (Chakravarti *et al.*, 1999; Hamamori *et al.*, 1999). In light of our observation concerning the requirement for p300 acetyltransferase activity at a step subsequent to chromatin disruption, we have focused on the effects of E1A on acetylation of substrates other than histones. We find that at high excesses of E1A to substrate (2-fold molar excess), acetylation of the histone is inhibited as reported (Chakravarti *et al.*, 1999; Hamamori *et al.*, 1999; A.Imhof, data not shown). The effects of E1A on acetyltransferase activity are more complex than simple inhibition, because under these same conditions the acetylation of MyoD is stimulated, whereas that of TR-RXR is inhibited (Figure 6B, lanes 1–4). At a more modest excess of E1A to substrate (1:5 ratio of E1A:histone), we find that E1A enhances acetylation of the core histones (Figure 6B, lanes 9–10). This same low ratio of E1A to other substrates has highly selective consequences for acetylation of the transcription factors TFIIE and TFIIIF. Acetylation of TFIIE β and TFIIIF (RAP 74) is severely inhibited at low ratios of E1A to substrate (Figure 6A and B, lanes 5–8). These results would be consistent with a model whereby the p300 acetyltransferase function is required to modify other substrates aside from

the histones. E1A might impair or facilitate modification of these substrates, altering their function and consequently the influence of p300 on minichromosome architecture and transcription.

The effects of E1A on transcriptional activation of the TR β A promoter by the histone deacetylase inhibitor trichostatin A (TSA)

As E1A influences the acetylation of multiple substrates in distinct ways depending on the nature of the substrate and the excess of E1A (Figure 6), we next approached the role of acetylation using a different reagent, the deacetylase inhibitor TSA. This deacetylase inhibitor has previously been shown to activate the TR β A promoter in *Xenopus* oocyte nuclei when assembled into chromatin during a replication-coupled reaction to an extent equivalent to the addition of ligand-bound TR-RXR (Wong *et al.*, 1998). We find that E1A inhibits basal transcription (Figure 7, compare lanes 1 and 2) and that TSA activates transcription (compare lanes 1 and 3). Interestingly, E1A substantially reduces but does not eliminate TSA-activated transcription (Figure 7, compare lanes 3 and 4). This implies that E1A acts to inhibit transcription through mechanisms in addition to any influence on histone acetylation. As TSA addition also interferes with the deacetylation of the basal transcriptional machinery components TFIIE β and TFIIIF (A.Imhof, unpublished observations), it is probable that E1A can influence transcription in this system through mechanisms independent of any change in protein acetylation status.

As E1A inhibits TR-RXR-induced chromatin remodeling in the presence of ligand (Figure 5), we next examined whether E1A would influence any effect of TSA on

Fig. 8. p300 and E1A regulate transcriptional activation and nucleosome remodeling from TR β A promoter. (A) Effects of p300 and E1A on TR-RXR-induced transcription. *Xenopus* oocytes were first injected with different mRNAs for protein synthesis. Two hours after the cytoplasmic injection, single-stranded TR β A promoter was injected into the nucleus in 9.2 nl (0.1 μ g/ μ l). The oocytes were then incubated in the presence or absence of 50 nM T₃ hormone at 18°C for 16 h. The microinjected oocytes (15–20) for each experimental group were collected for the transcription activity by primer extension. Lane 1, TR and RXR mRNA (1 fmol) without hormone induction; lane 2, TR and RXR mRNA (1 fmol) with T₃ induction; lane 3, TR, RXR mRNA (1 fmol) and p300 mRNA (2 fmol) in the presence of T₃; lane 4, TR, RXR mRNA (1 fmol), p300 mRNA (2 fmol) and E1A mRNA (2 fmol) with T₃ hormone induction. (B) Quantification of the experiment in (A) by PhosphorImager analysis. The endogenous H4 signal is used as a loading control. The transcription signals are plotted as fold induction relative to control [lane 1 in (A)]. (C) DNase I-hypersensitivity analysis of the experiment in (A). Twenty oocytes from each experimental group were used for the DNase I-hypersensitivity assay. DNase I digestion was carried out at 9–18 U/100 μ l at 37°C for 2 min. The purified DNA was then digested with *Eco*RI restriction enzyme, separated on a 1% agarose gel, transferred to Hybond+ membrane and hybridized with random primed probe from a 268 bp CAT segment (*Bgl*III–*Eco*RI, from +306 to +584). Lanes 1 and 2 as lane 1 in (A); lanes 3 and 4 as lane 2 in (A); lanes 5 and 6 as lane 3 in (A); and lanes 7 and 8 as lane 4 in (A). (D) E1A prevents nucleosome remodeling. The organization of minichromosome was analyzed by MNase digestion of the same batch of oocytes as in (A). The microinjected oocytes (20–25) were used for each experimental group. The oocytes were homogenized and digested with 4–120 U of micrococcal nuclease at 25°C for 2 min. The DNA was then purified, separated on 2% agarose gel, transferred onto Hybond+ membrane and hybridized with random primed probe from the promoter region of the TR β A plasmid (a 108 bp *Pst*I–*Rsa*I fragment from –255 to –147). Lanes 1–3 as lane 1 in (A); lanes 4–6 as lane 2 in (A); lanes 7–9 as lane 3 in (A); and lanes 10–12 as lane 4 in (A). (E) E1A synthesis in the oocytes by [³⁵S]methionine labeling. The oocytes were injected with either wild-type E1A (WT, lane 1) or the N-terminus deletion of E1A (Δ N, lane 2). Lane 3 is a control for no exogenous protein expression. (F) Effects on nucleosome remodeling by E1A and Δ N E1A. The experimental procedure is as in (D). Lanes 1–3, TR and RXR mRNA (1 fmol) without hormone induction; lanes 4–6, TR and RXR mRNA (1 fmol) with T₃ induction; lanes 7–9, TR, RXR mRNA (1 fmol), p300 mRNA (2 fmol) and wild-type E1A mRNA (20 fmol) with T₃ induction; lanes 10–12, TR, RXR mRNA (1 fmol), p300 mRNA (2 fmol) and mutant E1A mRNA (2 fmol) with T₃ induction. (G) Influence of E1A and Δ N E1A on transcription. The experimental procedure is as in (A). *Xenopus* oocytes were first injected with different mRNAs for protein synthesis as indicated. Two hours after cytoplasmic injection, single-stranded TR β A was injected into the nucleus in 9.2 nl (0.1 μ g/ μ l). The oocytes were then incubated in the presence or absence of T₃ hormone at 18°C for 16 h as indicated. The microinjected oocytes (15–20) were then collected for transcription assay by primer extension. Lane 1, TR, RXR mRNA (1 fmol) without hormone induction; lane 2, TR and RXR mRNA (1 fmol) with T₃ induction; lane 3, TR, RXR mRNA (1 fmol), p300 mRNA (2 fmol) and WT E1A (2 fmol) with hormone induction; lane 4, TR, RXR mRNA (1 fmol), p300 mRNA (2 fmol) and Δ N E1A (2 fmol) with hormone induction. The positions of TR β A transcripts and the H4 loading control are shown. Quantitation of transcription (not shown) reveals a 40% reduction in transcription of TR β A in lane 2 relative to lane 4 when normalized to the H4 loading control. (H) Influence of E1A and Δ N E1A on topological change induced by ligand-bound TR-RXR. DNA from the same batch of oocytes as in (G) was purified and analyzed for its topology on a 1.2% agarose gel in 1 $\times$ TPE buffer with 90 μ g/ml chloroquine. The detection of DNA was done by conventional Southern analysis. Lanes 1–4 as lanes 1–4 in (G). The arrow indicates the direction of increase in negative superhelicity (i.e. nucleosomes). NC shows the migration of nicked circular DNA. Densitometric scans are shown for ease of comparison between topoisomers.

chromatin disruption. As previously reported (Wong *et al.*, 1998), we find that the addition of TSA has relatively little effect on the topology of DNA (Figure 7C and D, lanes 1 and 3) in spite of the robust activation of transcription (Figure 7A, lanes 1 and 3). The addition of E1A leads to small changes in the distribution of topoisomers with a slight increase in superhelicity in the absence of TSA and a decrease in the presence of TSA (Figure 7C and D, lanes 1–4). Using micrococcal nuclease to assay nucleosomal spacing and accessibility, we find that the addition of TSA leads to a disruption of the nucleosomal array (Figure 7B, compare lanes 1–6 with 7–12). This disruption is larger than we have reported previously (Wong *et al.*, 1998); however, we are using twice the concentration of TSA used previously. The addition of E1A improved the quality of the nucleosomal array in the absence of TSA, but less so in the presence of the deacetylase inhibitor (Figure 7B, compare lanes 1–3 with 4–6, and 7–9 with 10–12).

These experiments show that E1A interferes with TSA-activated transcription (Figure 7A), but in contrast to the complete inhibition of transcription induced by TR-RXR plus ligand (Figure 5A), E1A does not completely inhibit activation of the TR β A promoter. Consistent with the effects on transcription, while E1A inhibits topological change induced by ligand-bound TR-RXR (Figure 5B and C), addition of E1A does not inhibit the more modest alterations in topology induced by TSA (Figure 7C and D). We conclude that E1A can impede transcriptional activation of the TR β A promoter by mechanisms that are auxiliary to any influence on protein acetylation.

E1A selectively restricts chromatin structural transitions instigated by ligand-bound TR-RXR

An alternative hypothesis for the action of E1A is that it impedes not only p300 function, but also that of other chromatin remodeling activities that are required for p300 to activate transcription or alter template topology. In fact, chromatin remodeling might be independent of p300 entirely, and the effects of E1A on the process might not require interaction with p300 at all. In addition, E1A prevents topological change due to ligand-bound receptor (Figure 5); however, it is possible that chromatin functional or structural transitions, as reflected by transcription or nuclease accessibility, might occur independently of major changes in minichromosome topology (see Figure 7; Pederson and Morse, 1990; Drabik *et al.*, 1997; Wong *et al.*, 1998). Minichromosomes including the TR β A gene promoter showed the same stimulation of transcription by exogenous p300 and inhibition of transcription by E1A as previously observed in the presence of ligand-bound TR-RXR (Figure 8A and B). DNase I-hypersensitive site analysis (Figure 8C) revealed that the addition of hormone to the chromatin-bound receptor enhances DNase I hypersensitivity as previously observed (Figure 8C, lanes 1–4; Wong *et al.*, 1997a,b). In contrast, neither p300 nor E1A had any major influence on hypersensitivity at the TR β A TRE. Densitometric analysis suggests that p300 enhances DNase I hypersensitivity slightly, whereas E1A represses DNase I cleavage (data not shown). However, whereas ligand-bound TR-RXR would disrupt the regular nucleosomal array obtained on micrococcal cleavage of repressed chromatin (Figure 8D, compare lanes 1–3 with 4–6),

E1A inhibited this chromatin disruption (lanes 10–12). Exogenous p300 did not enhance or inhibit chromatin disruption using the micrococcal nuclease cleavage assay (Figure 8D, lanes 7–9). The inhibition of chromatin disruption by E1A, induced by ligand-bound TR-RXR as assayed by micrococcal nuclease and minichromosome topology (Figures 5, 8C and D), is in contrast to the failure to inhibit the effects of the histone deacetylase inhibitor TSA completely (Figure 7). We conclude that whereas a DNase I-hypersensitive site can be established on the TR β A promoter in chromatin independently of exogenous p300 or E1A, the chromatin disruption assayed by changes in DNA topology (Figure 5) or micrococcal nuclease cleavage (Figure 8D) is inhibited by E1A. We suggest that E1A must be targeting a chromatin remodeling activity independently of p300. To test this hypothesis further, we made use of a mutant form of E1A (Δ N) that lacks the N-terminal domain important for association with p300 (Arany *et al.*, 1995). We find that expression of both wild-type E1A and the Δ N variant impairs chromatin disruption over the TR β A promoter instigated by ligand-bound TR-RXR (Figure 8E and F, compare lanes 2, 5, 8 and 11). However, examination of the micrococcal cleavage patterns with densitometry suggests that the Δ N mutation of E1A does not restore the regular nucleosomal array as effectively as wild-type E1A. In agreement with Chakravarti *et al.* (1999), we also find that the Δ N mutant of E1A inhibits transcription from the TR β A promoter weakly in comparison with wild-type E1A (Figure 8G, compare lanes 3 and 4). Examination of the topological change of the minichromosome shows that while wild-type E1A gives a complete inhibition of the topological change induced by ligand-bound TR-RXR, the Δ N mutant gives only a partial inhibition (Figure 8H, compare lanes 3 and 4). Taken together, these observations suggest that while p300 may have a facilitating or stabilizing role in chromatin disruption, other activities will have significant roles in the remodeling events necessary to activate transcription at the TR β A promoter.

Discussion

The major conclusion from this work is that p300 itself does not disrupt chromatin, but stimulates transcription from a disrupted chromatin template. This transcriptional stimulation requires acetyltransferase activity. Moreover, overexpression of a p300 mutant deficient in acetyltransferase activity leads to association with TR-RXR but does not inhibit chromatin disruption or transcription. This provides further evidence that p300 has no essential role in chromatin disruption and transcriptional activation induced by ligand-bound TR-RXR. E1A eliminates chromatin disruption by ligand-bound TR-RXR; however, E1A can inhibit but not eliminate transcription from a chromatin template in the presence of the deacetylase inhibitor TSA. This indicates that protein acetylation can stabilize promoter activity such that any inhibitory effect of E1A on acetyltransferase function can not completely inactivate the TR β A promoter. A mutant of E1A that has severely reduced binding to p300 impairs disruption and transcriptional activation but does so less efficiently than wild-type E1A. We conclude that p300 does not have an essential role in disrupting chromatin on the TR β A pro-

moter as targeted by the ligand-bound TR-RXR. Instead, p300 acts primarily to stimulate transcription at a subsequent step by acetylation of proteins associated with the TR β A promoter.

Chromatin remodeling and transcription

Canonical nucleosomes can provide a major impediment to transcription. There are two general pathways for alleviating this impediment: histones can be post-translationally modified to destabilize chromatin, especially by acetylation (reviewed by Hansen *et al.*, 1998); and nucleosomes can be disrupted through the activity of ATP-driven machines such as DNA polymerase (Sogo *et al.*, 1986), RNA polymerase (Studitsky *et al.*, 1997) or the SWI-SNF complex (Coté *et al.*, 1994; Kwon *et al.*, 1994). In yeast, these histone acetyltransferases and SWI-SNF activities have overlapping functional roles (Pollard and Peterson, 1997, 1998; Holstege *et al.*, 1998). Recent results suggest that the SWI-SNF complex acts before histone acetyltransferases to activate transcription (Cosma *et al.*, 1999). In animal cells these relationships have not yet been assessed. There is accumulating evidence that nuclear receptors can interact with vertebrate homologs of the SWI-SNF complex (Yoshinaga *et al.*, 1992; Muchardt and Yaniv, 1993; Chiba *et al.*, 1994; Fryer and Archer, 1998). The largest subunit of the mammalian SWI-SNF complex contains key receptor interaction motifs (Heery *et al.*, 1997; Dallas *et al.*, 1998). These interactions might account for the stimulation of receptor-dependent transcription in the presence of SWI-SNF complex (Yoshinaga *et al.*, 1992; Muchardt and Yaniv, 1993). Fryer and Archer (1998) have shown that nuclear receptors can target the vertebrate SWI-SNF complex, and Di Croce *et al.* (1999) have shown comparable topological changes to those we report here, which are targeted by the progesterone receptor and mediated by ISWI-containing complexes. We have begun to characterize the *Xenopus* BRG1-BAF complex (Gelius *et al.*, 1999). However, whether other coactivators are required to facilitate the transcription process from a disrupted chromatin template has not been determined.

In *Drosophila* extracts, the NURF, CHRAC and ACF chromatin remodeling complexes each contain the ISWI member of the SWI2-SNF2 superfamily that disrupts chromatin (Corona *et al.*, 1999) and facilitates both DNA replication (Alexiadis *et al.*, 1998) and transcription (Ito *et al.*, 1997; Mizuguchi *et al.*, 1997). These experiments show that chromatin disruption mediated by ISWI complexes can be an important determinant of template activity. Likewise, chromatin assembly has been shown to be important in revealing transcriptional synergy between ligand-bound nuclear receptors and p300 (Kraus and Kadonaga, 1998). In this case, chromatin disruption was not assayed; however, p300 was determined to facilitate transcription complex assembly but not reinitiation by RNA polymerase. The available evidence suggests that chromatin disruption would have to occur in order to allow the basal transcriptional machinery to gain access to DNA (Workman and Roeder, 1987; Meisterernst *et al.*, 1990; Imbalzano *et al.*, 1994; Godde *et al.*, 1995; Li *et al.*, 1998). Our results indicate that p300 itself does not disrupt chromatin (Figures 1, 2, 5 and 8); in addition, neither SRC-1 nor PCAF, which are also transcriptional coactivators

for nuclear receptors (Figure 2; Glass *et al.*, 1997; Spencer *et al.*, 1997), can disrupt chromatin, while PCAF can stimulate transcription >3-fold in the presence of ligand-bound TR-RXR (Figures 3 and 5). This indicates that chromatin remodeling activities other than histone acetyltransferases are required. This conclusion is consistent with recent results in yeast (Cosma *et al.*, 1999) which indicate that SWI-SNF proteins need to act before histone acetyltransferases.

p300 stimulates transcription from a disrupted chromatin template (Figure 1). The PCAF coactivator also facilitates transcription in the absence of further chromatin disruption (Figures 3 and 5). This indicates that chromatin disruption itself, while rendering a chromatin template competent for transcription, does not lead to maximal transcriptional efficiency. p300 and PCAF may enhance transcription from a disrupted chromatin template by facilitating recruitment of the basal transcriptional machinery (Barlev *et al.*, 1995; Nakajima *et al.*, 1997a,b; Kraus and Kadonaga, 1998). However, our results indicate that acetyltransferase activity is required for transcriptional stimulation from a disrupted chromatin template (Figure 4). This implies that this acetyltransferase activity is required either to maintain directly a disrupted chromatin structure or to modify other transcriptional regulators to facilitate transcription. Consistent with the requirement for chromatin disruption, there is evidence that the acetyltransferase activity of coactivators operates more effectively on free histone than nucleosome histone (Ogryzko *et al.*, 1996; Ohba *et al.*, 1999). It should be noted that hyperacetylated histones have relatively small effects on chromatin topology even when incorporated into less stable nucleosomal arrays, while still facilitating transcription (Norton *et al.*, 1989; Lutter *et al.*, 1992; Nightingale *et al.*, 1998; Tse *et al.*, 1998). This conclusion is further reinforced by our results on transcriptional activation by the histone deacetylase inhibitor TSA (Figure 7; Wong *et al.*, 1998). In these experiments, transcription is induced to the same levels as in the presence of ligand-bound TR-RXR, but topological changes are relatively minor. Thus, acetylation itself can not account for all of the characteristics of disrupted chromatin, suggesting that other activities such as SWI-SNF, which stably modify chromatin, will be important (Snitzler *et al.*, 1998; Di Croce *et al.*, 1999).

It is possible that histone acetylation might inhibit SWI-SNF-mediated remodeling, leading to a partial recovery of nucleosomal architecture and superhelicity (Figure 1C and D, compare lanes 5 and 6). The histone tails are important for the activity of *Drosophila* NURF, an ISWI-containing chromatin remodeling complex (Georgel *et al.*, 1997), and for the efficiency of chromatin remodeling by the human SWI-SNF complex (Guyon *et al.*, 1999). In this respect, it is also interesting to note that a robust biochemical connection exists between chromatin deacetylation and nucleosome remodeling by other SWI-SNF superfamily members (Tong *et al.*, 1998; Wade *et al.*, 1998a). Evidence consistent with functional roles connected with the modification of transcription factors is less direct; however, it is clear that E1A inhibits the acetylation of TFIIE β , TFIIF and the TR itself with much greater sensitivity than acetylation of histones (Figure 6). Both TFIIE β and TFIIF have important roles in pre-initiation complex assembly and function (Tan *et al.*,

1994; Yokomori *et al.*, 1998). Alterations in pre-initiation complex assembly could also indirectly influence chromatin structure (Gregory *et al.*, 1998). Interestingly, E1A will only partially inhibit transcription from a chromatin template assembled in the presence of high concentrations of TSA. Under these conditions, chromatin never has the opportunity to mature to a deacetylated repressed state (Ura *et al.*, 1997). This implies that either E1A can act to interfere with the acetylation of substrates that are deacetylated by enzymes that are TSA resistant, or E1A can also interfere with transcription at levels other than those dependent on protein acetylation (see below). Future experiments will explore these possibilities.

The nature of chromatin disruption

The extent of chromatin disruption assayed by change in DNA topology that can be achieved on a small minichromosome (5 kb or ~28 nucleosomes) is remarkable. The addition of saturating ligand-bound TR-RXR leads to the loss of negative superhelicity equivalent to the displacement of eight nucleosomes or approximately one-third of the minichromosomal chromatin. This would slightly exceed the entire domain of chromatin containing the TR β A regulatory DNA, where the upstream TREs begin 800 bp upstream from the start site of transcription and the most distal 3'TRE lies 264 bp downstream (Machuca *et al.*, 1995; Ranjan *et al.*, 1995; F.Urnov, manuscript in preparation). This domain would include five to six nucleosomes. Each TRE has the capacity to target ligand-bound TR-RXR and alter the organization of two to three nucleosomes (Wong *et al.*, 1997a). Although the loss of regular nucleosomal arrays occurs selectively over the promoter (Wong *et al.*, 1995, 1997a), there is not a huge increase in the rate of cleavage by micrococcal nuclease or DNase I (Figure 8; F.Urnov, manuscript in preparation). Thus, we favor a model in which histones remain associated with DNA. What then could account for a large change in DNA topology? We suggest that rather than a displacement of nucleosomes, a relatively minor alteration in the exit angles of DNA as it wraps around the histone octamer could account for our results. Each nucleosome whose entry and exit paths of the DNA helix crossover on wrapping around the histone octamer introduces one complete negative superhelical turns into DNA. The simple alteration of the path of DNA at the entry and exit of the nucleosome such that the helix does not crossover itself immediately reduces the number of negative superhelical turns introduced by each nucleosome to zero (Worcel *et al.*, 1981). This type of structural transition should also uncoil the linker DNA consistent with a transition from coiled (Yao *et al.*, 1991) to straight configuration (Bednar *et al.*, 1998). The mononucleosome core crystals suggest that DNA has a trajectory leaving the core that could accommodate DNA being either crossed or not crossed without difficulty (Luger *et al.*, 1997). Thus, we suggest that TR-RXR introduces a change in nucleosomal DNA consistent with a movement of DNA from crossed to not crossed within two to three nucleosomes in the vicinity of the TRE. This would lead to a local disruption of the chromatin higher-order structure, which in turn may facilitate transcription. In the case of the 3'TRE at +264, binding of the TR-RXR at the dyad axis of a positioned nucleosome could assist this alteration

in DNA path (Wong *et al.*, 1995, 1997b). In this model, p300 might direct a topological recovery by stabilizing aspects of pre-existing higher-order structure perhaps through direct contacts with histones (see Ornaghi *et al.*, 1999). Finally, this type of model, where nucleosome conformational change accounts for changes in topology, offers the potential to reconcile the continued presence of mobile nucleosomes on minichromosomal templates treated with ISWI-containing complexes (Hamiche *et al.*, 1999; Langst *et al.*, 1999), with the large topological changes observed with SWI-SNF-treated minichromosomes (Imbalzano *et al.*, 1994; Kwon *et al.*, 1994). ISWI-directed nucleosome mobility should promote transcription factor access to DNA (Ura *et al.*, 1995, 1997), and if it occurs in conjunction with a change in higher-order structures, as reflected in topological transitions that uncoil linker DNA, this will further facilitate nucleosome mobility and transcription (Di Croce *et al.*, 1999).

The effect of E1A on chromatin remodeling and transcription

The adenovirus E1A gene encodes potent oncoproteins that function to regulate both cellular and viral transcription. E1A physically interacts with Rb, p300, PCAF and other key regulatory proteins, thereby altering their function and disrupting the normal cellular transcriptional program (Dyson and Harlow, 1992; Bayley and Mymryk, 1994; Reid *et al.*, 1998; Chakravarti *et al.*, 1999). In earlier work, E1A has been shown to bind directly to TBP (Meyer *et al.*, 1996) and to contact nuclear receptors (Meyer *et al.*, 1996; Wahlstrom *et al.*, 1999), perhaps facilitating transcription by direct bridging (Berkenstam *et al.*, 1992; Wahlstrom *et al.*, 1999). These experiments largely make use of transient transfections and model reporter constructs that are unlikely to assemble chromatin fully with the same configuration that occurs in a natural chromosomal environment (Archer *et al.*, 1992). In contrast, we have focused on the impact of E1A in a chromatin environment to explore the role of chromatin remodeling in transcription. We have made use of E1A to explore chromatin remodeling and transcriptional stimulation by p300. Mutational analysis has demonstrated that the N-terminus of E1A (amino acids 1–76) plays an important role in p300 binding (Arany *et al.*, 1995) and that this interaction is important in constraining p300 function (Chakravarti *et al.*, 1999). The exact mechanisms by which E1A interferes with p300 function are unclear. E1A can disrupt p300 interaction with PCAF (Yang *et al.*, 1996; Kurokawa *et al.*, 1998); however, PCAF is absent from *Xenopus* oocytes, which excludes this as a regulatory mechanism in our system (Li *et al.*, 1998). In earlier studies it was found that PCAF acetyltransferase activity was essential for gene activation by the RAR, whereas the acetyltransferase activity of CBP/p300 was not required (Korzus *et al.*, 1998). Our results in the *Xenopus* oocyte differ in that p300 acetyltransferase activity is essential for maximal transcription efficiency (Figure 4). This difference might be due to more efficient chromatin assembly in our system or to the absence of PCAF from oocytes (Li *et al.*, 1998). Alternatively, the earlier study (Korzus *et al.*, 1998) made use of blocking antibodies to inhibit enzymatic activity, while we make use of p300 mutants deficient in acetyltransferase. E1A does not

influence TR-RXR interaction with p300 in our system or in others (Kurokawa *et al.*, 1998); however, E1A does influence the acetyltransferase activity of p300 (Chakravarti *et al.*, 1999; Perissi *et al.*, 1999). Consistent with the inhibition of acetyltransferase model (Chakravarti *et al.*, 1999), we find that E1A inhibits p300 acetyltransferase activity, but does so much more effectively on the transcription factors TFIIE β , TFIIF and TR-RXR than on the histones (Figure 6). This selectivity suggests a mechanism for transcriptional stimulation by p300 where the transcriptional machinery represents preferential targets for interaction and acetylation rather than the histones. Acetylation of TFIIE β and TFIIF has only a low 2-fold stimulatory effect on the transcription of naked DNA (Wade *et al.*, 1998b), so other targets such as the TR and RXR might be important. However, E1A has other consequences with respect to chromatin remodeling.

E1A does not lead to major changes in the assembly of a DNase I-hypersensitive site on the TR β A promoter (Figure 8C). This might be explained by the capacity of the receptor in purified form to associate with chromatin substrates due to features of nucleosome architecture over the TR β A promoter (Wong *et al.*, 1997b). However, E1A blocks the disruption of nucleosomal arrays as assayed by micrococcal nuclease or the alterations in minichromosome topology instigated by ligand-bound TR (Figures 5 and 8). This block in these aspects of chromatin remodeling does not absolutely require interactions with p300 since the N-terminal deletion mutant of E1A still impedes these events (Figure 8E and F). E1A may function in these assays by inhibiting chromatin remodeling by interfering with the activity of vertebrate homologs of the SWI-SNF complex. In yeast, E1A inhibits SWI-SNF functions (Miller *et al.*, 1996). Moreover, the C-terminus of E1A is known to determine interactions with Rb family members (Dyson and Harlow, 1992; Moran, 1993), and Rb physically interacts with hBRG1 and hBRM, two human SNF homologs (Dunaief *et al.*, 1994; Strober *et al.*, 1996). Thus, it is possible that the N-terminal deletion of E1A used in our experiments is impeding the activity of the vertebrate SWI-SNF complex. This would provide an alternative indirect mechanism by which E1A could interfere with p300 function. In this model, the inhibition of chromatin remodeling by E1A would prevent the p300 acetyltransferase from stimulating transcription.

Materials and methods

Plasmid DNA

T7TSp300 expression constructs generating M2-tagged p300 were described previously (Li *et al.*, 1998). T7TSE1A and T7PCAF expression plasmids were constructed by *Bam*HI–*Hind*III digestion from mammalian expression clones (Yang *et al.*, 1996) and blunt end ligation into the *Eco*RV site of pT7TS vector (Zorn and Krieg, 1997). SP6 expression constructs for murine SRC-1a and derivatives containing amino acids 1107–1441 (SRC-1a, 1107–1441) and 1216–1441 (SRC-1a, 1216–1441) were the kind gift of Dr Daniel Robyr, Université de Lausanne, Switzerland. *Xenopus* HSF expression clones have been described (Landsberger and Wolffe, 1997).

Microinjection of *Xenopus* oocytes

Defolliculated *Xenopus* stage VI oocytes were prepared as described previously (Almouzni and Wolffe, 1993). The amount or combination indicated of mRNA (TR, RXR, p300 and E1A) was injected into oocyte cytoplasm in 27.6 nl volume. Protein synthesis was allowed by incubating the oocytes at 18°C for 16 h. The nuclear injection of single-strand

TR β A reporter DNA (1–2 ng in 9.2 nl) was routinely done 2 h after mRNA injection. T₃ (50 nM) was used for hormone induction.

Protein extraction

Protein from oocytes was prepared by homogenizing the oocytes in 20 mM HEPES buffer containing 70 mM KCl, 5% sucrose and 1 mM dithiothreitol (DTT). The homogenate was centrifuged at 10 000 r.p.m. at 4°C for 10 min and separated on 4–20% Tris-glycine gradient gel (Novex).

'Histone' acetyltransferase (HAT) assay

Purified p300 (25 ng) was incubated with 1 μ g of histone or other substrates in 1 $\times$ HAT buffer [50 mM Tris pH 8.0, 0.1 mM EDTA, 10% glycerol, 1 mM DTT and 1 mM phenylmethylsulfonyl fluoride (PMSF)] in the presence of [³H]acetyl-CoA for 30 min at 37°C in a volume of 20 μ l. The reaction was stopped by addition of 20 μ l of 2 $\times$ SDS sample buffer and subjected to 4–20% SDS-PAGE analysis. The gel was stained by Coomassie Blue R for 10 min and destained overnight. The gel was then vacuum dried, exposed to film and scanned using a PhosphorImager (Molecular Dynamics) (Kodak). For *in vitro* acetylation reactions the following protocol was used. Recombinant human p300 (40 fmol) was used to acetylate core histones purified from chicken erythrocytes or other substrates. Reactions were done in a total volume of 100 μ l in 1 $\times$ HAT buffer (50 mM Tris-HCl pH 8.0, 10% glycerol, 1 mM DTT, 1 mM PMSF, 50 mM NaCl) in the presence of 3.5 μ l of [³H]acetyl-CoA (Amersham, 0.25 mCi/ml) at 25°C. Inhibition assays were done by preincubating glutathione *S*-transferase (GST)–E1A with the respective enzyme for 5 min at room temperature (22°C) before assembling the acetylation reaction. A fixed concentration of 3 μ M substrate was used and GST–E1A concentration was varied. Reactions were loaded on a 12% SDS-polyacrylamide gel, stained with Coomassie Blue, destained, treated with Amplify (Amersham) for 15 min, dried and exposed for 24 h before quantitation in the PhosphorImager.

Immunoprecipitation assay

Ten oocytes equivalent of protein were used for each pull-down assay. Monoclonal antibody against p300 (a kind gift from Dr Betty Moran) and polyclonal antibody against TR were coupled to protein G- or A-Sepharose (Pharmacia Biotech). The [³⁵S]methionine-labeled oocytes extract was incubated with either beaded p300 antibody or TR antibody in 500 μ l of 1 $\times$ binding buffer (100 mM NaCl, 20 mM Tris pH 8.0, 5 mM MgCl₂, 0.5 mM EDTA, 10% glycerol, 1 mM DTT and 1 mM PMSF) for 1 h at 4°C. The reaction was washed three times with 1 ml of washing buffer (250 mM NaCl, 20 mM Tris pH 8.0, 5 mM MgCl₂, 0.5 mM EDTA, 10% glycerol, 1 mM DTT, 1 mM PMSF, 0.05% NP-40). Pellet from the last wash was dissolved in 20 μ l of SDS sample buffer and analysis on 4–20% SDS-polyacrylamide gel (Li *et al.*, 1998).

mRNA preparation and primer extension

The total RNA from oocytes was prepared using RNA STAT-60 as instructed (Tel-Test, Inc.). Primer extension was performed on two oocytes equivalent of RNA. Primer I (ATCCTTATAAACGGTGAGTAGTGATGTCATCAG) was used to detect the specific TR β A transcript. H4 primer (GGCTTGGTGATGCCCTGGATGTTATCC) was employed to probe the endogenous H4 mRNA as an internal control for sample loading. Annealing of primer was carried out in 1 $\times$ first strand buffer (Gibco-BRL) at 65°C for 10 min, then at 55°C for 25 min and finally at 42°C for 10 min in a volume of 10 μ l. Primer extension was performed at 42°C for 1 h in 20 μ l with Superscript II as recommended (Gibco-BRL). The reaction was stopped by 15 μ l of denaturing loading buffer and 5 μ l of the sample was analyzed on 6% denaturing sequencing gel (Wong *et al.*, 1995).

DNase I hypersensitivity analysis

Stage VI oocytes were injected and incubated as described above. Groups of 20 healthy oocytes were collected and homogenized with the following buffer: 10 mM Tris-HCl pH 8.0, 50 mM NaCl, 1 mM EDTA, 1 mM DTT, 5% glycerol and 5 mM MgCl₂. The DNase I (Worthington) digestion was carried out at 9–18 U/100 μ l for 2 min at 37°C. The reaction was stopped by addition of an equal volume of stop solution containing 0.5% SDS and 20 mM EDTA. DNA was then purified by RNase A treatment, proteinase K digestion, phenol-chloroform extraction and isopropanol precipitation. For indirect end labeling, the purified DNA was restricted with *Eco*RI before resolution on 1% agarose gel, transfer to a filter and hybridization with a *Bgl*II–*Eco*RI DNA fragment that had been radiolabeled by random priming (Wong *et al.*, 1997b).

Micrococcal nuclease mapping

Stage VI oocytes were injected and incubated as described previously. Groups of 25 healthy oocytes were collected and homogenized with the following buffer: 10 mM Tris-HCl pH 8.0, 50 mM NaCl, 1 mM EDTA, 1 mM DTT, 5% glycerol, 3 mM CaCl₂ and 5 mM MgCl₂. The Mnase (Pharmacia) digestion was carried out at 4–120 U/100 µl for 2 min at 25°C. The reaction was stopped by addition of an equal volume of stop solution containing 0.5% SDS and 20 mM EGTA. DNA was then purified by RNase A treatment, proteinase K digestion, phenol-chloroform extraction and isopropanol precipitation. The purified DNA was resolved on 1% agarose gel, transferred to a filter and hybridized with random primed probe containing a segment of the promoter (a 108 bp *Pst*I–*Rsa*I fragment from –255 to –147) from the TRβA plasmid (Wong *et al.*, 1997a).

Supercoiling assay of DNA topology

Purified DNA (1 ng) from injected oocytes was separated on 1% agarose gel in 1× TPE buffer (40 mM Tris, 30 mM NaH₂PO₄ and 10 mM EDTA) containing 30–90 µg/ml freshly prepared chloroquine at 3.5 V/cm in the dark (Clark and Wolffe, 1991). The gel was then washed with distilled water for 1 h and the DNA was detected by conventional Southern blotting as for the micrococcal nuclease mapping.

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