16. C. H. Jones, H. Kanamori, S. W. Roecker, *ibid.*, in press.
17. P. Bird and R. W. Rosenstock, *Geol. Soc. Am. Bull.* **95**, 946 (1984).
18. L. N. Howard, in *Proceedings of the 11th International Congress of Applied Mechanics, Munich, Germany, 1964*, H. Gortler, Ed. (Springer-Verlag, Berlin, 1966), p. 1109.
19. J. Elder, *The Bowels of the Earth* (Oxford Univ. Press, London, 1976); the concept of a local Rayleigh number was developed for the analysis of transient convection from a thermal boundary layer. Later experimental and numerical analysis have confirmed the general validity of this type of analysis [C. R. Carrigan, *Science* **215**, 965 (1982); (*21*)].
20. D. L. Turcotte and G. Schubert, *Geodynamics* (Wiley, New York, 1982).
21. G. Houseman and D. McKenzie, *Geophys. J. R. Astron. Soc.* **68**, 133 (1982).
22. The thermal evolutions in both the oceanic problem and our problem are nearly identical after 5 million years, our temperature profile taking on that of a 30-million-years-old oceanic lithosphere. We expressed this equivalency between time in our problem, t, and time in the oceanic problem, t', with

$$t = t' - 25 \text{ million years} \quad (3)$$

where $t' > 30$ million years. The onset time for the oceanic problem of $t'_0 = 90$ million years, corresponding to a viscosity of $\mu^* = 2 \times 10^{20}$ Pa s (*21*), is equivalent to an onset time of $t_0^* = 65$ million years in our problem. This onset time is clearly too long to satisfy the geologic time constraint. The most uncertain and variable parameter is the viscosity, so we used Eqs. 1 and 2 to relate the viscosity to onset time

$$\mu \propto \Delta T(t_0)^{3/2} \quad (4)$$

and used this relation to find the relation between ratios of viscosities and onset times

$$\mu/\mu^* = (t_0/t_0^*)^{3/2} \quad (5)$$

Using Eqs. 2 and 5, we calculated the critical thickness, δ_c, and the corresponding μ (for both free and no-slip boundary conditions) for a range of $t_0 <$ 25 million years (since the opening of the slab window at the latitude of the anomaly) (Table 1).
23. The estimated temperature contrast represented by the seismic anomaly provides an important distinction between a sinking thermal blob and a sinking lithospheric plate. Many studies [(*15*); P. M. Davis, *Tectonophysics* **197**, 309 (1991)] have used values for the temperature dependence of compressional velocity, $\partial V_P/\partial T$, of -0.5 m s^{-1} °C^{-1} [O. L. Anderson, E. Schreiber, R. C. Liebermann, N. Soga, *Rev. Geophys.* **6**, 491 (1968)]; temperature derivatives for aggregates are based on various theoretical models combined with single mineral laboratory measurements]. Such values imply that a 1% velocity anomaly in the upper mantle corresponds to a 160°C temperature contrast. However, recent experimental data for peridotite gives a value of $\partial V_P/\partial T$ of -2.9 m s^{-1} °C^{-1} for temperatures from 1040° to 1300°C, corresponding to those in our problem [H. Sato, I. S. Sacks, T. Murase, *J. Geophys. Res.* **94**, 5689 (1989)]. For this temperature derivative, a 1% velocity anomaly corresponds to only a 28°C temperature contrast. If we consider the smoothing effect in the seismic tomography, the observed magnitude of the SGV anomaly of about 5% is a minimum estimate, and hence we estimate that the corresponding temperature variation is 150° to 200°C. This estimate is somewhat low but consistent with the upper bound of ΔT of the predicted temperature contrast of a sinking thermal blob (286°C in our model). It is also within the range of temperature contrast, about 200° to 300°C, of sinking thermal sheets in the numerical models of boundary layer instability (*21*). Values of $\partial V_P/\partial T$ intermediate between the end-members cited here can be accommodated in our hypothesis if the interior mantle temperature is increased and the transition temperature from viscous to rigid behavior is decreased; these effects increase ΔT by up to a factor of 2. However, if ΔT associated with the SGV anomaly is significantly greater than 500° to 600°C, it supports the idea that it represents delamination of a cold lower lithosphere as opposed to the instability of a thermal boundary layer.
24. L. M. Cathles III, *The Viscosity of the Earth's Mantle* (Princeton Univ. Press, Princeton, NJ, 1975).
25. Q. R. Passey, *J. Geophys. Res.* **86**, 11701 (1981).
26. F. Sigmundsson and P. Einarsson, *Geophys. Res. Lett.* **19**, 2123 (1992).
27. W. R. Peltier, in (*4*), pp. 389–478.
28. W. F. Haxby and J. K. Weissel, *J. Geophys. Res.* **91**, 3507 (1986).
29. W. R. Buck and E. M. Parmentier, *ibid.*, p. 1961.
30. J. Phipps Morgan and W. H. F. Smith, *Nature* **359**, 524 (1992).
31. L. Fleitout and D. A. Yuen, *J. Geophys. Res.* **89**, 9227 (1984).
32. D. A. Yuen and L. Fleitout, *Nature* **313**, 125 (1985).
33. C. Jaupart and B. Parsons, *Phys. Earth Planet. Inter.* **39**, 14 (1985).
34. G. T. Jarvis, *ibid.* **36**, 305 (1984); G. F. Davies, *Geology* **20**, 963 (1992).
35. M. S. Rentschler and R. B. Bloch, in *Studies of the Geology of the San Joaquin Basin*, S. A. Graham and H. C. Olsen, Eds. [Pacific Section of SEPM (Society for Sedimentary Geology), Tulsa, OK, 1988], pp. 29–52.
36. We thank A. Kersting, S. L. Beck, P. M. Davis, G. Schubert, and E. D. Humphreys for comments on an early draft; E. D. Humphreys, K. G. Dueker, C. H. Jones, H. Kanamori, and S. W. Roecker for sharing preprints; and J. Sweeney and J. Phipps Morgan for referring us to important references. We also thank the reviewers and editor for help in focusing our arguments. This research was supported by laboratory-directed research and development funds to the Institute of Geophysics and Planetary Physics at Lawrence Livermore National Laboratory (LLNL) and the Geoscience Research Program in the Department of Energy (DOE) Office of Basic Energy Sciences. This work was done under the auspices of the U.S. DOE by the LLNL under contract W-7405-ENG-48.

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Transcription Factor TFIIB Sites Important for Interaction with Promoter-Bound TFIID

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Transcription initiation factor TFIIB recruits RNA polymerase II to the promoter subsequent to interaction with a preformed TFIID-promoter complex. The domains of TFIIB required for binding to the TFIID-promoter complex and for transcription initiation have been determined. The carboxyl-terminal two-thirds of TFIIB, which contains two direct repeats and two basic residue repeats, is sufficient for interaction with the TFIID-promoter complex. An extra 84-residue amino-terminal region, with no obvious known structural motifs, is required for basal transcription activity. Basic residues within the second basic repeat of TFIIB are necessary for stable interaction with the TFIID-promoter complex, whereas the basic character of the first basic repeat is not. Functional roles of other potential structural motifs are discussed in light of the present study.

Transcription of eukaryotic protein-encoding genes requires at least five general transcription initiation factors (TFIIB, TFIID, TFIIE, TFIIF, and TFIIH) in addition to RNA polymerase II (*1*). The first step in preinitiation complex formation involves TFIID binding to the TATA box in the promoter region, a process that may be facilitated by TFIIA. Subsequently, TFIIB binds to the TFIID-promoter complex and acts as a bridging factor to incorporate RNA polymerase II into the complex and to specify the transcription initiation site (*2*). It has been proposed that TFIIB (*3*), as well as TFIID (*1*), are targets for the acidic activation domain on the transcriptional activator VP16. Therefore, exploring the mechanism by which TFIIB interacts with the TFIID-promoter complex is necessary for understanding transcription initiation and regulation.

The isolation and characterization of cDNAs encoding TFIIB from human (*4*), *Xenopus* (*5*), yeast (*2*), and *Drosophila* (*6, 7*) showed that TFIIB contains a Zn(II)-finger, imperfect direct repeats, bacterial σ-factor sequence similarities, and basic repeats. To understand the functional significance of these structural motifs, we constructed a series of TFIIB mutants and tested each mutant for basal transcription activity and the ability to interact with the TFIID-promoter complex.

A series of NH_2-terminal deletions were constructed (Fig. 1A) (*8*) and the ability of the mutant proteins (Fig. 1D) (*9*) to support basal transcription was tested in a TFIIB-dependent reconstituted transcription sys-

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tem (Fig. 1B) (*10*). TFIIB lacking residues 2 to 8 from the NH_2-terminus (Δ2-8) had full activity, whereas TFIIB lacking NH_2-terminal residues 2 to 21 (Δ2-21) retained about one-half of the activity of intact TFIIB. Because the potential Zn(II)-finger domain is found between residues 14 and 36, these data suggest that this domain is not essential for basal transcription. Deletions extending through or beyond residue 25 eliminated basal transcription, indicating that residues 22 to 25 are critical for basal transcription. The same series of TFIIB mutants were tested for interactions with the TATA box binding subunit of TFIID (TFIIDτ-TBP) in a promoter complex by gel retardation analysis (Fig. 1C) (*11*). All mutants with deletions extending to residue 105 (including Δ2-105) still formed a TFIIB-TFIIDτ-promoter complex, whereas a further deletion extending into the first basic repeat (Δ2-126) eliminated complex formation (Fig. 1C). Thus, the NH_2-terminal 105 amino acids that just precede the first basic repeat are not essential for interactions with the TFIIDτ-promoter complex, although the NH_2-terminal region is required for subsequent events leading to transcription initiation.

To determine the requirement for COOH-terminal residues for both activities, we constructed a series of COOH-terminal deletions (Fig. 2A). The deletion of 11 COOH-terminal residues (Δ305-315) had no effect on basal transcription, whereas elimination of 21 residues (Δ295-315) decreased activity to about 20% of the original value (Fig. 2B). Deletions extending into the direct repeat (Δ285-315, Δ275-315, and Δ265-315) completely eliminated transcription activity. The 11-residue deletion mutant (Δ305-315) stabilizes TFIIB-TFIIDτ-promoter complex formation, indicating that the COOH-terminal region (305-315) inhibited interaction with the TFIIDτ-promoter complex (Fig. 2C). Construct Δ295-315, which still retains significant transcription activity, yielded almost no complex (Fig. 2C). In several mutants, including Δ295-315, the ability of TFIIB to interact stably with TFIIDτ-promoter complex did not quantitatively reflect transcription activity, although both activities were qualitatively correlated. This may reflect the formation of a complex that is too unstable to be detected by gel retardation analysis but still functional as a result of stabilizing interactions with other factors that enter the preinitiation complex subsequent to TFIIB (*12*). Further deletions extending into the direct repeats (Δ285-315, Δ275-315, and Δ265-315) resulted in the loss of both activities. Together with the results from the NH_2-terminal deletion experiments, our data suggest that 198 amino acids (from positions 106 to 304) of TFIIB are sufficient to produce an efficient interaction with the TFIIDτ-promoter complex, whereas an additional 84 NH_2-terminal residues, which do not contain any recognizable motifs, are required for transcription.

The NH_2-terminal deletion extending

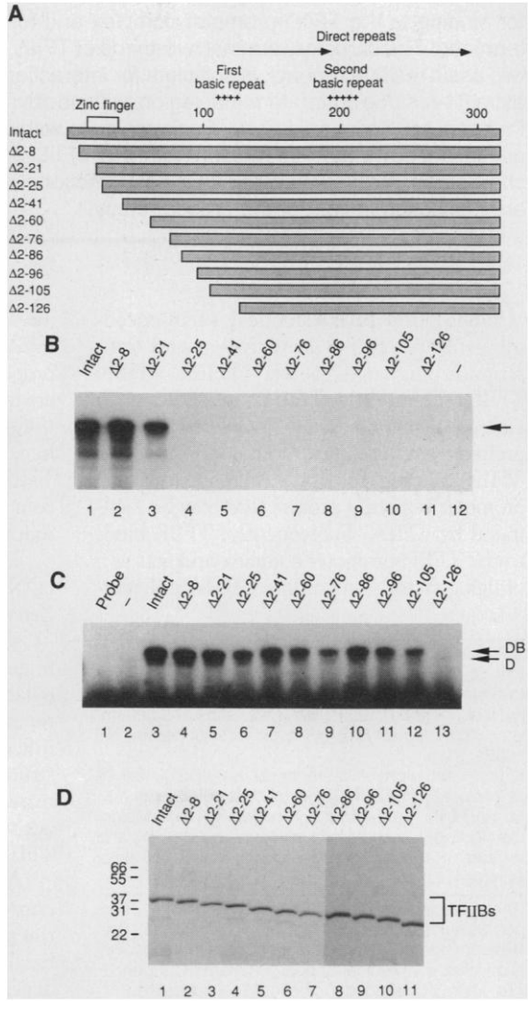

Fig. 1. Analysis of the NH_2-terminal region of TFIIB. (**A**) Construction of NH_2-terminal deletions. The Zn(II)-finger, first and second basic repeats, and imperfect direct repeats are indicated for the *Drosophila* TFIIB sequence. Numbers indicate the amino acid position from the NH_2-terminus. Bars represent the portion of TFIIB retained in the deletion mutants. Numbers on the left indicate the residues deleted. (**B**) Analysis of basal transcription activity. Transcription activity was determined in a TFIIB-dependent reconstituted transcription system with intact TFIIB (lane 1), mutant TFIIB (lanes 2 to 11), or no TFIIB (lane 12). A plasmid containing the adenovirus major late core promoter attached to the 380-bp G-less cassette was used as a template. The position of the accurately initiated transcripts is indicated by an arrow. (**C**) Analysis of TFIIB-TFIIDτ-promoter interactions by gel retardation assay. All lanes except lane 1 contained TFIIDτ. Reaction mixtures contained TFIIDτ (lanes 2 to 13) and intact (lane 3) or mutant (lanes 4 to 13) TFIIB. The positions of the TFIIDτ-TFIIB-DNA complex (DB) and TFIIDτ-DNA complex (D) are indicated. Note that TFIIDτ-DNA complex can be observed faintly in the buffer system used. (**D**) SDS–polyacrylamide gel electrophoresis (PAGE) analysis of mutant TFIIB proteins. Mutant proteins in this figure were analyzed by SDS-PAGE and stained with Coomassie brilliant blue R250. The positions of the TFIIB species are shown.

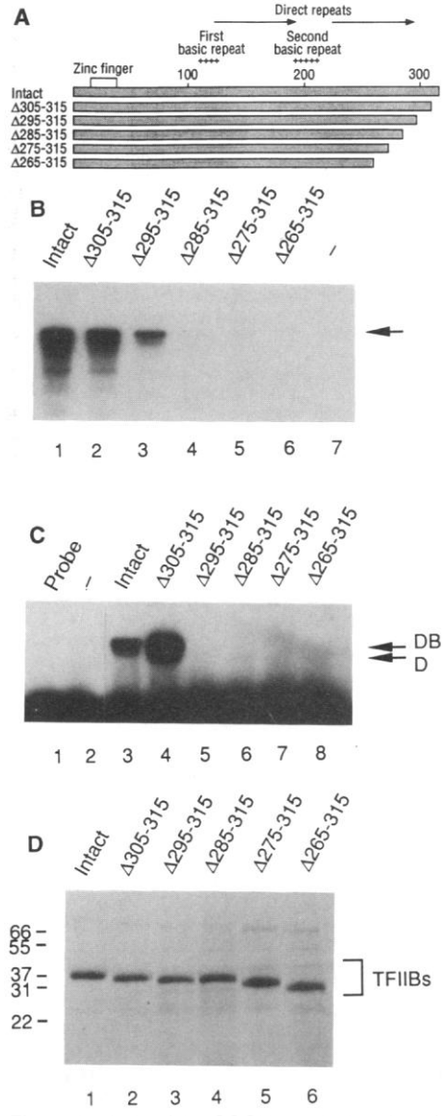

Fig. 2. Analysis of the COOH-terminal region of TFIIB. Structures of COOH-terminal deletion mutants (**A**), basal transcription activity (**B**), TFIIB-TFIIDτ-promoter interaction analysis by gel retardation experiments (**C**), and SDS-PAGE analysis (**D**) of mutant TFIIB proteins are represented as in Fig. 1.

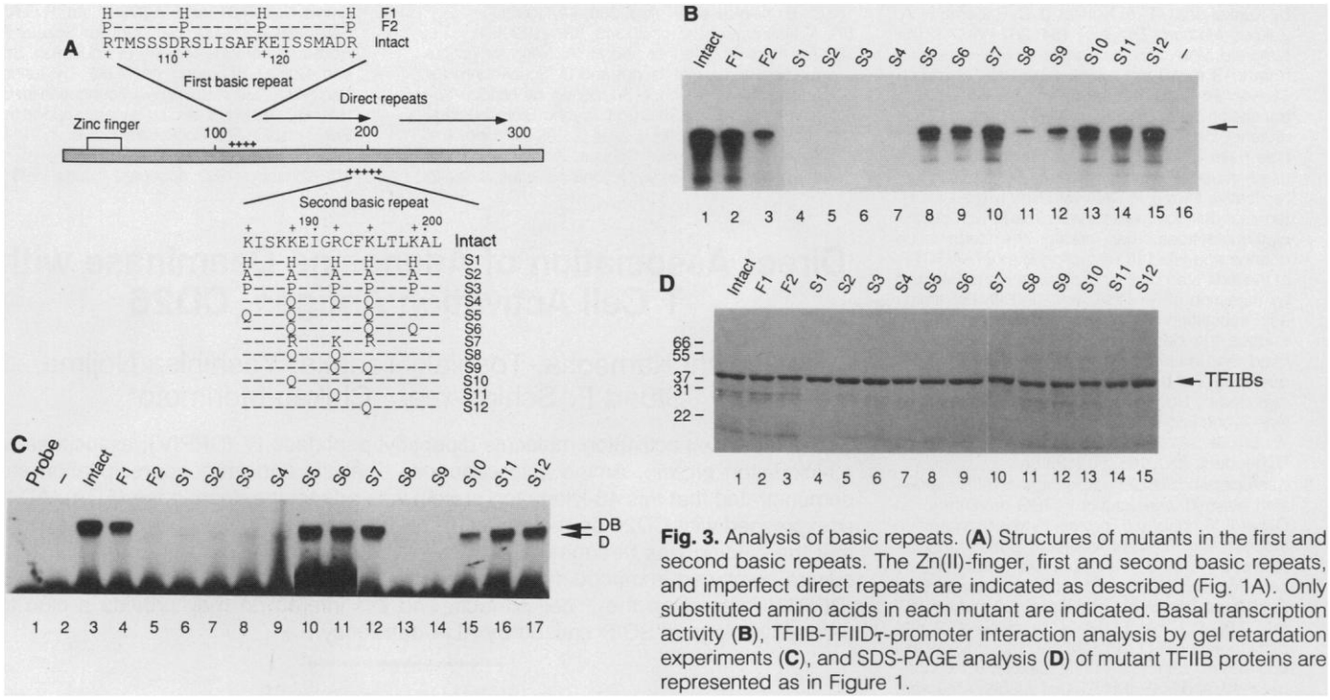

Fig. 3. Analysis of basic repeats. (**A**) Structures of mutants in the first and second basic repeats. The Zn(II)-finger, first and second basic repeats, and imperfect direct repeats are indicated as described (Fig. 1A). Only substituted amino acids in each mutant are indicated. Basal transcription activity (**B**), TFIIB-TFIIDτ-promoter interaction analysis by gel retardation experiments (**C**), and SDS-PAGE analysis (**D**) of mutant TFIIB proteins are represented as in Figure 1.

just through the first basic repeat region (Δ2-126) abolished formation of a TFIIB-TFIIDτ-promoter complex (Fig. 1C). Amino acid substitution mutants were constructed for a more detailed analysis of this region (Fig. 3A). The four basic amino acids in the first basic repeat region were replaced completely with either His (F-1) or Pro (F-2). No significant effect on either transcription (Fig. 3B) or complex formation (Fig. 3C) was observed for F-1, suggesting that a strong basic character such as Arg and Lys in the first basic region is not necessary for TFIIB activities. The F-2 mutant decreased transcription activity (Fig. 3B) and almost completely abolished complex formation (Fig. 3C). With the NH_2-terminal deletion experiments, these data suggest that a structural feature of the first basic repeat region, but not its basic character, contributes to a stable interaction with the TFIIDτ-promoter complex as monitored by gel retardation assay.

To test the requirement for the second basic repeat, we replaced all five basic amino acids located on the same side of the putative α helix (*4*) with either His (S-1), Ala (S-2), or Pro (S-3). Each replacement substantially reduced both basal transcription and TFIIB-TFIIDτ-promoter complex formation (Fig. 3, B and C). These results suggest that the basic side chains in the second basic repeat are essential for these functions.

To determine which basic amino acid residues in the second basic repeat are necessary for TFIIB activities, we constructed various triple mutants (Fig. 3A, S4-S6). The basic amino acids were replaced with Gln, because its hydrophobicity and accessible surface area are relatively close to those for Arg and Lys. Significant inhibition of both basal transcription and TFIIB-TFIIDτ-promoter complex formation was observed in mutant S-4, whereas there was only a minor diminution of these activities with mutants S-5 and S-6. This indicates that the particular positions of the basic amino acids, rather than the total number, are important for both activities (Fig. 3, B and C). To test the contribution of each of the altered basic amino acids in mutant S4 to the two TFIIB activities, we prepared single and double mutants (Fig. 3A, S8-S12). The double mutants, S-8 and S-9, had less inhibitory effect on the two activities as compared with the triple mutant S4 (Fig. 3, B and C). Moreover, the single mutants S9, S10, and S11 had almost no effect on either activity. These data suggest that of the three basic amino acids changed in mutant S4, any two basic residues are sufficient for both TFIIB activities.

To test whether the TFIIB activities require a particular basic residue or whether any positively charged residue would suffice, we constructed mutant S-7 (Fig. 3A). Because there was no significant decrease of activities with S-7 (Fig. 3, B and C), we concluded that positive charges at positions 188, 192, and 195, rather than particular basic residues, are important for both basal transcription and for TFIIB-TFIIDτ-promoter complex formation activities.

TFIIB interacts with the downstream region of the transcription initiation site in a sequence-independent manner (*13*, *14*), and this interaction is essential for formation of the TFIIB-TFIIDτ-promoter complex (*14*). Pinto *et al.* (*2*) suggested that this TFIIB-DNA interaction may be mediated by a potential Zn(II)-finger domain (Cys-X^2-His-X^{15}-Cys-X^2-Cys; between residues 14 and 36). However, our results showed that removal of the entire potential Zn(II)-finger domain (Δ2-41, Δ2-60, Δ2-76, Δ2-86, Δ2-96, and Δ2-105 in Fig. 1) has no effect on complex formation. It is therefore unlikely that the Zn(II)-finger domain is important for TFIIB-TFIIDτ-promoter complex formation. As a possible function of this domain, its interaction with a transcriptional activator *fushi-tarazu* has recently been proposed (*15*).

X-ray crystallography of $TFIID_\tau$ (*16*) showed that the COOH-terminal core domain, which includes both the direct repeats and the basic repeat (*17*), forms a highly symmetric structure that contains a DNA binding structure, resembling a molecular saddle that sits astride the DNA. Because both TFIIDτ and TFIIB have similar structural motifs and function as monomers, TFIIB might have a similar structure to TFIIDτ.

REFERENCES AND NOTES

1. R. G. Roeder, *Trends Biochem. Sci.* **16**, 402 (1991).
2. I. Pinto, D. E. Ware, M. Hampsey, *Cell* **68**, 977 (1992).
3. Y. S. Lin and M. R. Green, *ibid.*, **64**, 971 (1991).
4. S. Malik, K. Hisatake, H. Sumimoto, M. Horikoshi, R. G. Roeder, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 9553 (1991); I. Ha, W. S. Lane, D. Reinberg, *Nature* **352**, 689 (1991).
5. K. Hisatake, S. Malik, R. G. Roeder, M. Horikoshi, *Nucleic Acids Res.* **19**, 6636 (1991).
6. S. Yamashita *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 2839 (1992).
7. S. L. Wampler and J. T. Kadonaga, *Genes Dev.* **6**, 1542 (1992).
8. All TFIIB mutants were generated by deoxyoligonucleotide-directed mutagenesis, as described

by Kunkel *et al.* [T. A. Kunkel, J. D. Roberts, R. A. Zakour, *Methods Enzymol.* **154**, 367 (1987)]. The template DNA for mutagenesis was prepared from M13 mp19 containing the cDNA encoding *Drosophila* TFIIB, harboring an Nde I site at the translation initiation site. For NH_2-terminal deletion mutants, deoxyoligonucleotides that introduce an Nde I site (CATATG) at the deletion points were used; mutants were confirmed by digestion of replicative form (RF) DNA with Nde I. The COOH-terminal deletion mutants were made with deoxyoligonucleotides that create the termination codons and Hind III restriction sites (TAAGCTT) at the indicated positions; mutants were detected by digestion of RF DNA with Hind III. For amino acid substitutions, deoxyoligonucleotides that introduce the desired amino acid changes were used, and mutants were confirmed by DNA sequencing. For bacterial expression, RF DNA was digested by Nde I and Eco RI and subcloned into Nde I– and Eco RI–digested 6His-pET-5a [(*16*); F. W. Studier, A. H. Rosenberg, J. J. Dunn, J. W. Dubendorff, *ibid.* **185**, 60 (1990)].

9. *Escherichia coli* BL21 harboring the TFIIB expression plasmid were grown in TBG medium to an OD at 600 nm of 0.8; protein synthesis was then induced with 0.5 mM isopropyl β-D-thiogalactoside at 37°C for 3 hours. Cells were collected by centrifugation (6000*g*, 10 min) and resuspended in 1/10 culture volume of buffer B [20 mM tris-HCl (pH 7.9), 0.2 mM EDTA, 10% glycerol, 2 mM dithiothreitol (DTT), 0.1 mM phenylmethylsulfonyl fluoride] containing 1 mM imidazole, 0.1% Nonidet P-40, and 500 mM KCl. Cell suspensions were incubated on ice for 15 min and then disrupted by sonication. After centrifugation (8000*g*, 20 min), supernatant was applied onto a Ni-agarose column (Qiagen, Studio City, CA), equilibrated with buffer B containing 10 mM imidazole and 100 mM KCl. After washing with the same buffer, protein was eluted with buffer B containing 100 mM imidazole and 100 mM KCl. The COOH-terminal deletion and the second basic repeat mutants, which are mostly insoluble under these conditions, were prepared as follows: The cell pellet was suspended in 1/10 culture volume of buffer B containing 6 M guanidine HCl and 500 mM KCl, and disrupted by a short pulse of sonication. After centrifugation (8000*g*, 20 min), supernatant was applied onto a Ni-agarose column. After washing with buffer B containing 6 M guanidinium-HCl, 500 mM KCl, and 10 mM imidazole, protein was eluted with the same buffer containing 100 mM imidazole. The eluate was then dialyzed against buffer B containing 2 M guanidine-HCl and 500 mM KCl for 2 hours at 4°C and then dialyzed against buffer B containing 1 M guanidine HCl and 100 mM KCl overnight at 4°C.
10. In vitro transcription was carried out as described (*6*), except that the native human TFIID fraction was used. The template plasmids used in each experiment are described in the appropriate figure legends.
11. ^{32}P–end-labeled double-stranded DNA with the adenovirus major late promoter sequence (−40 to +10 of the transcription initiation site) was used as a probe. Each complete system contained 5 × 10^5 dpm of probe (about 50 fmol), poly(dGdC) (10 μg/ml) (Pharmacia), 35 mM Hepes-KOH buffer (pH 8.0), 7.5 mM $MgCl_2$, 6% (v/v) glycerol, 60 mM KCl, 6 mM DTT, 60 μM EDTA, *Drosophila* TFIIDτ (0.5 μg/ml), and TFIIB (1.2 μg/ml) in a total volume of 25 μl. After incubation at 30°C for 40 min, products were analyzed on a 4% polyacrylamide gel (59:1) containing TBE buffer [89 mM tris, 89 mM boric acid, and 2 mM EDTA (pH 8.0)] and 3% (v/v) glycerol at 100 V with the use of TBE buffer as a running buffer.
12. T. Yamamoto *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 2844 (1992).
13. S. Buratowski, S. Hahn, L. Guarante, P. A. Sharp, *Cell* **56**, 549 (1989).
14. E. Maldonado *et al.*, *Mol. Cell. Biol.* **10**, 6335 (1990).
15. J. Colgan, S. Wampler, J. L. Manley, *Nature* **362**, 549 (1993).
16. D. B. Nikolov *et al.*, *ibid.* **360**, 40 (1992).
17. A. Hoffmann *et al.*, *ibid.* **346**, 387 (1990).

18. We thank M. Brenner and H. A. Nash for critical reading of the manuscript and D. Schoenberg for editing the manuscript. Supported by Nippon Suisan Kaisha Ltd. (S.Y.) and Toyobo Biotechnology Foundation (K.H.). M.H. was an Alexandrine and Alexander Sinsheimer Scholar. A portion of this study was supported by National Institutes of Health grants CA42567 and AI27397 (to R.G.R.) and GM45258 (to M.H.), by Grant for Special Project Research from the Ministry of Education, Science, and Culture of Japan (to M.H.), by funds from Sankyo Co. Ltd. and Sagawa Foundation for Cancer Research (to M.H.), and by general support from the Pew Trusts to The Rockefeller University.

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Direct Association of Adenosine Deaminase with a T Cell Activation Antigen, CD26

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CD26, the T cell activation molecule dipeptidyl peptidase IV (DPPIV), associates with a 43-kilodalton protein. Amino acid sequence analysis and immunoprecipitation studies demonstrated that this 43-kilodalton protein was adenosine deaminase (ADA). ADA was coexpressed with CD26 on the Jurkat T cell lines, and an in vitro binding assay showed that the binding was through the extracellular domain of CD26. ADA deficiency causes severe combined immunodeficiency disease (SCID) in humans. Thus, ADA and CD26 (DPPIV) interact on the T cell surface, and this interaction may provide a clue to the pathophysiology of SCID caused by ADA deficiency.

CD26, a T cell activation molecule (*1*, *2*), is a 110-kD glycoprotein that is also present on epithelial cells of various tissues, including the liver, kidney, and intestine. CD26 is identical with dipeptidyl peptidase IV (DPPIV) (*3*), which can cleave NH_2-terminal dipeptides from polypeptides with either L-proline or L-alanine at the penultimate position. No physiological substrates have yet been identified. We isolated cDNA encoding human CD26 and established CD26-transfected Jurkat T cell lines (*4*). Functional analysis of these Jurkat transfectants showed that cross-linking of the CD26 and CD3 antigens with their respective antibodies (Abs) resulted in enhanced intracellular calcium mobilization and interleukin-2 production, providing direct evidence that the CD26 antigen plays an integral role in T cell activation. The cDNA sequence of CD26 predicted a type II membrane protein with only six amino acids in the cytoplasmic region (*4*, *5*), suggesting that other association molecules are involved in CD26-mediated signal transduction. CD26 associates with CD45 (*6*), which might be involved in regulating the $p56^{lck}$ activity through its protein phosphatase activity. Another candidate for the signal transduction molecule was a 43-kD protein, p43, which can be coprecipitated by antibody to CD26 (anti-CD26) from ^{125}I-labeled T cells, phytohemagglutinin (PHA) blast cells, and from CD26-transfected Jurkat cell lines (*4*).

To identify p43, we purified the protein by immunoaffinity chromatography from CD26-transfected Jurkat cells. The purified protein was separated by SDS–polyacrylamide gel electrophoresis (PAGE), transferred to a nitrocellulose membrane, and stained by Ponceau S, resulting in a single band of 43 kD in addition to the CD26 110-kD protein (Fig. 1). The 43-kD protein was then digested with trypsin, separated by reversed-phase high-pressure liquid chromatography (rpHPLC), and subjected to amino acid sequencing. According to the homology search, the amino acid sequences of the two peptides derived from p43 were completely identical to those of residues 35 to 64 and 172 to 206 of the human adenosine deaminase (ADA) (*7*).

ADA is a 41-kD protein, expressed in all tissues (highest expression in lymphocytes), that catalyzes the conversion of adenosine and deoxyadenosine to inosine and deoxyinosine, respectively. ADA is present on the cell surface, as well as in the cytoplasm, of human fibroblasts, rabbit renal tubular cells, and human mononuclear blood cells (*8*). ADA deficiency causes severe combined immunodeficiency disease (SCID) in humans (*9*), yet no direct interaction between ADA and T cell surface molecules has been identified.

The possibility that CD26 is associated with ADA was investigated by biochemical analysis with polyclonal rabbit Ab to ADA (anti-ADA) (*10*). Immunoblotting with anti-ADA after immunoprecipitation from CD26 transfectants by various Abs demon-

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