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DNA topoisomerase I is involved in both repression and activation of transcription

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Reconstituted transcription reactions containing the seven general transcription factors, in addition to RNA polymerase II, respond poorly to transcriptional activators. Two factors, Dr_2 and ACF, necessary for high levels of transcription in response to an activator have been identified. ACF can enhance basal and activated transcription. Dr_2 represses basal transcription, but this can be overcome by transcriptional activators or TFIIA. Dr_2 is human DNA topoisomerase I. The DNA relaxation activity of topoisomerase I is dispensable for transcriptional repression. The effect of Dr_2 is specific for TATA-box-containing promoters and is mediated by the TATA-binding protein.

EARLY studies established that certain proteins can activate transcription[1]. Although the mechanism(s) of this activation is unclear, it has been presumed that activator proteins work by communicating with and influencing the basal transcription machinery[2]. Recent studies have shown specific and direct interactions between regulatory factors and some of the general transcription factors (GTFs). Specifically, a direct interaction between an acidic activator and TFIIB[3], TFIIH (D.R. and J. Greenblatt, unpublished result) and the TATA-binding protein (TBP) subunit of TFIID[4] have been demonstrated. These interactions appear to be part of the activation process, as mutations in the acidic domain of an activator, which weaken or eliminate activation of transcription, also reduce the extent of interaction with the particular GTF[3,4]. Moreover, mutations of specific residues in TFIIB, which eliminate the interaction with an acidic activator, abolish activated transcription, but have little effect on the ability of TFIIB to participate in basal transcription[5]. Despite these findings, physiological levels of activation cannot be reproduced in a reconstituted transcription system composed of an activator, TFIID, and the remaining GTFs. Thus, contact between components of the basal machinery and an activator is not sufficient to stimulate transcription; additional factors appear to be necessary.

To elucidate the mechanism(s) operating to activate transcription, we analysed the factors necessary for activation *in vitro*. We determined that the response to an acidic activator requires two protein fractions, ACF and Dr_2, in addition to TFIID, the GTFs and RNA polymerase II. In the absence of an activator, Dr_2 represses basal transcription, but in the presence of an activator, Dr_2 stimulates this response. Dr_2 was purified to homogeneity from HeLa cells and identified as topoisomerase I.

Dr_2 and ACF necessary for optimal transcription

To analyse the factors required for activation of transcription *in vitro*, reactions were reconstituted as follows: a circular DNA template containing five GAL4-DNA-binding sites upstream of the TATA motif of the adenovirus major late promoter (Ad-MLP), was mixed with a second template lacking GAL4-DNA-binding sites. The addition of increasing amounts of a fusion protein composed of the GAL4-DNA-binding domain and an acidic domain (GAL4-AH)[6] to a transcription assay containing both templates, TFIID, the GTFs and RNA polymerase II resulted in low, non-physiological levels of activated transcription from the template containing the GAL4-DNA-binding sites (Fig. 1*a*, compare lane 1 with 2–4). The template lacking GAL4 sites was unaffected. The addition of the Dr_2 protein fraction (see Fig. 2 legend) resulted in repression of basal transcription and stimulation of activated transcription (Fig. 1*a*, compare lane 5 with 1 and 3). The addition of the ACF protein fraction resulted in further stimulation of transcription from the activator-responsive template (lanes 6 and 7). Under these conditions Dr_2 repressed basal transcription.

The individual effects of Dr_2 and ACF in transcription, in the presence and absence of activator and with an excess amount of GTFs are shown in Fig. 1*b*. The addition of increasing amounts of Dr_2 to a transcription assay devoid of an activator resulted

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in repression (lanes 1–4). With activator, Dr_2 repressed only transcription from the DNA lacking the GAL4 recognition elements, whereas the template containing the GAL4 sites was stimulated (compare lane 9 with 10–12). Under these conditions the net effect of the activator was about a 20-fold stimulation of transcription (compare lane 4 with 12). The addition of ACF to reactions lacking Dr_2 and activator resulted in a modest stimulation of transcription at low ACF concentrations and a moderate inhibition from both templates at higher concentrations (Fig. 1*b*). The addition of both Dr_2 and ACF repressed transcription from both templates in the absence of the activator (lane 8), yet in its presence the activator-responsive template was stimulated, with the net effect of GAL4-AH being a 40-fold enhancement of transcription (compare lanes 8 and 17).

We demonstrated that the GAL4(1–94) DNA-binding domain by itself could not overcome repression of transcription (Fig. 1*c*, lane 4), indicating that an activating domain is required. Furthermore, the antirepression phenomenon was not specific for GAL4-AH, as GAL4-VP16 (lane 8), or glutamine-rich (lane 10) and proline-rich (lane 12) activators were capable of neutralizing the repressing effect of Dr_2.

Dr_2 contains DNA topoisomerase I activity

Dr_2 activity was purified to apparent homogeneity and found to reside in a polypeptide of apparent M_r 97K (Fig. 2*a*). Renaturation experiments with the 97K polypeptide eluted from SDS–PAGE confirmed this observation (data not shown). Highly purified Dr_2 was transcriptionally active (Fig. 2*b*).

Digestion of Dr_2 with trypsin yielded eight polypeptides whose amino-acid sequences are indicated in Fig. 2. Comparison of these sequences with those in the Genebank database revealed that all the peptides are present in human topoisomerase I (hTopo I)[7]. This led us to investigate whether the purified preparation of Dr_2 contained Topo I activity. Consistently, Dr_2 could relax supercoiled DNA (Fig. 2*c*).

Dr_2 and topoisomerase I activities

To analyse further whether Dr_2 and Topo I activities were contained in the same polypeptide, a recombinant form of hTopo I was purified from baculovirus-infected insect cell extracts. Silver staining of a SDS–PAGE revealed the presence of 97K and 77K polypeptides in this sample. These polypeptides were absent in the extract derived from wild-type baculovirus-infected cells (Fig. 3*a*). The 97K rhTopo I polypeptide comigrated on SDS–PAGE with Dr_2 (Fig. 3*a*) and both polypeptides reacted with

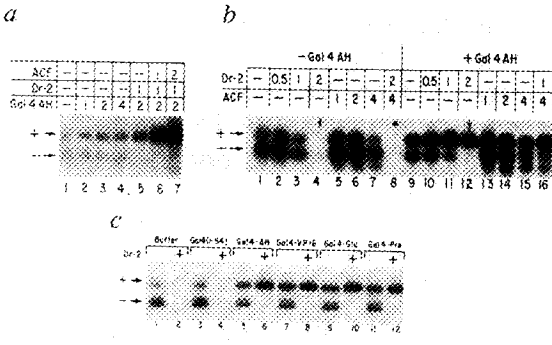

FIG. 1 ACF and Dr_2 are necessary to achieve optimal response to an activator. *a*, Transcription reactions contained a DNA template with five GAL4-binding sites upstream of the TATA motif directing transcription of a 380 nucleotides G-less cassette (+ transcripts) and a second DNA template lacking GAL4-binding sites and producing a 330-nucleotide transcript (– transcript). Reactions also contained the different GTFs and RNAPII. In addition, reactions contained GAL4-AH (50 ng), Dr_2 (200 ng), and ACF (275 ng as 1 × concentration) as indicated. *b*, The individual effect of increasing concentrations of ACF and Dr_2 was analysed in the absence and presence of the activator GAL4-AH. Reactions were as described in *a*, but the concentration of the GTFs was doubled. *c*, Different activators, as indicated, were analysed for their ability to overcome Dr_2-mediated inhibition. The amount of activator added to the reactions was standardized by measuring DNA-binding activity (data not shown).
METHODS. Transcription reactions were as previously described[38] and contained 200 ng of each of the DNA molecules, RNAPII and the different GTFs: IIA/IIJ, rIIB, IID, rIIE, IIF, and IIH, as previously described[38]. The RNA products were analysed on a polyacrylamide–urea gel. The synthetic transcriptional activators GAL4-AH, GAL4-VP16, GAL4-Glu, GAL4-Pro and the DNA-binding domain of GAL4(1–94) were purified as described[6,39–41]. All proteins were kept in buffer C (20 mM Tris–HCl, pH 7.9, 10% glycerol, 1 mM ethylenediamine tetra-acetic acid, 0.01% Tween20, 0.2 mM phenylmethylsulphonyl fluoride and 5 mM β-mercaptoethanol) containing 0.1 M KCl. Dr_2 and ACF were purified from the previously described phosphocellulose 1 M fraction (see Fig. 2 legend).

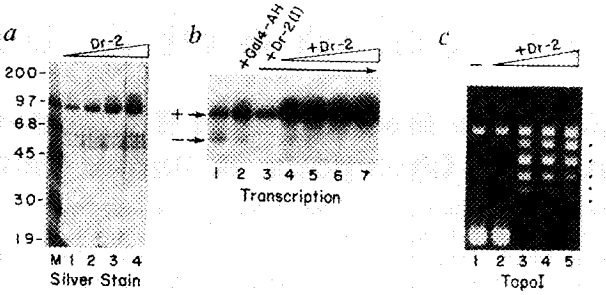

FIG. 2 Identification of Dr_2. Aliquots (75, 100, 150 and 200 ng) of the μ-Mono S Dr_2 protein pool were analysed by SDS–PAGE followed by silver staining (*a*) and transcription (*b*). Transcription reactions were as described in Fig. 1*b*. Lane 3 indicates a reaction containing an aliquot of the Dr_2-phenyl-Superose protein pool (400 ng). The Topo I activity of the μ-Mono S pool was examined (*c*). The dots to the right of *c* indicate DNA topoisomers. The μ-Mono S Dr_2 fraction was further purified on a μ-reverse-phase chromatography (RPC) column and a single peak was recovered. This material was used for amino-acid analysis and trypsin digestion. The peptides obtained after trypsinization were separated on an RPC column and sequenced by Edman degradation as described[42].
METHODS. The 1.0 M phosphocellulose fraction derived from HeLa cell nuclear extracts was fractionated on a CM-Sepharose column as previously described[17]. Fractions containing TFIID and Dr_2/ACF activities were pooled (315 mg), dialysed against butter C (50 mM KCl) and loaded onto a 60-ml DEAE-52 column. About 80% of the Dr_2/ACF activities passed through the column. This fraction also contained ~30% of the input TFIID activity. The DEAE-52 flow-through (163 mg) was loaded onto a MonoQ column (Pharmacia, HR10/10). The flow-through (51 mg) containing Dr_2/ACF was further purified by chromatography on a phenyl-Superose column (Pharmacia, HR10/10). The column was equilibrated with buffer C containing 1.2 M ammonium sulphate ($(NH_4)_2SO_4$). Proteins were eluted with a linear gradient (1.0–0 M) of $(NH_4)_2SO_4$ in buffer C. This resulted in the separation of Dr_2 and ACF. Dr_2 activity eluted at 0.7 M $(NH_4)_2SO_4$, whereas ACF activity eluted at 0.3 M $(MH_4)_2SO_4$. The phenyl-Superose Dr_2 fraction (810 µg) was further purified on a μ-MonoS column (SMART-Pharmacia-LKB) equilibrated with buffer C containing 50 mM KCl. The Dr_2 activity was eluted with a linear gradient between 50–500 mM KCl. The fractions containing Dr_2 activity (250–300 mM KCl) were pooled (9 µg) and further purified on a μ-RPC column (SMART-Pharmacia-LKB). The column was developed with a linear gradient of acetonitrile (0–100%) containing 0.1% trifluoroacetic acid; Dr_2 polypeptide elutes at 65% acetonitrile. The Dr_2 polypeptide (95 pmol) was digested with trypsin and the amino-acid sequence of eight peptides were as follows (in single-letter code): (1) AVALYFIDKLALR, (2) TYNASITLQQQLK, (3) AVQRLEEQLMK, (4) NIITNLSK, (5) AVAILCNHQR, (6) IKGEKDWQKYETAR, (7) SMMNLQTK, (8) NFFK. Topoisomerase I assays were as follows: supercoiled DNA (0.3 µg) was incubated with 1, 2, 4 and 8 µl of Dr_2 μ-MonoS (diluted 1/3,200) protein fraction for 5 min at 30 °C. The 10-µl reactions contained 20 mM HEPES, pH 7.9, 8 mM $MgCl_2$, 2.5 mM $(NH_4)_2SO_4$, 2% polyethylene glycol 8000 and 50 mM EDTA. These reactions were stopped with 2 µl 1% SDS, 30% glycerol, 0.25% xylene xienol and bromophenol blue, and the products were analysed on a 1% agarose gel followed by staining with ethidium bromide.

anti-Topo I antibodies (Fig. 3*b*). The 77K rhTopo I polypeptide also reacted with the antibodies. A similar polypeptide has been found in human cell extracts and was shown to be a proteolysed form of hTopo I lacking N-terminal residues[8]. Fractions containing the recombinant 97K and 77K polypeptides, but not fractions derived from wild-type baculovirus-infected cells, contained Dr_2 activity. This was analysed by measuring the ability of the fraction to repress basal transcription (Fig. 3*c*). Both the 77K and 97K rhTopo I polypeptides contained Dr_2 activity (data not shown).

It was important to analyse whether the DNA relaxation activity of hTopo I was necessary for Dr_2 function. Tyrosine at position 723 in the active site of hTopo I is essential for DNA relaxation activity[9]. A mutant hTopo I gene was engineered with tyrosine 723 substituted by phenylalanine (Y723F). This baculovirus-expressed form of hTopo I was purified and analysed for Dr_2 activity. In agreement with previous studies[9–11], the mutant rhTopo I protein was inactive in DNA relaxation (data not shown), yet this protein was still capable of repressing basal transcription (Fig. 3*d*, lane 4) and yielding high levels of activated transcription in the presence of ACF (lane 6). ACF, at the concentration used and in the absence of Dr_2, failed to stimulate the response to the activator (lane 7).

Gene-specific repression of transcription

Having found that hTopo I is a repressor of basal transcription, we analysed the specificity of these observations. It is possible that the observed effect was a result of the DNA-binding activity associated with hTopo I[12]. We thus analysed whether Dr_2 activity is present in topoisomerases derived from different sources. Vaccinia virus, *Escherichia coli*, and yeast Topo I proteins were unable to repress basal transcription (Fig. 4*a*), suggesting that the Dr_2 activity present in hTopo I is unlikely to result solely from DNA binding. This agrees with our analysis indicating that optimal Dr_2 activity requires about five molecules of hTopo I per molecule of DNA, a quantity insufficient to cover the DNA completely and account for total repression of basal transcription.

The specificity of Dr_2 was further scrutinized by analysing whether different class II promoters could be repressed by Dr_2. The promoters chosen were the Ad-E4 and the TATA-less β-DNA polymerase promoters. The minimal elements required for transcription from these promoters were placed downstream of five GAL4 recognition sites. Transcription used nuclear extracts supplemented with GAL4-AH and Dr_2. Basal transcription from these promoters was below detectable levels; however, the addition of GAL4-AH resulted in transcription (Fig. 4*b*, compare lanes 1 and 2). The addition of increasing concentrations of Dr_2 resulted in inhibition of transcription from the Ad-E4 promoter; however, transcription directed by the β-DNA polymerase promoter was unaffected (lanes 3–5). We reason that these promoters respond differently to Dr_2 because they use different pathways to promote preinitiation complex formation[13]. In the case of the Ad-E4 promoter, transcription is directed by the TATA motif. Transcription from the TATA-less β-DNA polymerase promoter is directed by the initiator (Inr), which results in assembly of a complex responsive to GAL4-AH yet unresponsive to Dr_2. To substantiate this observation further, the prototype Ad-MLP with (pG_6TI) and without (pG_6I) a TATA motif and five Sp1-binding sites was analysed. Consistently, TATA-dependent transcription, but not Inr-directed transcription, was inhibited by topoisomerase I (Fig. 4*c*). These results indicate that the effect of Dr_2 is not a consequence of DNA binding, and more importantly, they suggest that the inhibitory function of Dr_2 is promoter-specific.

TBP mediates Dr_2 effect on transcription

To analyse the step at which Dr_2 functions during complex formation, we attempted to isolate a preinitiation complex intermediate refractory to Dr_2 inhibition. Subsets of GTFs required to direct formation of transcription-competent complexes (TFIID, -11A and -11B) were incubated with DNA before the addition of Dr_2, as illustrated in Fig. 5*a*. The incubation of TFIID, TFIIA, TFIIB and Dr_2 with DNA resulted, as expected, in inhibition of basal transcription (lane 3). However, inhibition could not be observed if Dr_2 was added after the formation of the D·A complex (lane 5). TFIIB was without effect (lane 4), and the incubation of TFIID and DNA, in the absence of TFIIA, was not sufficient to overcome Dr_2 inhibition (lane 6).

Although TFIIA is not required for basal transcription when TBP is used in a reconstituted system[14], our previous observations demonstrated that TFIIA was necessary to obtain comparable levels of transcription when the TFIID complex was implemented in the assay[14]. This result led us to suggest that TFIIA may function to remove negative regulators present in the TFIID complex[14]. To test this hypothesis, we investigated whether Dr_2 is present in the TFIID complex.

TFIID was purified from HeLa cells and from a cell line containing a stably integrated epitope-tagged TBP (eTFIID)[15]. Both forms of TFIID were fractionated through a protein A column containing antibodies recognizing the epitope of eTFIID. Proteins were eluted from the columns with increasing salt washes. The presence of Dr_2 was determined by measuring Topo I enzymatic activity and by western blot using anti-Topo I antibodies. The presence of epitope-tagged TFIID was also investi-

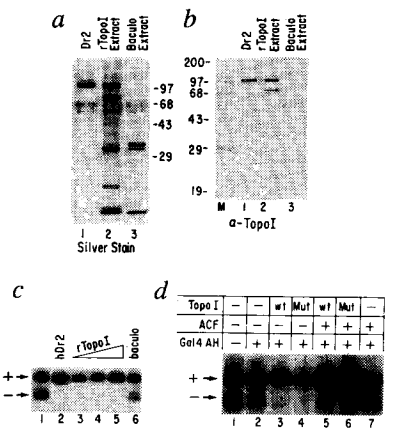

FIG. 3 Recombinant hTopo I contains Dr_2 activity. *a*, Silver staining of an SDS–polyacrylamide gel representing highly purified Dr_2 (lane 1), proteins isolated from SF9 insect cells infected with a hTopo I recombinant baculovirus (lane 2), or a wild-type baculovirus (lane 3). *b*, Western blot analysis using anti-hTopo I antibodies; lanes as in *a*, with BRL prestained M_r markers (lane M). *c*, Dr_2 (μ-Mono S protein fraction, 200 ng, lane 2), and hTopo I isolated from hTopo I-baculovirus-infected SF9 cells (200, 300 and 400 ng, lanes 3–5), and proteins (200 ng) from the wild-type (wt)-baculovirus-infected SF9 cells (lane 6), were analysed for their ability to repress basal transcription, as described in Fig 1. *d*, Recombinant wild-type and mutant (Y723F) hTopo I proteins (400 ng each) were analysed in basal and activated transcription, in the presence of ACF (550 ng), as indicated on the top of the panel. Transcription assays are described in the legend to Fig. 1.

METHODS. Recombinant hTopo I was generated using a baculovirus expression system. Extracts were prepared from hTopo I-baculovirus- or wild-type baculovirus-infected SF9 insect cells and non-rhTopo I proteins were precipitated with polyethyleneglycol in high salt (K.R.M. *et al.*, manuscript in preparation). The supernatant fraction was dialysed against buffer C containing 100 mM KCl, resulting in the precipitation of rhTopo I. rhTopo I was recovered by centrifugation at 10,000g for 10 min and resuspended in 6 M GuHCl. The denatured proteins were renatured by dilution to 0.1 M guanidinium-HCl with buffer C containing 100 mM KCl. A similar extract was prepared with recombinant baculovirus carrying a single substitution (Y723F) in the Topo I gene (K.R.M. *et al.*, manuscript in preparation). Because the mutant rhTopo I is deficient in DNA relaxation[9], silver staining of SDS–PAGE and western blot analysis[9], were used to analyse the integrity and the concentration of the recombinant proteins (data not shown).

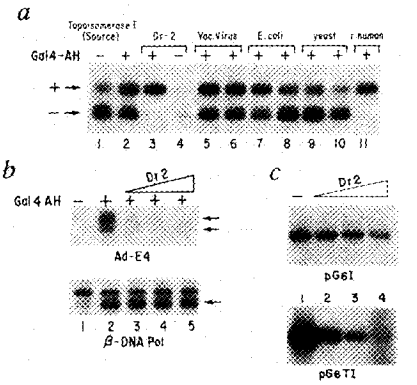

FIG. 4 Dr_2/hTopo I-dependent repression of transcription is species- and promoter-specific. *a*,Topoisomerases from different sources were used as source of Dr_2 in transcription. Similar (odd lanes) or twice the amounts (even lanes) of vaccinia virus[43], *E. coli*[44] and *S. cerevisiae*[11] topoisomerases, relative to the hTopo I DNA-relaxing activity in Dr_2, were tested on transcription as described for Fig. 1 (lanes 5–10). Positive controls included Dr_2 (μ-MonoS, 200 ng) (lanes 3 and 4) and recombinant hTopo I (400 ng, Lane 11). The quantification of the topoisomerase DNA-relaxing activity was done using serial dilutions of the different samples, as shown in Fig. 2*c* with the Dr_2 fraction. *b*, Primer extension analysis of transcription reactions directed by the Ad-E4 (pG5D38E4CAT[45]; gift from M. Green) and the β-DNA polymerase (pG5β-polCAT; L. Weis and D.R., manuscript in preparation) promoters, both containing 5 GAL4 DNA-binding sites upstream from the TATA motif. Reactions were done as previously described[46] using HeLa nuclear extracts (210 μg) supplemented with GAL4-AH (1.8 μg, lanes 2–5) and increasing amounts of Dr_2 (lanes 3–5, 0.6, 0.9 and 1.2 μg, respectively). *c*, Reactions as in *b*, but containing the Ad-MLP with six Sp1-binding sites upstream of the TATA motif (pG6TI), or a similar DNA construct in which the TATA motif was mutated (pG61) and thus transcription was directed by the initiator. These DNA templates directed transcription of a G-less cassette[47]. The nuclear extract was supplemented with Dr_2 as described in *b* (lanes 2–4).

gated using anti-epitope antibodies. Topo I was detected in the 1.0 M KCl phosphocellulose TFIID and eTFIID protein fractions (Fig. 5*b*, *c*, lanes 1 and 2), as well as in the flow-through of the protein A columns (Fig. 5*b*, *c*, lanes 3 and 4). However, Topo I from the eTFIID fraction was also retained by the protein A column and was eluted with 0.2 and 0.5 M KCl washes (Fig. 5*b*, *c*, lanes 8 and 10). Topo I present in the non-epitope tagged TFIID fraction was not retained by the protein A column (Fig. 5*b*, *c*, lanes 7 and 9). These results indicate that Dr_2/hTopo I is associated with the TFIID complex. To analyse whether hTopo I directly interacts with TBP, a FAR-western analysis was done using rhTopo I. The interaction was analysed using human biotinylated TBP. These studies demonstrate that there is a direct interaction of Topo I with TBP (Fig. 5*d*, lane 2). The interaction is specific, as it was not evident in samples lacking rhTopo I (lanes 3 and 4) nor in samples blotted with avidin-alkaline phosphatase without biotinylated TBP (data not shown).

The results presented above indicate that the effect of Dr_2 in transcription is mediated by an interaction with TBP. Moreover, the functional analysis (Fig. 5*a*) suggests that TFIIA and Dr_2 may counteract each other's function. In light of these results and our previous analysis of the role of TFIIA in transcription[14], we analysed the effect of Dr_2 and TFIIA in reactions reconstituted with TBP. Transcription activity could be demonstrated in the absence of TFIIA, provided that TBP was used instead of TFIID (Fig. 6*a*, lane 1). The addition of Dr_2 resulted in an inhibition that could be overcome by TFIIA (Fig. 6*a*). Moreover, the preincubation of TBP and TFIIA, before addition of Dr_2, resulted in a reaction resistant to inhibition (Fig. 6*b*). Thus,

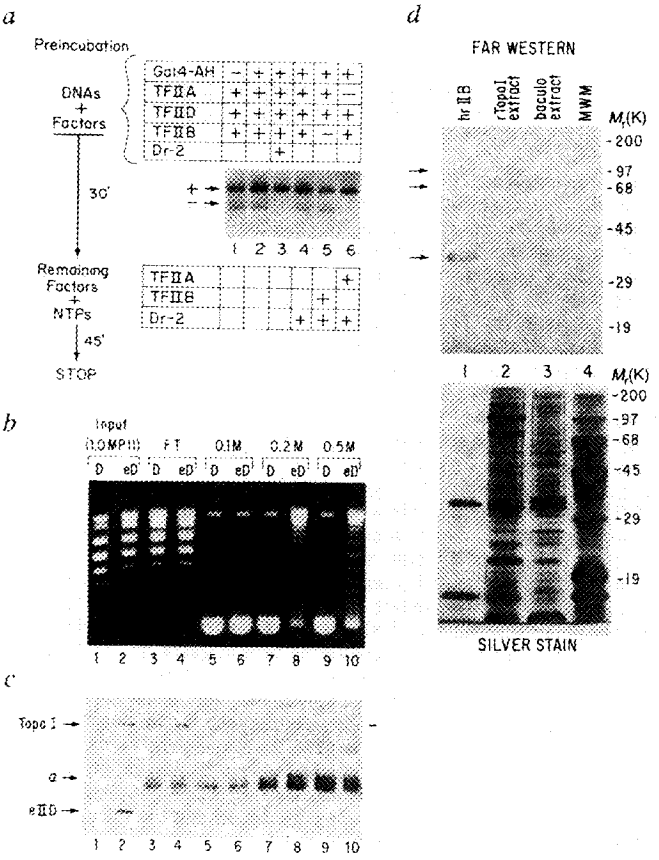

FIG. 5 Dr_2/hTopo I interacts with TBP preventing the formation of a transcription-competent complex. *a*, The DA complex is refractory to Dr_2 inhibition. Transcription reactions were done as described for Fig. 1, but modified as shown on the left-hand side of the panel. A combination of GTFs and GAL4-AH (as shown on the upper part of the panel) were preincubated with DNA for 30 min at 30 °C before addition of NTPs and the rest of the GTFs (as described in the lower part of the panel). hTopo I was added to the transcription reactions during (lane 3) or after preincubation (lanes 4–6). *b*, *c*, Dr_2/hTopo I interaction with TFIID in solution. Nuclear extracts from normal and stably transformed HeLa cells containing the epitope-tagged TFIID[15], were purified on a phosphocellulose column. The 1.0 M phosphocellulose fractions were then loaded onto independent columns containing a monoclonal antibody directed against the epitope tag of TBP (YPYDVPDYA). The columns were washed extensively with buffer C (100 mM KCl) and eluted by increasing the concentration of KCl to 200 and 500 mM. The fractions obtained were assayed for DNA relaxation activity as in Fig. 2 (*b*), and by western blot analysis using antibodies against the epitope tag of eTFIID and hTopo I[9] (*c*); *α* represents the anti-mouse IgG reacting material leaking from the immunoaffinity column. *d*, FAR-western analysis or rhTopo I using biotinylated human TBP. Proteins present in recombinant TFIIB[42] (lane 1), extracts derived from hTopo I-baculovirus (lane 2), wt-baculovirus-infected cells (lane 3), and markers (lane 4) were separated by SDS–PAGE. One gel was silver-stained (lower part of the panel) and a duplicate was transferred to nitrocellulose.

METHODS. Anti-epitope tag IgG monoclonal antibody, mAb12CA5 (50 μg; Berkeley Antibody Co., Inc.), were immobilized on 50 μl of protein A-Sepharose (Pharmacia). The FAR-western analysis was done as described[31]; however, the blocking agent was changed from 5% milk to 3% gelatin. TFIIB and Topo I (~1 μg each) were blotted with 2 μg ml^{-1} biotinylated hTBP overnight at room temperature.

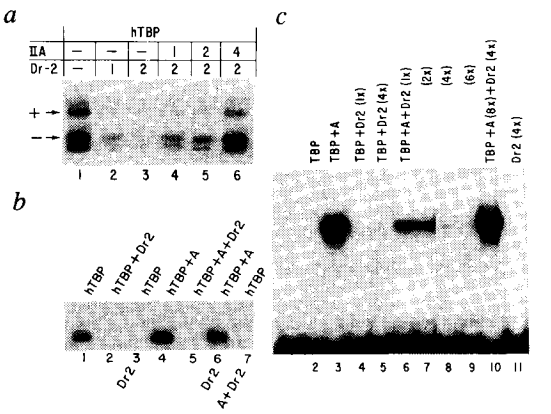

FIG. 6 TFIIA overcomes Dr_2/hTopo I-mediated repression. *a*, Transcription reactions were done as described for Fig. 1 with the following changes. hTBP (40 ng) was used instead of TFIID. Increasing amounts of a hydroxylapaptite TFIIA fraction[14] (50, 125 and 200 ng) were added to the reactions containing the highest amount of Dr_2 (150 ng, μ-MonoS). *b*, The TBP-A complex is refractory to Dr_2 inhibition. Transcription reactions were done as described for Fig. 1*a*, but modified as shown on the left-hand side of Fig. 5*a*. A combination of TBP, TFIIA and Dr_2 (as shown on the upper part of the panel) was preincubated with DNA for 30 min at 30 °C before addition of NTPs and the rest of the GTFs. Dr_2 was added to the transcription reactions during (lanes 2 and 5) or after preincubation (lanes 3, 6 and 7). *c*, Mobility shift assay measuring the effect of Dr_2 on the DA complex. DNA-binding assays were done using a ^{32}P-labelled Ad-MLP DNA fragment (−55 to +40) and contained hTBP (20 ng), TFIIA (25 ng, hydroxylapaptite fraction) and Dr_2 (50 ng), all as 1 × concentrations, or as indicated at the top of the panel.
METHODS. Binding assays were done in 10 µl containing 20 mM HEPES, pH 7.9, 8 mM $MgCl_2$, 2.5 mM $(NH_4)_2SO_4$, 2% polyethylene glycol 8000, 1 µg poly-dG/poly-dC (Pharmacia). The DNA–protein complexes were separated on a non-denaturing polyacrylamide gel as previously described [16,17].

TFIIA neutralizes the effect of Dr_2. This was further analysed using the gel-mobility shift assay. In agreement with previous observations[16,17], the addition of TBP and TFIIA to DNA-binding assays using a TATA-containing fragment resulted in the formation of the D·A complex (Fig. 6*c*, lane 3). In the absence of TFIIA, TBP could not produce a stable complex with the TATA motif (lane 2). Although Dr_2 interacts directly with TBP (Fig. 5*d*), a Dr_2-TBP-DNA complex could not be demonstrated under these conditions (Fig. 6*c*, lanes 4, 5); nor could Dr_2 bind stably to DNA (lane 11). Nonetheless, the simultaneous addition of Dr_2 and TFIIA to TBP-containing reactions resulted in inhibition of D·A complex formation; the extent of inhibition was proportional to the amount of Dr_2 added (lanes 6–9). This inhibition was specific, as excess TFIIA could restore the D·A complex (lane 10). These results not only present a possible mechanism for Dr_2/topo I-mediated repression of transcription, but also argue against a generic inhibition due to the nonspecific DNA-binding activity associated with Topo I[12].

Discussion

We have analysed the factors necessary to mediate activation of *in vitro* transcription mediated by RNA polymerase II. We find that in addition to the activator, TFIID, and the remaining GTFs, two other components, ACF and Dr_2, are necessary to achieve levels of activation approaching those observed *in vivo*. ACF is poorly defined and probably includes factors previously described as mediators or adaptors[18–21]. Dr_2 was identified as hTopo I. This factor not only augmented the response to the activator, but in the absence of the activator, repressed basal transcription. The repressing activity associated with Dr_2 cannot be explained by the nonspecific DNA-binding activity associated with hTopo I[12], as our experiments demonstrate that: (1) topoisomerases isolated from sources other than human are unable to repress the human transcription system; (3) Dr_2/hTopo I is part of the TFIID complex and specifically interacts with TBP; and (3) Dr_2 activity is specific for those promoters that contain a TATA motif. A promoter lacking this motif, such as the β-DNA polymerase promoter, or the TdT-Inr-mediated transcription, is resistant to Dr_2-mediated repression. Our observations agree with recent studies demonstrating that the tumour-suppressor p53 protein interacts with TBP to repress transcription[22]. Moreover, the studies of Mack and co-workers found that p53-mediated repression requires a TATA motif and that Inr-directed transcription is resistant to inhibition[23]. Our studies also agree with the *in vivo* studies of Choder[24], who observed that when yeast cells reached stationary phase, topoisomerase I (yTopo I) can repress transcription of most, but not all, genes. We found that the DNA-relaxation activity associated with hTopo I was not required for repression of transcription, because hTopo I(Y723F), a mutant lacking DNA relaxation activity[9], was active in repression, as well as in enhancing the response to the activator.

Despite the observations that the human polypeptide and yeast Topo I gene can repress transcription, we found that yeast Topo I was not able to affect transcription in the human system. This is surprising, because hTopo I could interact with yeast TBP (data not shown). The molecular basis for the functional differences remains to be determined; however, precedent for this kind of observation does exist. For example, human TFIIB can interact with yeast TBP (and vice versa), but the factors (human and yeast TFIIB) are not functionally interchangeable[2]. These observations suggest that there may be other steps during formation of a transcription-competent complex where hTopo I may play a role. This step appears to be species-specific. Indeed, our preliminary studies indicate that hTopo I can interact with RNAPII in solution and, moreover, it appears that hTopo I travels with RNAPII during elongation, as we have been able to detect topoisomerase I in ternary complexes (unpublished results).

Previous studies have demonstrated that topoisomerase I is associated with transcriptionally active class II genes[25]. Moreover, the studies of Stewart *et al.*, suggested a functional interaction between Topo I and RNAPII during transcription elongation of the *fos* gene[26]. It is tempting to postulate that hTopo I is loaded onto the transcription complex by interacting with the TFIID complex. In the absence of the activator, this interaction results in repression of transcription. But, in the presence of the activator, hTopo I is translocated from the TFIID complex to the elongating complex. This might permit effective elongation by removing the superhelical tension induced by the elongation process[27]. Although this model is attractive, it is not supported by studies in yeast, as the deletion of the Topo I gene had no effect on cell viability[28,29]. Studies in higher eukaryotes, however, have found that the Topo I gene is essential for development in *Drosophila*[30].

We have demonstrated that Dr_2 represses transcription *in vitro*, and that in the absence of an activator, TFIIA can overcome this repression. Moreover, we found that the requirement for TFIIA is dictated by the presence of Dr_2/hTopo I. These observations agree with studies by Cortes *et al.*, who postulated that a function of TFIIA in transcription is to remove repressors present in the TFIID complex[14]. Our observations also agree with the demonstration that TFIIA can overcome the negative effect on transcription of Dr1[31], NC1, NC2 and DBF4[32,33]. But Dr_2 differs from the above repressors in its failure to form a complex with TBP and DNA on mobility shift assays. The functional relationship between these repressors of RNAPII transcription remains to be elucidated. It is clear, however, from

this and other studies[34–37] that the process of transcription activation involves at least two independent, but interrelated steps. The initial step involves the removal of molecules that maintain genes in a silent state, a process known as antirepression[36]. The second step represents true activation, in which the levels of expression of particular genes are increased well above basal levels. It is likely that these distinct events are coupled and that a family of ubiquitous activators operates to remove repressors, thereby setting specific genes into a state responsive to gene-specific activators. □

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LETTERS TO NATURE

Supernova 1993J as a spectroscopic link between type II and type Ib supernovae

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SUPERNOVA 1993J in the nearby galaxy M81 is one of the closest—and hence brightest—supernovae to be witnessed this century. The early spectrum of SN1993J showed[1–3] the characteristic hydrogen signature of type II supernovae, but its subsequent evolution is atypical for this class of supernova. Here we present optical and infrared spectra of SN1993J up to 43 days after outburst, which reveal the onset of the helium absorption and emission features more commonly associated with hydrogen-free type Ib supernovae. Corresponding model spectra show that the progenitor star must have possessed an unusually thin (for type II supernovae) hydrogen-rich envelope overlying a helium-rich mantle. Moreover, the supernova ejecta must have remained compositionally stratified, with little transport of the hydrogen-rich material down into the underlying helium layer, or mixing of heavier elements (such as radioactive ^{56}Ni) outwards. SN1993J therefore represents a transition object between hydrogen-dominated type II supernovae, and hydrogen-free, helium-dominated type Ib supernovae.

Optical spectra of SN1993J taken at 5:30 UT on 29 April and 4:05 on 9 May 1993 are shown in Fig. 1 together with a spectrum taken 20 April 1993 (ref. 1). These correspond to 32, 43 and 23 days, respectively, after the explosion (assuming that shock break-out occurred 28 March[2]). All spectra show the hydrogen Balmer series in absorption. The spectrum of 20 April, in which the helium features are weak or absent, shows an apparent flat-topped Hα profile in both absorption and emission. Between 20 and 29 April He I lines appeared as reported earlier[3]. The time for this event is probably constrained more closely by the changes in the shape of the Na I 5893-Å line observed between 22 and 26 April[4] as the He I 5876 absorption became stronger. After the helium lines become prominent, the He I 6678-Å line appears on the flat-topped Hα emission feature. The strength of the helium absorption lines increased in comparison to the strength of the hydrogen features in the ~10 days elapsed between 29 April and 9 May. Further details of the observed spectra are presented in Table 1.

Infrared spectra shown in Fig. 2 were taken at 3:50 on 3 May 1993 (37 days after shock break-out). The spectrum shows He I 1.083-μm emission with a normal P-Cygni profile, and weak He I 2.058-μm emission with an asymmetric absorption trough. Hydrogen (Paschen) Pa-β 1.282-μm emission is observed with a flat-topped P-Cygni profile.

Figures 1 and 2 also present the results of a model atmosphere of the supernova at 40 days after explosion. The model is derived from the unmixed 4.0-$M_\odot$ (solar masses) hydrodynamic model of Shigeyama *et al.*[5]. In this model, the supernova is originally compact, lacking an extended hydrogen-rich envelope, and hence the model will not reproduce the observed initial peak of the SN1993J light curve[2]; but comparison with dynamical models with low-mass extended envelopes show that it is a reasonable representation of the density structure at later times.

The parameters that we varied to obtain the best agreement with the observations are: the mass assigned to the hydrogen-