AIF pathway (which would be independent of DEVDases) can also be excluded. For example, CED-3 protease is absolutely required for programmed cell death in *Caenorhabditis elegans* (*37*), and caspase-3 (CPP32) is obligatory for cell death in areas of the developing murine brain (*38*).

Bcl-x_L is structurally related to certain bacterial pore-forming proteins (*39*), and Bcl-2 probably has a similar structure (*40*). Thus, Bcl-2 could conceivably block cytochrome c efflux directly. Or, Bcl-2 may prevent this event indirectly by regulating the flow of ions, including Ca^{2+}, across the mitochondrial and ER membranes (*41–46*). Such a scenario could explain the ability of Bcl-2, in some cell types, to block apoptosis even when its location is restricted to the ER (*47*). In the cell-free system described here, ER membranes are not required for activation of DEVD-specific proteases (Fig. 4), and thus a possible effect of Bcl-2 on this compartment may not be discernible.

Our results show that Bcl-2 acts on the mitochondria with which it is associated (Fig. 4). This localized action of Bcl-2 may depend on the ability of Bcl-2 to target the kinase Raf-1 to mitochondrial membranes (*48*). The Bcl-2–dependent mitochondrial sequestration of Raf-1 could be a mechanism underlying our finding that, when Bcl-2 was preincubated in cytosol with one aliquot of mitochondria, it failed to protect a second portion of mitochondria added later (Fig. 4, C and D).

Our observations have identified the mitochondrial release of cytochrome c as a major target for the anti-apoptotic effects of Bcl-2. The ability of cytochrome c to activate CPP32-like proteases and cell death appears to be distinct from this protein's life-sustaining role in respiration. Cytochrome c is highly conserved in eukaryotes (*49*). If its function in apoptosis is also conserved, this would explain how Bcl-2 or similar molecules can effectively regulate most forms of apoptosis.

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Role for the Amino-Terminal Region of Human TBP in U6 snRNA Transcription

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Basal transcription from the human RNA polymerase III U6 promoter depends on a TATA box that recruits the TATA box–binding protein (TBP) and a proximal sequence element that recruits the small nuclear RNA (snRNA)–activating protein complex ($SNAP_c$). TBP consists of a conserved carboxyl-terminal domain that performs all known functions of the protein and a nonconserved amino-terminal region of unknown function. Here, the amino-terminal region is shown to down-regulate binding of TBP to the U6 TATA box, mediate cooperative binding with $SNAP_c$ to the U6 promoter, and enhance U6 transcription.

The TATA box–binding protein is a central transcription factor required for transcription by all three RNA polymerases. The highly conserved COOH-terminal domain performs all of the TBP functions examined so far, including binding to the TATA box and interacting with TBP-associated factors, general transcription factors, and activators (*1*). In vivo, this domain can functionally replace the full-length protein for all promoters tested in mammalian systems (*2*); and in yeast, strains carrying a TBP missing the NH_2-terminal domain are viable (*3*). The role of the nonconserved NH_2-terminal domain is unknown.

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Most RNA polymerase III promoters consist of gene-internal elements and recruit TBP as part of the TBP-containing transcription factor IIIB (TFIIIB), through protein-protein interactions with the DNA-binding TFIIIC (*4*). However, in some unusual cases, exemplified by the human U6 snRNA promoter, the promoter elements are located upstream of the transcription start site (*5*) and appear to recruit neither TFIIIC (*6*) nor the same TFIIIB complex recruited by RNA polymerase III promoters with gene-internal elements (*7*). Instead, the U6 promoter contains two basal promoter elements, a proximal sequence element (PSE), which recruits a multisubunit complex referred to as the SNAP complex ($SNAP_c$) or PSE transcription factor (*8*) and a TATA box,

which recruits TBP (*9*).

To test whether $SNAP_c$ and TBP might bind to the U6 promoter cooperatively, as suggested by previous results (*10*), we performed an electrophoretic mobility shift analysis (EMSA) (*11*) with a probe corresponding to the basal human U6 promoter but containing the mouse U6 PSE, which is a higher affinity binding site for $SNAP_c$. A barely detectable complex formed when full-length human TBP fused to glutathione-S-transferase (G-hTBP), was incubated with the probe, but a prominent complex formed when a fraction highly enriched in $SNAP_c$ was incubated with the probe (Fig. 1A). When G-hTBP and $SNAP_c$ were co-incubated with the probe, a prominent complex of slower mobility was observed, and its formation was dependent on an intact PSE and an intact TATA box (Fig. 1A). This complex contained both G-hTBP and $SNAP_c$, as determined by antibody supershift experiments (*12*). Similar results were obtained when hTBP was used instead of the G-hTBP fusion protein (*12*). Thus, $SNAP_c$ appeared to enhance binding of TBP to the U6 promoter probe. The COOH-terminal core of TBP lacking the NH_2-terminal region (G-hTBPΔN) bound much more efficiently to the TATA box than did G-hTBP on its own, and G-hTBPΔN binding was not substantially increased in the presence of $SNAP_c$ bound to the PSE (Fig. 1A). Thus, although both factors could co-occupy the U6 probe, in this case they did not influence each other's ability to bind DNA. Similarly, in assays with the TATA box of the adenovirus 2 major late (Ad2 ML) promoter, G-hTBP bound more weakly than did G-hTBPΔN (*12*). These results suggest that the NH_2-terminal region of human TBP down-regulates binding to the TATA box and allows $SNAP_c$ to mediate recruitment of TBP to the U6 TATA box.

To determine whether the same effect could be observed at equilibrium in solution, we performed a deoxyribonuclease I (DNase I) footprinting experiment (*13*). Addition of increasing amounts of human G-hTBP to the U6 probe did not result in any detectable footprint, indicating that under the conditions used, full-length G-hTBP did not bind stably to the probe (Fig. 1B). In contrast, in the presence of $SNAP_c$, which gave a footprint over the high-affinity mouse U6 PSE on its own, a clear footprint over the TATA box was observed, confirming that $SNAP_c$ recruits and stabilizes TBP on the TATA box. Recruitment of TBP by $SNAP_c$ was also observed with non–glutathione-S-transferase (GST) TBP purchased from Promega (*12*). In contrast, G-hTBPΔN generated a clear footprint over the TATA box in the absence of $SNAP_c$, and this footprint was not increased in the presence of $SNAP_c$ (Fig. 1C). The results confirm that G-hTBPΔN binds more efficiently to the TATA box than does G-hTBP and cannot be further stabilized on the DNA by $SNAP_c$.

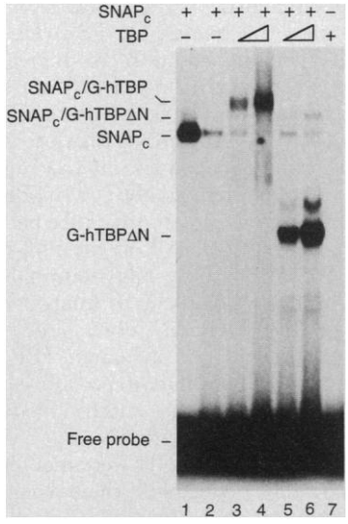

Fig. 2. Enhanced recruitment of $SNAP_c$ to a low-affinity human PSE by full-length hTBP but not hTBPΔN. The EMSA was performed with a U6 probe containing either the high-affinity mouse U6 PSE (lane 1) or the low-affinity human U6 PSE (lanes 2 through 7) and a TATA box. Either G-hTBP (lanes 3, 4, and 7) or G-hTBPΔN (lanes 5 and 6) and a constant amount of $SNAP_c$, as indicated above the lanes, was used. Each titration contained 40 and 100 ng of TBP.

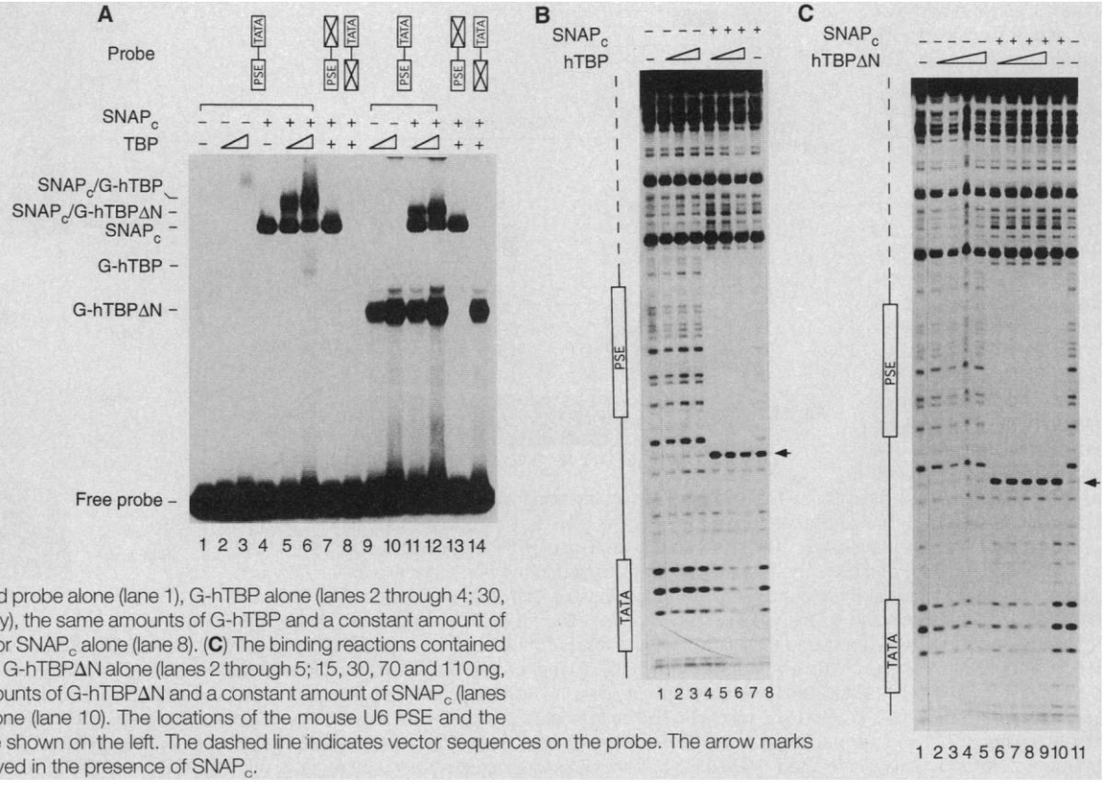

Fig. 1. $SNAP_c$-enhanced binding of full-length human TBP but not TBPΔN to the TATA box. (**A**) The EMSA was performed with a probe containing a high-affinity PSE (mouse U6 PSE) and a TATA box, or mutated versions thereof, and G-hTBP (lanes 2, 3, 5, and 6), or G-hTBPΔN (lanes 9 through 12) or $SNAP_c$ or both, as indicated above the lanes. Proteins were obtained as described (*11*). Each titration contained approximately 80 and 160 ng of TBP. The positions of the free probe and the protein-probe complexes are indicated. (**B**) The binding reactions for the DNase I footprinting assay contained probe alone (lane 1), G-hTBP alone (lanes 2 through 4; 30, 70, and 110 ng, respectively), the same amounts of G-hTBP and a constant amount of $SNAP_c$ (lanes 5 through 7) or $SNAP_c$ alone (lane 8). (**C**) The binding reactions contained probe alone (lane 1 and 11), G-hTBPΔN alone (lanes 2 through 5; 15, 30, 70 and 110 ng, respectively), the same amounts of G-hTBPΔN and a constant amount of $SNAP_c$ (lanes 6 through 9), or $SNAP_c$ alone (lane 10). The locations of the mouse U6 PSE and the TATA box on the probe are shown on the left. The dashed line indicates vector sequences on the probe. The arrow marks a hypersensitive site observed in the presence of $SNAP_c$.

To test whether TBP stabilized $SNAP_c$ on the PSE, we assayed binding of $SNAP_c$ and G-hTBP to the wild-type human U6 promoter, which contains a PSE with very low affinity for $SNAP_c$. The $SNAP_c$ bound efficiently to the mouse U6 PSE but not to the human U6 PSE (Fig. 2). However, upon addition of increasing amounts of G-hTBP, which did not bind effectively to the probe on its own, we observed very efficient formation of a $SNAP_c$/G-hTBP complex on the human U6 promoter. Remarkably, G-hTBPΔN bound very efficiently to the probe but was unable to recruit $SNAP_c$ to the PSE (Fig. 2). Yeast TBP, whose NH_2-terminal domain bears no homology to human TBP, behaved like hTBPΔN (*12*). Thus, the NH_2-terminal region of human TBP not only down-regulates binding of full-length TBP to the TATA box, but also mediates cooperative binding with $SNAP_c$.

The nonconserved NH_2-terminal region of TBP can be divided into three segments: (i) a run of glutamines at position 55 through 95 (segment II), (ii) the segment that precedes it (segment I, amino acids at position 1 through 54), and (iii) the segment that follows it (segment III, amino acids at position 96 through 158) (Fig. 3A). We first generated two constructs that either contained only the third segment (ΔN+III) or only the first two segments (ΔN+I+II) of the NH_2-terminal region, and compared them with full-length TBP and hTBPΔN in their ability to recruit $SNAP_c$ to the low-affinity human PSE. We tested both GST TBP fusion proteins and non-GST TBP proteins (Fig. 3B). $SNAP_c$ alone bound relatively weakly to the PSE, and full-length G-hTBP or hTBP did not show detectable binding. However, $SNAP_c$ and G-hTBP together or $SNAP_c$ and hTBP together bound very efficiently to the human U6 promoter. Both G-hTBPΔN and hTBPΔN bound to the probe on their own but did not recruit $SNAP_c$ to the PSE. G-TBP and TBP proteins containing only the third segment of the NH_2-terminal region (ΔN+III) also bound to the TATA box on their own and also failed to enhance recruitment of $SNAP_c$ to the PSE. In contrast, proteins containing only the first two segments (ΔN+I+II) did not bind efficiently on their own but were able to bind cooperatively with $SNAP_c$ (Fig. 3B). The TBP molecules lacking the GST moiety were also tested for their ability to bind to the Ad2 ML TATA box, and in this assay too, full-length hTBP and ΔN+I+II bound less efficiently to the TATA box than did hTBPΔN and ΔN+III (*12*). These results suggest that the first two segments down-regulate binding of human TBP to the TATA box and recruit $SNAP_c$ to the PSE.

We then tested two GST fusion constructs containing either segment I or segment II fused directly to the COOH-terminal domain (constructs ΔN+I and ΔN+II; Fig. 3A). Whereas G-hTBPΔN+I was unable to bind effectively to the U6 TATA box on its own (like full-length and ΔN+I+II TBP), G-hTBPΔN+II could bind to the TATA box (Fig. 3B). These results suggest that segment I down-regulates binding of TBP to the TATA box. Both proteins, however, were capable of binding cooperatively with $SNAP_c$ to the U6 promoter, although less efficiently than either G-hTBP or G-hTBPΔN+I+II (Fig. 3B). This suggests that segments I and II both contribute to cooperative binding with $SNAP_c$.

To determine whether the NH_2-terminal

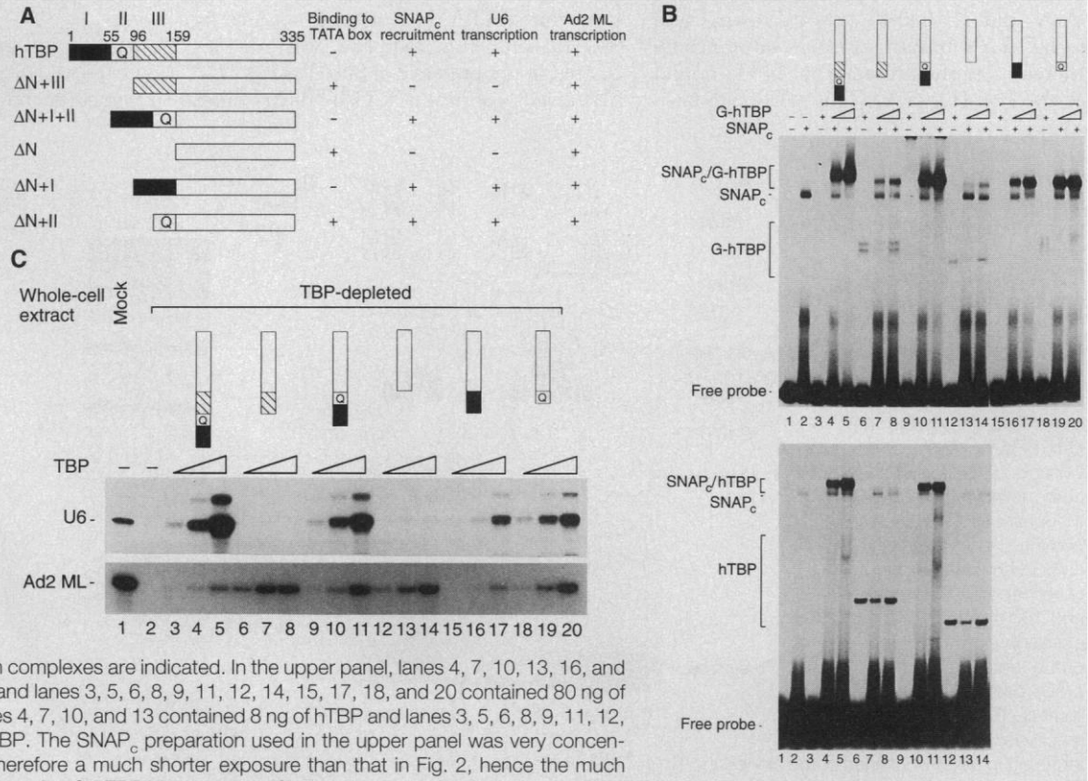

Fig. 3. (**A**) Structure of NH_2-terminal deletion mutants of hTBP and their effect on DNA binding, ability to interact with $SNAP_c$, and basal U6 and Ad2 ML transcription. The positions of segments I, II, and III in wild-type TBP and the various TBP mutants are indicated. The EMSA and transcription results in (B) and (C) are summarized on the right. (**B**) The first two segments of the NH_2-terminal domain of TBP can recruit $SNAP_c$ to the PSE. The upper panel shows an experiment performed with GST fusion proteins and the lower panel shows an experiment performed with TBP molecules lacking the GST moiety. The NH_2-terminal deletion mutants of hTBP used are indicated above the lanes. The positions of the free probes and of the various DNA-protein complexes are indicated. In the upper panel, lanes 4, 7, 10, 13, 16, and 19 contained 40 ng of G-TBP and lanes 3, 5, 6, 8, 9, 11, 12, 14, 15, 17, 18, and 20 contained 80 ng of G-TBP. In the lower panel, lanes 4, 7, 10, and 13 contained 8 ng of hTBP and lanes 3, 5, 6, 8, 9, 11, 12, and 14 contained 16 ng of hTBP. The $SNAP_c$ preparation used in the upper panel was very concentrated; the autoradiogram is therefore a much shorter exposure than that in Fig. 2, hence the much weaker signals corresponding to the G-hTBPΔN complex. (**C**) The first two segments of the NH_2-terminal domain of hTBP are required for efficient in vitro transcription from the human U6 but not from the Ad2 ML promoter. Mock-depleted (lane 1) or TBP-depleted (lanes 2 through 20) whole-cell extracts (10 μl) were programmed with the U6 template hU6/Hae/RA.2 (*22*) containing a mutated enhancer (upper panel) or with the p119ML(C2A) template (*22*) containing the Ad2 ML promoter in front of a G-less cassette (lower panel). Where indicated, the reactions were supplemented with 20, 60, and 180 ng of G-hTBP or truncated versions thereof, as illustrated above the lanes. RNA derived from the U6 template was analyzed by ribonuclease T_1 protection with the U6/RA.2/143 probe, according to Lobo *et al.* (*7*). A correctly initiated RNA signal from the U6 promoter and from the Ad2 ML promoter is indicated. Transcription from U6 was severely reduced by mutations in the PSE or the TATA box (*12*).

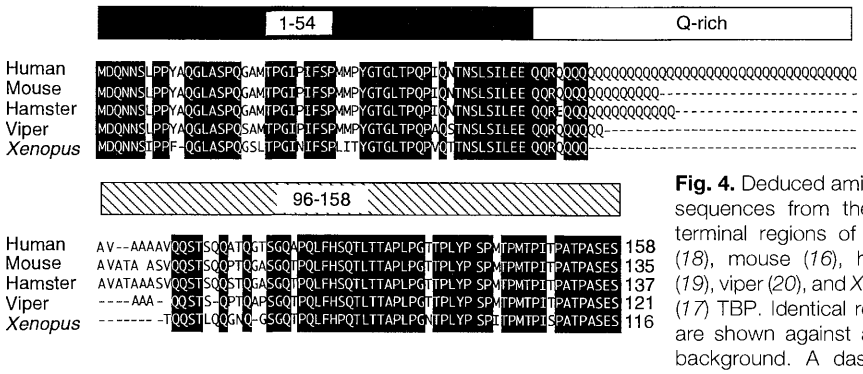

Fig. 4. Deduced amino acid sequences from the NH_2-terminal regions of human (*18*), mouse (*16*), hamster (*19*), viper (*20*), and *Xenopus* (*17*) TBP. Identical residues are shown against a black background. A dash indicates a gap. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

region of TBP plays a role in snRNA gene transcription, we compared the ability of G-hTBP and the various NH_2-terminal truncations to direct basal RNA polymerase III transcription from the human U6 promoter, which has a low-affinity PSE, and basal RNA polymerase II transcription from the Ad2 ML promoter, in an extract immunodepleted of TBP (*14*). Depletion of TBP inhibited both U6 and Ad2 ML transcription, but addition of increasing amounts of G-hTBP or any of its mutant derivatives restored transcription from the Ad2 ML promoter (Fig. 3C). This indicates that none of the TBP mutants were inherently defective, including those that bound only weakly to the TATA box, and also suggests that the TBP mutants that do not bind DNA effectively can be stabilized on the Ad2 ML TATA box by other members of the Ad2 ML initiation complex, such as TFIIA or TFIIB. Indeed, all TBP mutants bound to the Ad2 ML TATA box in an EMSA in the presence of TFIIA or TFIIB (*12*). Transcription from the U6 promoter could be restored very efficiently by addition of increasing amounts of G-hTBP or G-TBPΔN+I+II and somewhat less efficiently by addition of increasing amounts of G-hTBPΔN+I or G-hTBPΔN+II. Remarkably however, neither G-hTBPΔN+III nor G-hTBPΔN was capable of restoring U6 transcription at any of the concentrations tested (Fig. 3C). Thus, the abilities of the different mutant TBPs to support basal transcription from the human U6 promoter correlate with their abilities to recruit $SNAP_c$ to the human PSE, and not with their intrinsic abilities to bind DNA or to restore basal RNA polymerase II transcription. These results suggest that recruitment of $SNAP_c$ by the NH_2-terminal domain of TBP is essential for basal transcription from the human U6 promoter, which has a low-affinity PSE. The results may also explain the observation of Lescure *et al.* that a monoclonal antibody directed against an epitope corresponding to the junction between regions I and II inhibits RNA polymerase III transcription from a U6 promoter but not from TATA-less promoters in vitro (*15*).

How does the NH_2-terminal domain of TBP down-regulate binding to the TATA box and recruit $SNAP_c$ to the PSE? Perhaps this domain masks part of the conserved COOH-terminal DNA-binding domain and undergoes a conformational change during cooperative binding of $SNAP_c$ and TBP to the U6 promoter, so that it becomes engaged in direct protein-protein interactions with $SNAP_c$. This putative interaction would be dependent on both factors binding to DNA, because both an intact PSE and an intact TATA box are required for formation of the $SNAP_c$/TBP complex. Are the functions we describe restricted to human TBP? Although the TBP NH_2-terminal domain has diverged much more than the COOH-terminal domain, it is highly conserved among vertebrate TBPs (*16*, *17*). Segments I and III are 78 and 73% identical, respectively, among humans (*18*), mice (*16*), hamsters (*19*), two different vipers (whose TBPs are identical except for the number of Gln residues in segment II) (*20*), and *Xenopus laevis* (*17*) TBPs, with large blocks of amino acids conserved 100% (Fig. 4). The main variation is the length of segment II, which indeed is polymorphic in humans (*21*). Similarly, snRNA promoters have diverged widely between, for example, yeast and humans, but are quite conserved among vertebrates (*5*). Perhaps the TBP-$SNAP_c$ interaction, like the NH_2-terminal region of TBP, has remained conserved among vertebrate species.

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11. EMSAs (20 μl) contained 100 mM KCl, 20 mM Hepes (pH 7.9), 5 mM $MgCl_2$, 0.2 mM EDTA, 10% glycerol, 1 mM dithiothreitol, 0.07% Tween-20 and 0.2 μg each of poly(dG-dC)(dG-dC) and pUC118. Reactions were incubated for 15 min at 4°C before addition of radiolabeled probe (20,000 cpm) followed by a 30-min incubation at 30°C. The binding reactions were fractionated on a 5% polyacrylamide gel (39:1 acrylamide:bisacrylamide) in 1× TGEM running buffer (50 mM tris base, 380 mM glycine, 2 mM EDTA, and 5 mM $MgCl_2$). EMSA probes were prepared by polymerase chain reaction (PCR) amplification with γ-^{32}P end-labeled primers. Full-length human TBP and its NH_2-terminal deletions were expressed with the pET11c expression system [F. W. Studier, A. H Rosenberg, J. J. Dunn, J. W. Dubendorff, *Methods Enzymol.* **185**, 60 (1990)] as GST fusions in *Escherichia coli* strain BL21(DE3) and purified on glutathione-agarose (Sigma). The non-GST TBP molecules were obtained by removal of the GST moiety with thrombin [V. Mittal, M. A. Cleary, W. Herr, N. Hernandez, *Mol. Cell. Biol.* **16**, 1955 (1996)]. All proteins were analyzed by SDS–polyacrylamide gel electrophoresis, and equivalent amounts of each purified protein were used in all assays. $SNAP_c$ was derived from a MonoQ peak fraction, which corresponds to the fourth step in the purification of $SNAP_c$ and a purification of approximately 2500 times [R. W. Henry, C. L. Sadowski, R. Kobayashi, N. Hernandez, *Nature* **374**, 653 (1995)].
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13. Probes for DNase I footprinting were prepared by PCR with one γ-^{32}P end-labeled primer. Proteins were incubated at 30°C for 30 min in a 50-μl reaction containing 100 mM KCl, 20 mM Hepes (pH 7.9), 5 mM $MgCl_2$, 0.2 mM EDTA, 10% glycerol, 1 mM dithiothreitol, 0.2 μg each of poly(dG-dC)(dG-dC) and pUC118, 2% polyvinyl alcohol, and 10,000 cpm of radiolabeled probe. DNase I digestion was carried out as described [M. C. Schmidt, Q. Zhou, A. J. Berk, *Mol. Cell. Biol.* **9**, 3299 (1989)], and the reaction products were analyzed on a 7% polyacrylamide-urea gel.
14. For in vitro transcriptions, whole-cell extracts were depleted of TBP with a mixture of polyclonal and monoclonal antibodies to TBP cross-linked to protein A–Sepharose beads. Mock-depleted extracts were processed identically but with beads contain-

ing no antibodies. Transcription reactions for U6 and Ad2 ML were carried out as described by Lobo *et al.* (*7*). Purified GST-hTBP proteins were added as indicated in the figure legends, and the transcription reactions were allowed to proceed for 30 (for U6) or 90 (for Ad2 ML) min at 30°C.
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Structural Convergence in the Active Sites of a Family of Catalytic Antibodies

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The x-ray structures of three esterase-like catalytic antibodies identified by screening for catalytic activity the entire hybridoma repertoire, elicited in response to a phosphonate transition state analog (TSA) hapten, were analyzed. The high resolution structures account for catalysis by transition state stabilization, and in all three antibodies a tyrosine residue participates in the oxyanion hole. Despite significant conformational differences in their combining sites, the three antibodies, which are the most efficient among those elicited, achieve catalysis in essentially the same mode, suggesting that evolution for binding to a single TSA followed by screening for catalysis lead to antibodies with structural convergence.

Convergent evolution is a frequent outcome of the process of natural selection. At present, we know very little about this process, particularly with respect to proteins and their function. Among the unanswered questions are concerns about how many different ways a protein pocket can effect a given chemical transformation, and what elementary processes once existed and have then been discarded along the evolutionary pathway to the efficient enzymes we see today. Catalytic antibodies that are induced experimentally in real time offer a way to reveal much about enzyme evolution including questions about convergence (*1*). The proposal that antibodies with catalytic activity (abzymes) could be generated to an analog of a transition state (TSA) of the reaction to be catalyzed (*2*) has proved widely applicable (*3*). Now, instead of reporting a singular event via a structure we report and compare the best solutions to catalysis in a family of antibodies, thereby offering insight into the power of selection, through a screening for catalytic activity, even when it is only allowed to operate once.

Most abzymes have been identified by screening the immune response for binding to the hapten and then testing the best scoring clones for catalytic activity. X-ray structures of abzymes generated following this general protocol have begun to define relationships between the hapten used to elicit catalytic antibodies and the residues of these antibodies which effect catalysis (*4*–*8*). With a more facile procedure, cat-ELISA, in which product-specific antibodies are used to detect the appearance of product after immobilized substrate is exposed to the supernatant of culture hybridoma cells, we were able to probe the entire hybridoma repertoire for catalytic antibodies (*9*). The catalytic antibodies D2.3, D2.4, and D2.5 were obtained by immunizing BALB/c mice with phosphonate **1**, an analog of the oxyanion intermediate in the hydrolysis of **2**, coupled to keyhole limpet hemocyanin (KLH) (Fig. 1), and identified with the use of catELISA. Among a total of 1570 hybrid clones derived from a single mouse, nine scored positive in this assay, a figure to be compared to 970 hapten-binding clones (*9*). Catalytic antibodies D2.3, D2.4, and D2.5 show specific and efficient hydrolysis, accelerated by up to five orders of magnitude, toward the *p*-nitrobenzyl ester **2** (Table 1) and are significantly more active than the other six catalytic clones that were identified (as determined by cat-ELISA).

The sequences of the variable domains of D2.3, D2.4, and D2.5 have been determined. The most extensive differences are found in the H3 loop (10 residues), which in D2.4 differs from those in D2.3 and D2.5 at four positions and by an insertion (*10*). Otherwise, the sequences present a high degree of identity, similar to that observed in families of catalytic antibodies elicited with the same hapten (*11*–*13*), as opposed to the extensive differences between se-

Table 1. Kinetic and structural data on antibodies D2.3, D2.4, and D2.5.

	D2.3	D2.4	D2.5
k_{cat} (min^{-1})*	3.6	1.0	0.07
K_m (μM)	280	300	340
$K_D S/K_D$ TSA	1.1×10^5	3.3×10^4	1.3×10^3
k_{cat}/k_{uncat}†	1.3×10^5	3.6×10^4	2.5×10^3
Resolution (Å)	1.9	3.1	2.2
Precision of atomic positions‡ (Å)	0.25	0.35	0.25
Interactions of TSA 1 with the Fab			
Buried surface§ ($Å^2$)	515	510	505
van der Waals contacts (nb)	86	96	76
Hydrogen bonds (nb)	5	5	5

*The catalytic activity (per site) and binding parameters were measured as described (*9*, *19*). Data for the most efficient of these antibodies, D2.3, have be reported (*9*); those for D2.4 and D2.5 are reported in a kinetic study of all three antibodies (*19*, *28*). †k_{uncat} is measured in the same buffer as used for measuring catalysis, the kinetic constant being extrapolated to zero buffer concentration, at constant ionic strength. ‡The precision of atomic positions is evaluated from the variation of the *R* factor with the resolution, according to the method of Luzzati (*20*). §An equivalent surface area is buried on the Fab side.

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