^{86}Sr ratios in the phenocrysts therefore requires that assimilation occurred at an early stage of magmatic evolution. Each subsequent recharge event added Sr with a lower $^{87}Sr/^{86}Sr$ ratio (Fig. 4). Although assimilation between recharge events may gradually increase the $^{87}Sr/^{86}Sr$ ratio of the magma (as may be illustrated by the Purico-Chascon example in Fig. 2), this question typically cannot be resolved at the scale of microdrilling. A progressive decrease in the $^{87}Sr/^{86}Sr$ ratio must reflect the frequency and relative amount of mafic recharge compared with assimilation. This effect may well be accentuated by the contribution of Sr from dissolving of plagioclase rims attendant with each injection of mafic magma (*18*). Recharge of the magma system must maintain a rather steady-state magma chamber (*19*), as the small variations in An content across dissolution surfaces and lack of unidirectional overall zoning preclude growth from magmas that vary greatly in composition. In the case of El Chichón, the uniformity of erupted products over 300,000 years of total activity (*16*) seems to support the operation of a steady-state magma system.

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11. At 950°C, a $^{87}Sr/^{86}Sr$ profile such as that determined in Fig. 1 would take approximately 10^5 to 10^6 years to form by diffusion, given the diffusion coefficients of B. J. Giletti and E. D. Casserly [*Geochim. Cosmochim. Acta* **58**, 3785 (1994)] and equations as in J. N. Christensen and D. J. DePaolo [*Contrib. Mineral. Petrol.* **113**, 100 (1993)]. An isolated body of molten magma at shallow levels in the crust could only be maintained over long periods of time if it were large {a 4-km^3 pluton emplaced in the crust would be expected to solidify in 50,000 years [F. Spera, *Science* **207**, 299 (1980)]}, yet the magmatic volumes produced by both lava dome systems are small (<2 km^3). Even if the Purico-Chascon domes were regarded as a residual magma system to the larger volume ignimbrite (1.3×10^6 years old), the preservation of mafic inclusions, many with quenched margins, requires that the inclusion-forming event and therefore the maximum amount of time in which the crystals are immersed in mafic magma was short. The inclusion-forming event most likely took place shortly before dome emplacement, allowing incomplete hybridization to be preserved and perhaps acting as a trigger for emplacement as described by R. S. J. Sparks, H. Sigurdsson, and L. Wilson [*Nature* **267**, 315 (1977)], J. C. Eichelberger [*ibid.* **288**, 446 (1980)], and J. S. Pallister, R. P. Hoblitt, and A. G. Reyes [*ibid.* **356**, 426 (1992)].
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Selective Use of TBP and TFIIB Revealed by a TATA-TBP-TFIIB Array with Altered Specificity

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Interaction between the TATA box–binding protein TBP and TFIIB is critical for transcription in vitro. An altered-specificity TBP-TFIIB interaction was rationally designed and linked in sequence to an altered-specificity TATA box–TBP interaction to study how TBP and TFIIB function together to support transcription in human cells. The activity of this altered-specificity TATA-TBP-TFIIB array demonstrated that many activators use the known TBP-TFIIB interaction to stimulate transcription. One activator, however, derived from a glutamine-rich activation domain of Sp1, activated transcription independently of this interaction. These results reveal that selectivity in activator function in vivo can be achieved through differential use of TBP and TFIIB.

In vivo analysis of the basal transcriptional machinery is difficult because the basal factors are highly conserved, ubiquitously expressed, and probably essential proteins. One way to circumvent these limitations is to exploit mutations that alter the specificity of interaction between two factors, allowing their activities to be measured in vivo in the presence of their wild-type counterparts. We have previously used a form of TBP with an altered TATA box–binding specificity (TGTA) (*1*) to probe the role of TBP in activated transcription in human cells (*2*). We found that TBP activity in vivo is sensitive to certain combinations of clustered point mutations across the surface of the molecule, and that in vivo activity correlates closely with the ability to associate in vitro with the largest TBP-associated factor, $hTAF_{II}250$ (*3*), suggesting that the TBP-associated factors (TAFs) are important for activated transcription in vivo, as they are in vitro (*4*).

Biochemical studies indicate that the interaction of TBP with TFIIB is also critical for activated transcription in vitro (*5*), but none of our original TBP mutations targeted residues involved in interaction with TFIIB (*2*, *6*, *7*). To probe the importance of the TBP-TFIIB interaction for transcription in vivo, we examined the effects of single or double amino acid substitutions (*8*) at three TBP residues critical for interaction with TFIIB in vitro (*7*) (Fig. 1A), measuring the response to two GAL4-fusion activators carrying either the

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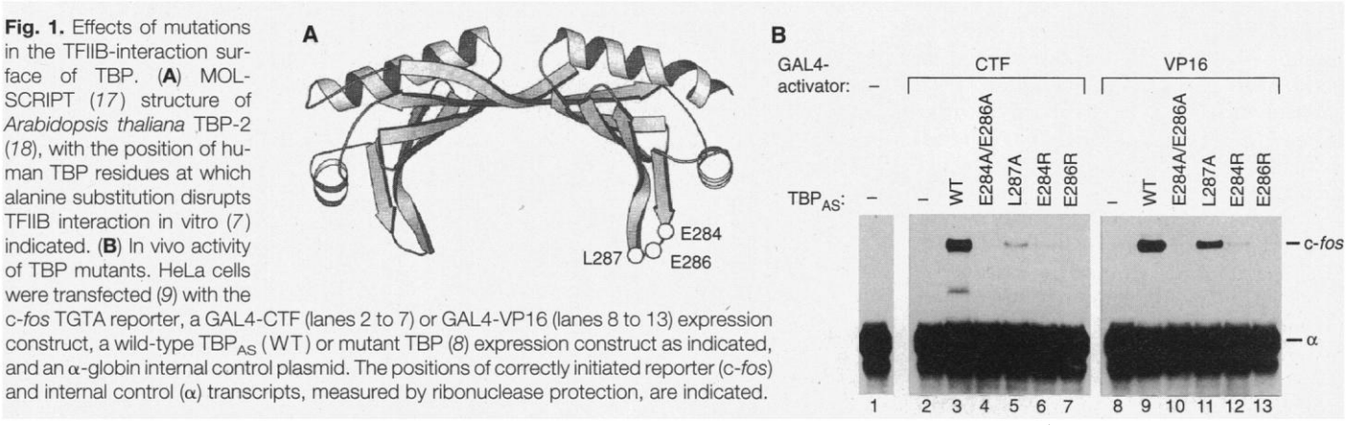

Fig. 1. Effects of mutations in the TFIIB-interaction surface of TBP. (**A**) MOLSCRIPT (*17*) structure of *Arabidopsis thaliana* TBP-2 (*18*), with the position of human TBP residues at which alanine substitution disrupts TFIIB interaction in vitro (*7*) indicated. (**B**) In vivo activity of TBP mutants. HeLa cells were transfected (*9*) with the c-*fos* TGTA reporter, a GAL4-CTF (lanes 2 to 7) or GAL4-VP16 (lanes 8 to 13) expression construct, a wild-type TBP_{AS} (WT) or mutant TBP (*8*) expression construct as indicated, and an α-globin internal control plasmid. The positions of correctly initiated reporter (c-*fos*) and internal control (α) transcripts, measured by ribonuclease protection, are indicated.

proline-rich CTF (GAL4-CTF) (*2*) or the acidic VP16 (GAL4-VP16) (*2*) activation domains (Fig. 1B). We assayed the effects of these mutations on the ability of altered-specificity TBP (TBP_{AS}) to support transcription from a c-*fos*–based reporter, carrying a "TGTA" box and four GAL4 DNA-binding sites, in human HeLa cells (9).

Each of these amino acid substitutions in TBP disrupted its function in vivo (Fig. 1B) (*10*). Three of the mutations, E284A/E286A, E284R, and E286R, had similar marked effects as measured by their response to both activators. In contrast, the L287A mutant both retained significant activity and displayed an activator-specific effect, responding better to the VP16 activator than to the CTF activator. This behavior contrasts with the effects of a previously described TBP mutation in which the opposite activator preference was observed (*2*). Together, these results suggest that these two activators use TBP differently to activate transcription.

To determine whether the TFIIB-contact mutants of TBP were defective because they failed to interact with TFIIB in vivo, we investigated whether a secondary mutation in TFIIB could suppress the defects of one of these TBP mutations. In the crystal structure of the TBP-TFIIB interaction (6), Glu^{284} of TBP (E284) participates in a polar side chain–side chain interaction with Arg^{169} of human TFIIB (R169). Because the $Glu^{284}\rightarrow Arg$ (E284R) substitution in TBP disrupted its function in vivo (Fig. 1B), we examined whether the reciprocal $Arg^{169}\rightarrow Glu$ (R169E) mutation in TFIIB (*11*) would rescue the activity of the E284R mutant TBP_{AS} in vivo. Indeed, the R169E human TFIIB molecule strongly stimulated the activity of the E284R mutant TBP_{AS}, resulting in the complete restoration of wild-type levels of c-*fos* reporter activity (Fig. 2). The in vivo rescue of mutant TBP activity by a secondary TFIIB mutant argues that, for response to the GAL4-CTF activator, the TBP-TFIIB interaction is critical for transcription in vivo.

To probe the specificity of this rescue, we determined whether in vivo complementation was specific to the particular amino acid substitutions we had made in TBP and TFIIB. We examined the ability of different pairs of TBP and TFIIB mutants, each of which carried a single radical amino acid substitution at a TBP-TFIIB contact residue (6), to function together to support a transcriptional response to GAL4-VP16 in HeLa cells (Fig. 3). As observed with GAL4-CTF (Fig. 2), the combination of E284R TBP_{AS} and R169E TFIIB resulted in levels of GAL4-VP16 activation considerably higher than that seen with the E284R mutant TBP alone: No other combination of TBP and TFIIB mutants resulted in transcriptional activity. In particular, changing Glu^{284} of TBP to a lysine instead of an arginine blocked the response to the R169E suppressor TFIIB, demonstrating that the rescue of transcription is highly specific, even for the precise nature of the basic side-chain at position 284 of TBP (*12*). This high degree of allele specificity suggests that the designed TBP-TFIIB interaction functions by creating a highly specific

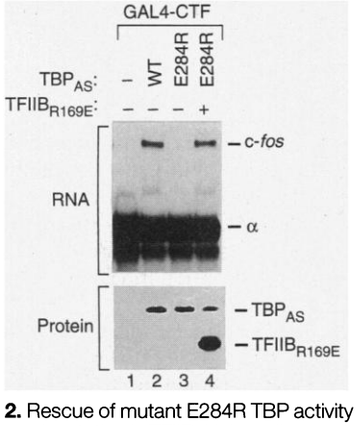

Fig. 2. Rescue of mutant E284R TBP activity by a designed mutation in TFIIB. HeLa cells were transfected with (i) GAL4-CTF, (ii) epitope-tagged wild-type (WT) or E284R mutant TBP_{AS}, and (iii) epitope-tagged R169E mutant TFIIB expression constructs as indicated (*9*). Levels of correctly initiated c-*fos* and α-globin transcripts (RNA) and epitope-tagged TBP and TFIIB molecules (Protein; measured by immunoblotting) are shown (see legend to Fig. 1 for details).

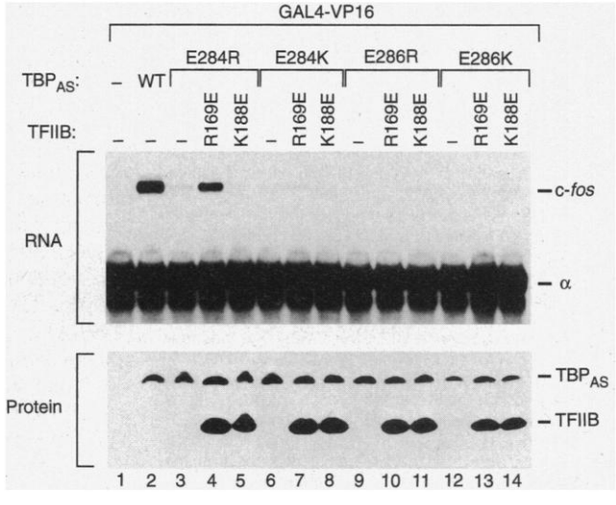

Fig. 3. Allele-specific rescue of transcription by TBP and TFIIB mutants. The altered-specificity TBP assay (*9*) was used to test the effects of pairwise combinations of TBP and TFIIB mutants on activation by GAL4-VP16 as indicated. TBP_{AS} residues 284 and 286 were individually substituted with either arginine (E284R and E286R) or lysine (E284K and E286K); TFIIB residues 169 or 188 were individually substituted with glutamic acid (R169E and K188E). Levels of correctly initiated c-*fos* and α-globin transcripts (RNA) and epitope-tagged TBP and TFIIB molecules (Protein) are shown (see legend to Fig. 1 for details).

reverse polarity interaction between residues 284 of TBP and 169 of TFIIB. We call the R169E TFIIB suppressor mutant "altered-specificity TFIIB" ($TFIIB_{AS}$).

This altered-specificity TBP-TFIIB interaction functions together with the altered-specificity TBP–TATA box interaction, resulting in a "sequential altered-specificity" TATA-TBP-TFIIB array that provides the opportunity to define, by mutagenesis, the regions of TFIIB that mediate transcriptional activation in vivo. Such an analysis has shown that the NH_2-terminal region of TFIIB is important for transcription in vivo (*13*). This sequential altered-specificity array also, however, directly probes how TBP and TFIIB are used by transcriptional activators. For example, transcriptional activation by both GAL4-CTF and GAL4-VP16 was sensitive to the E284R mutation in TBP_{AS} and was rescued by $TFIIB_{AS}$ (Figs. 2 and 3), demonstrating that the known TBP-TFIIB interaction is used by these activators in vivo.

To probe how other activators use TBP and TFIIB, we assayed the response of two other activators to the E284R mutation in TBP_{AS} and to rescue by $TFIIB_{AS}$. A GAL4-fusion activator carrying the acidic p53 activation domain (GAL4-p53) (*14*) behaved similarly to GAL4-CTF and GAL4-VP16 (Fig. 4). In contrast, a GAL4-fusion activator carrying a glutamine-rich Sp1 activation domain (GAL4-$Sp1^Q$) (*2*) displayed a markedly different response (Fig. 4, lanes 5 to 8). First, activation was less sensitive to the E284R mutation in TBP_{AS}, being reduced by a factor of only 3 to 4 (Fig. 4). This activator-specific difference is analogous to those observed with other mutations in TBP [(*2*) and this study] (Fig. 1B), but in this instance the TBP mutation does not discriminate between the CTF and VP16 activators as before, but rather between

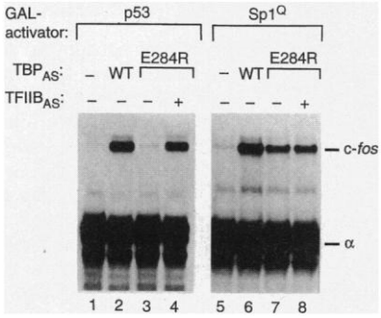

Fig. 4. Differential use of the TBP-TFIIB interaction by activators in vivo. HeLa cells were transfected (*9*) with (i) GAL4-p53 or GAL4-$Sp1^Q$ activator, (ii) wild-type or E284R mutant TBP_{AS}, and (iii) $TFIIB_{AS}$ expression constructs as indicated. Levels of correctly initiated c-*fos* and α-globin transcripts are shown (see legend to Fig. 1 for details).

the $Sp1^Q$ and other activators. The repeated observation of activator-specific defects resulting from mutations in TBP suggests that activators commonly use different mechanisms to activate the basal transcriptional machinery.

The molecular basis of activator-specific defects in TBP has not been clear. $TFIIB_{AS}$, however, provides a strategy to explain the differential effect of the E284R mutation. We envision two mechanisms for the relative insensitivity of GAL4-$Sp1^Q$ to the E284R TBP mutation. In the first mechanism, GAL4-$Sp1^Q$ uniquely stabilizes binding of endogenous TFIIB to the E284R TBP_{AS} in vivo. In this instance, $TFIIB_{AS}$ would be expected to increase the response of the E284R TBP_{AS} to GAL4-$Sp1^Q$, as it does with the other activation domains. In the second mechanism, GAL4-$Sp1^Q$ activates transcription independently of the known TBP-TFIIB interaction. In this instance, the diminished activity of E284R TBP_{AS} would not result from loss of interaction with TFIIB and would not respond to $TFIIB_{AS}$. Indeed, with GAL4-$Sp1^Q$, E284R TBP_{AS} did not respond to $TFIIB_{AS}$ (Fig. 4), suggesting that this Sp1-derived activator can activate transcription independently of the known TBP-TFIIB interaction. These results demonstrate selective use of TBP and TFIIB by activators in vivo.

The differential use of TBP and TFIIB by GAL4-$Sp1^Q$ may indicate that TFIIB does not participate in activation by this activator. Alternatively, TFIIB may be required for activation by GAL4-$Sp1^Q$, but the structure of the basal machinery is flexible, and TBP and TFIIB adopt a different arrangement at the promoter when responding to GAL4-$Sp1^Q$ compared with other activators. In either instance, the selective use of TBP and TFIIB provides a mechanism by which activators can selectively activate transcription from different promoters, where the basal transcriptional machinery may be arranged differently. For example, on a promoter where TBP and TFIIB do not associate as they do on a TATA-containing promoter, the glutamine-rich $Sp1^Q$ activation domain may be more potent than the other activation domains we have analyzed. Such a difference could explain the preferential ability of GAL4-$Sp1^Q$ and related Sp1 activators to activate transcription at certain TATA-less promoters (*15*).

In addition to TBP and TFIIB, the ability to link altered-specificity interactions in sequence may provide the opportunity to study other components of the basal transcriptional machinery in vivo, or, more generally, other biological machines including those involved in DNA replication, RNA splicing, and translation.

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8. The mutations were a single alanine substitution at Leu^{287} (L287A), single arginine substitutions at Glu^{284} (E284R) or Glu^{286} (E286R), or a double alanine substitution of residues 284 and 286 (E284A and E286A). Mutant TBP molecules were generated by site-directed mutagenesis (*16*) of the full-length TBP_{AS} expression construct $pCGNhTBP_{AS}$ (*2*).
9. Altered-specificity TBP was assayed in transiently transfected HeLa cells (*2*). Calcium phosphate coprecipitation was used to transfect HeLa cells with plasmid DNAs: (i) 2 μg of c-*fos* (−56)[4xGAL] reporter; (ii) between 80 and 640 ng of each pCGGAL4-activator expression plasmid; (iii) 160 ng of either wild-type or mutant $pCGNhTBP_{AS}$ expression plasmid; (iv) 160 ng of the α-globin internal control plasmid pα4x(A+C); and (v) pUC119, bringing the total amount of DNA transfected to 20 μg. In experiments involving TFIIB, between 1.6 and 6 μg of TFIIB expression construct was also included. Ribonuclease protection analysis was used to quantitate correctly initiated transcripts from the c-*fos* and α-globin plasmids. Because the potency of the GAL4-fusion activators differs (*2*), c-*fos* ribonuclease protection probes of various specific activities were used to measure reporter transcript levels for different activators. TBP and TFIIB expression was monitored by quantitative immunoblot analysis, with an antibody (12CA5) specific for the hemagglutinin A (HA)–epitope tag and enhanced chemiluminesence detection (Pierce). In all experiments, the amount of TBP_{AS} and $TFIIB_{AS}$ expression constructs used was adjusted to give equivalent expression for each TBP and each TFIIB mutant as shown or as in (*13*).
10. The in vivo effects of mutations in these three TFIIB-interaction residues of TBP are generally consistent with those observed by Bryant *et al.* [G. O. Bryant, L. S. Martel, S. K. Burley, A. J. Berk, *Genes Dev.* **10,** 2491 (1996)] except for the behavior of the E286R mutant TBP_{AS}, which showed negligible activity in our assay but considerable activity (50% of wild-type TBP_{AS}) in the Bryant *et al.* study. Differences in the cell lines used (HeLa versus COS-1) may explain this disparity.
11. A pCGN expression construct of full-length human TFIIB ($pCGNhTFIIB_{WT}$) was modified by removal of the initiator methionine of TFIIB to minimize translation initiation after the HA-epitope tag (*13*). The mutant TFIIB molecules in which Arg^{169} and Lys^{188} were changed individually to glutamic acid (R169E and K188E) were derived by site-directed mutagenesis (*16*) of $pCGNhTFIIB_{WT}$.
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