cene time is regionally heterogeneous and is partitioned into six domains that have independent histories and styles of late Cenozoic deformation (*4*). These domains are separated by zones of extension and strike slip. Tectonic rotation and internal deformation of the MDB domains are regarded as produced within a belt of regionally distributed right shear that Dokka and Travis termed the Eastern California shear zone. At least 65 km of right slip is believed to have occurred across this zone; this accounts for 9 to 14% of the motion between the North American and Pacific plates since 10.6 Ma (*4*). Because of kinematic and temporal similarities, this shear zone is thought also to include the southern Death Valley and the adjacent Furnace Creek fault zones (Fig. 1).

This heterogeneous strain model is based on field mapping, which shows that most northwest-striking faults in the MDB lack continuity across the block; that with only one exception the faults fall into two distinct groups separated across an east-oriented strip of terrain that passes through Barstow; and that there is a major discrepancy in the slip values on individual faults to the south and their projected counterparts to the north. This model differs substantially from regionally distributed simple shear models (*9*) that require continuity of the faults, and uniform slip across the entire MDB.

The newly observed faults provide supporting evidence for the heterogeneous strain model. The Broadwell Lake fault is limited in extent, in common with other right-slip faults in the area to the northeast (Bristol Mountains and Granite Mountains faults) and west (Ludlow fault). The fault is situated in an area of dominant right shear that reportedly shows no evidence of rotation (*20*). A net slip of 0.5 km is predicted for this fault from the combined right-slip offset that is believed to have been accommodated since 10 Ma in the central and eastern Mojave Desert. The model also predicts about 35 km of late Cenozoic right shear along the nearby Bristol Mountains and Granite Mountains faults.

The Cady Mountains and the SBF are situated in an area of dominant left slip. The SBF marks the southern limit of this area, as predicted by the heterogeneous strain model. Paleomagnetic declination studies from the Cady Mountains indicate contrasting rotations of crustal blocks in this region. Whereas most blocks were rotated clockwise an average of ~50° prior to about 18 Ma, the southwest Cady Mountains appear to have rotated about 124° clockwise (7). Deformation during rotation was accomplished in part by left-slip motion on a number of internal faults that include the north strand of the Cady fault.

In summary, the enhanced TM images have allowed the recognition of important faults overlooked by field geologists, the field investigation of faults so recognized, the regional analysis of their significance, and the associated implications for models of tectonic evolution in the MDB. These results show effective use of multispectral image analysis for tectonic studies in complexly deformed terrain at local and regional map scales. They also show that previous mapping of faults in the study area was inadequately representative. Continued field mapping guided by remote sensing image analyses over wider areas of the Mojave Desert can improve understanding of deformation in this region.

REFERENCES AND NOTES

1. R. K. Dokka, *Geology* **11**, 305 (1983); ———, M. McCurry *et al.*, in *This Extended Land, Geological Journeys in the Southern Basin and Range*, D. L. Weide and M. L. Faber, Eds., *Geol. Soc. Am. Cordilleran Sect. Field Trip Guidebook* (Department of Geoscience, University of Nevada, Las Vegas, NV, 1988), pp. 21–44.
2. R. K. Dokka, *Geol. Soc. Am. Spec. Pap.* **208**, 75 (1986); ——— and M. O. Woodburne, *Louisiana State Univ. Publ. Geol. Geophys. Tecton. Sediment. 1* (1986); A. F. Glazner, J. M. Bartley, J. D. Walker, *Geology* **17**, 50 (1989).
3. R. K. Dokka, *Tectonics* **8**, 363 (1989).
4. ——— and C. J. Travis, *ibid.*, **9**, 311 (1990).
5. M. Golombek and L. Brown, *Geology* **16**, 126 (1988).
6. B. J. McFadden, M. O. Woodburne, N. D. Opdyke, *J. Geophys. Res.* **95**, 4597 (1990).
7. T. M. Ross, B. P. Luyendyk, R. B. Haston, *Geology* **17**, 470 (1989); T. M. Ross and R. K. Dokka, *Geol. Soc. Am. Abstr. Progr.* **22** (no. 3), 79 (1990).
8. T. W. Dibblee, Jr., *U. S. Geol. Surv. Prof. Pap. 424-B* (1961), p. B107.
9. Z. Garfunkel, *Geol. Soc. Am. Bull.* **85**, 1931 (1974); J. N. Carter *et al.*, *ibid.* **98**, 199 (1987).
10. S. G. Wesnousky, *J. Geophys. Res.* **91**, 12587 (1986).
11. C. W. Jennings (compiler), *Geologic Data Map No. 2.* (Calif. Div. Mines Geol., Sacramento, CA 1977).
12. D. F. Hewett, *Calif. Div. Mines Geol. Bull.* **170** (part 2), 15 (1954).
13. A. M. Bassett and D. H. Kupfer, *Calif. Div. Mines Geol. Spec. Rep.* **83** (1964); plate 1 of Bassett and Kupfer was previously published as *U.S. Geol. Surv. Misc. Inv. Field Studies Map MF-205* (1962). The geology was mapped in 1953.
14. T. W. Dibblee, Jr., *U.S. Geol. Surv. Misc. Geol. Inv. Map I-478* (1967); ——— and A. M. Bassett, *ibid. I-467* (1966); larger scale maps of parts of the area appear in an unpublished survey by the Southern Pacific Minerals Company (now Santa Fe Mining, Inc).
15. T. W. Dibblee, Jr., and A. M. Bassett, *U.S. Geol. Surv. Misc. Geol. Inv. Map I-461* (1966); T. W. Dibblee, Jr., *ibid., I-430* (1964); *ibid., I-472* (1966); *ibid. I-477* (1967).
16. D. Miller, K. Howard, B. John, in *Geologic Excursions in the California Desert*, J. D. Cooper, Ed., *Geol. Soc. Am. Cordilleran Sect. Meeting Guidebook* (Pacific Section, Society of Economic Paleontologists and Mineralologists, Bakersfield, CA, 1982), pp. 91–100; M. Woodburne and R. Tedford, *ibid.*, pp. 65–76; A. F. Glazner, *Geol. Soc. Am. Bull.* **100**, 424 (1988); D. Miller, *Geol. Soc. Am. Abstr. Progr.* **20** (no. 3), 215 (1988).
17. R. E. Crippen, Ph.D. thesis, University of California, Santa Barbara (1989).
18. M. H. Podwysocki, M. S. Power, O. D. Jones, *Adv. Space Res.* **5** (no. 5), 13 (1985).
19. D. M. Morton, F. K. Miller, C. C. Smith, *U.S. Geol. Surv. Misc. Field Studies Map MF-1051* (1980); J. Sauber, W. Thatcher, S. Solomon, *J. Geophys. Res.* **91**, 12683 (1986); S. Goter (compiler), *Seismicity of California, 1808–1987, U.S. Geol. Surv. Open-File Rep. 88-286* (1988).
20. R. E. Wells and J. W. Hillhouse, *Geol. Soc. Am. Bull.* **101**, 846 (1989).

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Reversal of Creatine Kinase Translational Repression by 3′ Untranslated Sequences

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A subline of U937 cells (U937D) was obtained in which creatine kinase B (CK-B) messenger RNA was present and bound to ribosomes, but CK activity was undetectable. Transformation of U937D cells with retrovirus vectors that contain the 3′ untranslated region (3′ UTR) of CK-B messenger RNA exhibited CK activity with no change in abundance of CK-B mRNA. The 3′ UTR formed a complex in vitro with a component of S100 extracts from wild-type cells. This binding activity was not detectable in S100 extracts from cells that expressed CK activity after transformation with the 3′ UTR–containing vector. These results suggest that translation of CK-B is repressed by binding of a soluble factor or factors to the 3′ UTR.

A COMMON FEATURE OF EUKARYOTIC mRNAs is a 3′ UTR of variable length. In some genes, the primary sequence of the 3′ UTR is conserved across species (*1*), implying that a function exists for these sequences. Stability of mRNA can be regulated by interaction of the 3′ UTR with soluble cellular proteins (*2*). The 3′

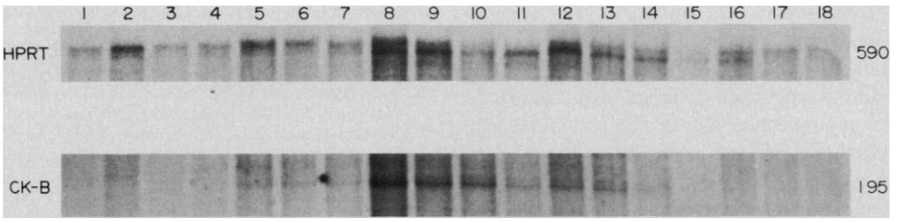

Fig. 1. Polysome profile of CK-B and HPRT mRNA in U937D cells. After centrifugation of cell extracts (10^8 cells; 38,000*g*, 45 min), supernatants of 10^8 cells were applied to a 10-ml 10 to 40% sucrose gradient (*7*). After fractionation, RNA was ethanol precipitated, phenol/chloroform extracted twice, and ethanol precipitated a second time (*13*). RNA from each fraction was assayed by RNase protection analysis (*14*) with ^{32}P-labeled antisense RNA probes complementary to CK-B (*4*) and HPRT mRNAs. The 960-nt CK-B probe was synthesized from a DNA template that contains part of exon 4, intron 4, exon 5, and 3′ flanking DNA (*7*). Two regions of this probe are protected by CK-B mRNA, a 195-nt fragment corresponding to exon 4, and a 405-nt fragment corresponding to exon 5 (*7*). The region of the autoradiogram shown illustrates the 195-nt CK-B fragment. The HPRT probe is complementary to the 5′ terminus of HPRT mRNA, and the 590-nt fragment of the probe protected by HPRT mRNA is illustrated. The bands for the respective probes are from the same autoradiogram, but the length of exposure is different for the two probes because HPRT is more abundant than CK-B in the U937D cell line. Fraction 1 is the top of the gradient.

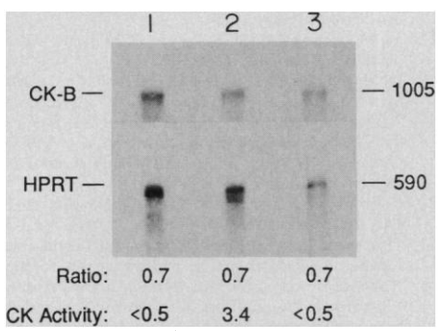

Fig. 2. CK-B mRNA abundance and enzyme activity in wild-type and transformed U937D cells. Total RNA was isolated (*15*), and 50 μg of RNA was used for each RNase protection analysis (*14*). CK-B mRNA was quantitated with a probe synthesized from a human CK-B cDNA (*16*). This probe is complementary to the 3′ terminal 1005 nt of CK-B mRNA. It was selected for these studies because of its ability to distinguish between the endogenous CK-B mRNA and the retroviral transcript. Endogenous CK-B mRNA gives a 1005-nt protected fragment with this probe, while the retroviral transcript yields protected fragments of 405 nt (exon 5) and 195 nt (exon 4). The HPRT probe, which serves as an internal control, gives a protected fragment of 590 nt. Lane 1, wild-type cells; lane 2, cells transformed with the retrovirus vector containing the 960-bp CK-B gene fragment (*8*); lane 3, cells transformed with the retrovirus vector alone. Transcript abundance was estimated by densitometry, and the ratio of CK-B signal to HPRT signal is tabulated at the bottom of the figure. CK enzyme activity in extracts from the respective cell lines was determined by a sensitive radiochemical assay (*17*), and the activity is reported in milliunits per milligram of protein. A milliunit is the amount of enzyme required to convert 1 nmol of creatine to creatine phosphate in 1 min.

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UTR may also participate in translational control (*3*), but little is known about the molecular mechanisms underlying such regulation. Expression of CK-B, a pivotal enzyme in cellular energy metabolism, is regulated by stage-specific, tissue-specific, and hormonal signals (*4*). Because the 3′ UTR of this gene is conserved (*5*), we sought to determine if the 3′ UTR is involved in posttranscriptional control of CK-B expression.

Posttranscriptional control of CK-B expression was initially suggested by the serendipitous finding of a subline of U937 cells (*6*) (U937D), which had undetectable CK activity (<0.5 mU/mg) yet had CK-B mRNA (Figs. 1, 2, and 3). The 1.6-kb CK-B mRNA in these cells was associated with polysomes, as determined by its sedimentation in sucrose gradients (Fig. 1). The peak of CK-B mRNA occurred at the same position in the gradient as observed previously with another U937 cell line in which CK-B mRNA is translated (*7*). These results suggest that expression of CK-B mRNA is blocked at a step subsequent to ribosome binding in the U937D subline.

To determine if a fragment of the CK-B gene that contains the 3′ UTR functions in translational control of CK-B mRNA, we transformed U937D cells with a retrovirus vector that expressed the 3′ UTR (*7, 8*). In three independently isolated populations of transformed cells, previously undetectable CK activity now ranged from 3.4 to 7.5 mU/mg. CK activity remained undetectable in cells transformed with the vector alone (Fig. 2). Two other enzyme activities, adenosine monophosphate (AMP) deaminase and hypoxanthine phosphoribosyltransferase (HPRT), remained unchanged in cells transformed with the vector containing CK-B sequences, suggesting that the effect was

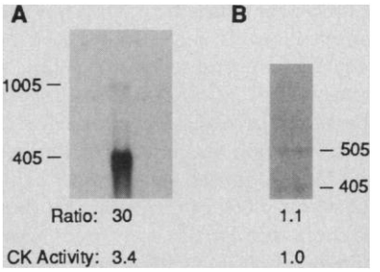

Fig. 3. Relative abundance of endogenous CK-B mRNA and retroviral transcripts. RNA was isolated and quantitated as described in the legend to Fig. 2. (**A**) RNA from cells transformed by the retroviral vector containing the 960-bp CK-B fragment. The probe used for these analyses is described in Fig. 2. The 1005-nt protected band is derived from the endogenous mRNA; the 405-nt protected band is derived from exon 5 of the retroviral transcript. See legend to Fig. 2 for the sizes of the protected bands expected for the respective transcripts. (**B**) RNA from cells transformed by the retroviral vector with the 505-bp CK-B fragment containing the 3′ UTR. The probe used to analyze RNA from these cells is described in Fig. 1. The retroviral transcript gives a 505-nt protected band. Endogenous CK-B mRNA gives two bands; the 405-nt band (exon 5) is shown. See legend to Fig. 1 for an explanation of the protected fragments expected for CK-B mRNA. The ratio of the retroviral transcript to endogenous CK-B mRNA, based on densitometric scans of the autoradiograms, is tabulated at the bottom of the figure. CK enzyme activity in extracts from the respective cell lines was determined with a radiochemical assay (*17*), and the activity is reported in milliunits per milligram of protein.

specific for CK activity. The abundance of CK-B mRNA was not increased in the transformed cells, which had an increase in CK activity (Fig. 2). In these transformants, the retroviral transcript was nearly 30 times as abundant as cellular CK-B mRNA (Fig. 3A).

To localize the region of the 960-nucleotide (nt) CK-B fragment that was responsible for increased CK activity, we subdivided this fragment into two segments. By cleaving at the Nci I restriction site 4 nt downstream of the stop codon in CK-B (*7, 8*), we obtained a 455-nt fragment that consisted of predominantly 3′ coding sequences, and a 505-nt fragment that contained the 3′ UTR. Each fragment was ligated into a retrovirus vector (*7, 8*). Multiple cell lines transformed with the 455-nt 3′ coding fragment expressed the retroviral transcript in amounts comparable to those shown in Fig. 3A; however, CK activity remained undetectable in these transformants. Only one of five lines transformed with the 505-nt fragment retained the portion of the retroviral transcript containing the CK-B 3′ UTR, and the retroviral transcript was produced in relatively low amounts (≈ a 1:1 ratio with the endogenous mRNA) (Fig. 3B). Measurement of CK activity in this cell line on two

occasions demonstrated CK activity that ranged from 0.8 to 1.0 mU/mg (Fig. 3B). The lower CK activity in these transformants as compared to cells transformed with the 960-nt CK-B fragment may be due to a lower abundance of the retroviral transcript relative to endogenous mRNA or to the deletion of 3′ coding sequences from this transcript. However, these results demonstrate that the 3′ UTR participates in posttranscriptional control of CK-B expression.

We suggest that relief of translational repression is responsible for the increase in CK activity that was observed following transformation of U937D cells with vectors containing the 3′ UTR of CK-B. We have not formally excluded that a full-length, inactive CK protein is activated posttranslationally or that another CK gene is induced. However, it would be unusual for the 3′ UTR to effect enzyme activity or transcription of another gene.

Translational repression could possibly be achieved by binding of a factor or factors to the 3′ UTR. The retroviral transcripts that contain CK-B 3′ UTR sequences may relieve translational inhibition by competing in trans for such a factor. Band shift analyses (*9*) were used to detect a 3′ UTR–binding activity. When the full-length, 960-nt CK-B RNA was incubated with S100 extract (*10*) from control U937D cells, a discrete ribonuclease (RNase)-resistant complex was observed (Fig. 4A, lane 2). When human HPRT RNA was incubated with the same S100 extract, this RNase-resistant complex was not observed (Fig. 4B). Addition of a tenfold excess of unlabeled, CK-B RNA diminished the intensity of the complex formed with CK-B RNA and S100 extract (Fig. 4A, lane 3). The S100 extracts from transformed cells that expressed the 960-nt CK-B RNA fragment and displayed CK activity, did not give rise to RNase-resistant, gel-retarded complex (Fig. 4A, lane 4). Therefore, the in vitro binding results correlated with the in vivo expression of CK activity. If a similar complex forms in vivo, this complex might sediment during preparation of the S100 extract from transformed cells, because the retroviral transcript that contains the CK-B sequences is polysome-associated (7).

When incubated with the 505-nt CK-B RNA fragment, which contained the 3′ UTR, S100 extracts from wild-type U937D cells produced an RNase-resistant complex that was similar in mobility to the complex formed with the 960-nt CK-B RNA fragment (Fig. 4A, lane 5). The 405-nt 3′ coding region of CK-B RNA did not generate this complex (Fig. 4A, lane 6), localizing the binding site to the 3′ UTR of CK-B RNA. The factor or factors that bound to CK-B RNA was probably proteinaceous, because complex formation was destroyed by treatment of the S100 extract with proteinase K (*11*).

Based on our results, we hypothesize that some portion of the 3′ UTR of CK-B mRNA, possibly one or more of the highly conserved regions, binds to soluble proteins in U937D cells. This complex may then block translation at a step subsequent to initiation. Because expression of the CK-B gene is extensively regulated (*4*), it may be that translational repression is utilized to control CK-B expression in some cells. Studies with CYC1 and pyruvate kinase from yeast, human interferon-β, frog ferritin, mouse protamine 1 (*3*), and clam ribonucleotide reductase (*12*) suggest that expression of other genes may be regulated by their 3′ untranslated sequences.

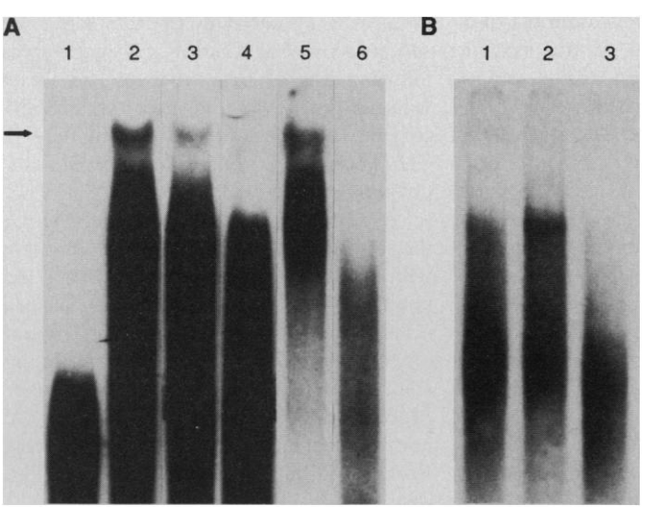

Fig. 4. Identification of a CK-B 3′ UTR binding activity. Sense RNA probes corresponding to the indicated regions of CK-B or HPRT were synthesized from the appropriate in vitro transcription plasmid, and the RNA was purified by denaturing polyacrylamide gel electrophoresis (*14*). Gel retardation analyses were performed as described (*9*). The probes were labeled with [^{32}P]UTP (10^5 dpm, ≈ 20 nM RNA), and incubated with 40 μg of S100 extract (*10*) at 4°C for 30 min. One unit of RNase T1 was added for 10 min, followed by heparin sulfate (5 mg/ml) for an additional 10 min. Protein/RNA complexes, indicated by the arrow, were resolved on a 4% non-denaturing polyacrylamide gel at 8V/cm for 3 hours. (**A**) Binding studies with CK-B RNA. Lanes 1 to 4, 960-nt CK-B RNA containing 3′ coding and noncoding sequences; lane 5, 505-nt 3′ UTR region of the 960-nt CK-B fragment; lane 6, 455-nt 3′ coding region of the 960-nt CK-B fragment. RNA probes were incubated with: lane 1, 960-nt RNA with no protein; lane 2, 960-nt RNA with S100 extract from wild-type cells; lane 3, 960-nt RNA with extract from wild-type cells plus a tenfold molar excess of unlabeled 960-nt CK-B RNA; lane 4, 960-nt RNA with S100 extract from cells transformed with the retrovirus vector containing the 960-bp CK-B fragment; lane 5, 505-nt 3′ UTR RNA with S100 extract from wild-type cells; lane 6, 455-nt 3′ coding RNA with S100 extract from wild-type cells. (**B**) Binding studies with HPRT RNA. A 590-nt HPRT probe was used for these studies. Lane 1, HPRT RNA with S100 extract from wild-type cells; lane 2, HPRT RNA with S100 extract from cells transformed with the retrovirus vector containing the 960-bp CK-B gene fragment; lane 3, HPRT RNA, no protein.

REFERENCES AND NOTES

1. D. Yaffee, U. Nudel, Y. Mayer, S. A. Neuman, *Nucleic Acids Res.* **13**, 3723 (1985).
2. G. Shaw and R. Kamen, *Cell* **46**, 659 (1986); R. D. Klausner and J. B. Harford, *Science* **246**, 870 (1989); J. S. Malter, *ibid.*, p. 664.
3. K. S. Zaret and F. Sherman, *J. Mol. Biol.* **176**, 107 (1984); I. J. Purvis, A. J. E. Bettany, L. Loughlin, A. J. P. Brown, *Nucleic Acids Res.* **15**, 7951 (1987); V. Kruys *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 6030 (1987); L. F. Dickey, Y. H. Wang, G. E. Shull, I. A. Wortman, E. C. Theil, *J. Biol. Chem.* **263**, 3071 (1988); R. E. Braun, J. J. Peschon, R. R. Behringer, R. L. Brinster, R. D. Palmiter, *Genes Dev.* **3**, 793 (1989).
4. G. H. Daouk, R. Kaddurah-Daouk, S. Putney, R. Kingston, P. Schimmel, *J. Biol. Chem.* **263**, 2442 (1988).
5. J. J. Billadello, D. P. Kelly, D. G. Roman, A. W. Strauss, *Biochem. Biophys. Res. Commun.* **138**, 392 (1986).
6. H. S. Koren, S. J. Anderson, J. W. Larrick, *Nature* **279**, 328 (1979).
7. J. L. C. Ch'ng, R. C. Mulligan, P. Schimmel, E. W. Holmes, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 10006 (1989).
8. A 960-bp fragment of the human CK-B gene (*4*) that includes the entire 3′ UTR and part of the 3′ coding region was ligated into the unique Bam HI site of the pWE vector (*18*). Constructs were transfected into ψ-2 cells, and the media from these cells was used to infect ψ-AM cells, resulting in an amphotropic vector (*19*). U937 cells (*6*) were infected by incubating 10^5 cells with 1 ml of amphotropic viral stock in the presence of 12 μg of Polybrene. Transformants were selected in medium containing G418 (1 mg/ml). Five or more clones were present in each independently isolated population of transformants. The 960-nt CK-B gene fragment was subcloned in an in vitro transcription plasmid for synthesis of one cRNA probe (Fig. 1). A cRNA probe complementary to the 1005 nt at the 3′ terminus of a CK-B cDNA (*16*) was used for other RNase protection assays (Figs. 2 and 3).
9. E. A. Leibold and H. N. Munro, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 2171 (1988); T. A. Rouault, M. W. Hentze, S. W. Caughman, J. B. Harford, R. D. Klausner, *Science* **241**, 1207 (1988).
10. J. D. Dignam, R. M. Lebovitz, R. D. Roeder, *Nucleic Acids Res.* **11**, 1475 (1983).
11. J. L. C. Ch'ng, unpublished data.
12. N. Standart and T. Hunt, personal communication.
13. N. Aziz and H. N. Munro, *Nucleic Acids Res.* **14**, 915 (1986).
14. P. A. Kreig and D. A. Melton, *Methods Enzymol.* **155**, 397 (1987).
15. J. M. Chirgwin, A. E. Przybyla, R. J. MacDonald, W. J. Rutter, *Biochemistry* **18**, 5294 (1979).
16. F. J. Kaye *et al.*, *J. Clin. Invest.* **79**, 1412 (1987).
17. A. R. Leech, I. Beis, E. A. Newsholme, *Anal. Biochem.* **90**, 561 (1978).

18. P. V. Choudray *et al.*, *Cold Spring Harbor Symp. Quant. Biol.* **51**, 1047 (1986).
19. R. D. Cone and R. C. Mulligan, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 6349 (1984).
20. We thank G. Daouk for the CK-B gene fragment and R. Mulligan for the pWE retrovirus vector. The human HPRT cDNA was provided by T. Palella. Technical assistance of M. Evans is appreciated. Supported by NIH grants R01-CA 47631-02 and GM34366.

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Blocking of the Initiation-to-Elongation Transition by a Transdominant RNA Polymerase Mutation

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RNA polymerase, the principal enzyme of gene expression, possesses structural features conserved in evolution. A substitution of an evolutionarily invariant amino acid (Lys^{1065} → Arg) in the β subunit of *Escherichia coli* RNA polymerase apparently disrupts its catalytic center. The mutant protein inhibited cell growth when expressed from an inducible promoter. The assembled holoenzyme carrying the mutant subunit formed stable promoter complexes that continuously synthesized promoter-specific dinucleotides but that did not enter the elongation step. The mutant polymerase inhibited transcription by blocking the access of the wild-type enzyme to promoters.

DNA-DEPENDENT RNA POLYMERase is a multifunctional enzyme that carries out the principal biochemical steps of gene expression, including specific promoter binding, melting of the DNA double helix, template-directed de novo initiation, processive elongation, and release of RNA at terminators. Although we have a fairly detailed phenomenological understanding of these steps of RNA polymerase functional cycle (*1*), our knowledge of the underlying molecular structures is limited (*2*). These structures, however, must be universal among living organisms, since the general multisubunit architecture of the enzyme (*3–5*) and the primary amino acid sequence of its subunits [(*6*), see Fig. 1A] are remarkably conserved.

We used the molecular genetic approach to study RNA polymerase structure function, focusing on the β subunit of the enzyme from *Escherichia coli*, which is encoded by the *E. coli rpoB* gene (*7*). The β subunit has a homolog in every living organism (Fig. 1A) and has been implicated in each basic step of the RNA polymerase functional cycle (*8*). Our strategy is to engineer in vitro *rpoB* mutations leading to the substitution of evolutionarily conserved amino acids in the hope to selectively inactivate individual basic functions of the enzyme. For example, a mutation might disrupt the polymerase catalytic center so that the enzyme binds to promoters but fails to make RNA. Obviously, such a mutation would be dominantly lethal because the mutant polymerase would in effect become a nonspecific repressor of transcription. For this reason, the mutations are engineered and maintained in a tightly repressed copy of *rpoB*. The second element of our experimental system is the in vitro assembly of a homogeneous preparation of the mutant RNA polymerase from individually overexpressed subunits, which is necessary for a biochemical analysis of the defect. The details of this technology are to be published elsewhere (*9*). Here we describe an *rpoB* mutation that blocks the "escape" of RNA polymerase from the initiation complex, interrupting the transcriptional cycle at an intermediate step.

The sites for mutagenesis were chosen on the basis of affinity cross-linking mapping of amino acids in the vicinity of the RNA polymerase active center (*10*) combined with the homology sequence alignment (Fig. 1A). Two Lys (K) to Arg (R) substitutions, K1065R and K1051R, were constructed in the *rpoB* gene cloned into the pMKA92 expression plasmid (Fig. 1, A and B).

In the genetic system for the analysis of the mutants' phenotype (Fig. 1C), the Rif-sensitive host strain AJ6207 carries amber mutations in its chromosomal *rpoB* and *lacZ* genes and also harbors the Lac repressor–producing plasmid pLacI^{Q}. Such cells are absolutely dependent on the presence of the *supU* suppressor tRNA specified by an F′ episome. Because of this dependence, the host strain never segregates Lac^{-} variants. When the mutant K1051R β subunit was expressed in this host from the pMKA92-derivative plasmid, the cells became rifampicin-resistant. Under these conditions, IPTG-dependent Lac^{-} segregants appeared with the frequency of about 2%, indicating that the plasmid-borne β could complement the chromosomal *rpoB* defect (IPTG, isopropyl-β-D-thiogalactopyranoside). Such a phenotype is identical to the one conferred by the induction of the wild-type β. Thus mutation K1051R did not affect the vital functions of RNA polymerase. In contrast, the AJ6207 cells, as well as other hosts carrying the mutation K1065R encoded in the pMKA92-derivative plasmid, failed to form colonies on IPTG plates altogether, indicating that the cell cannot tolerate the mutate β subunit. Thus we classify the K1051R and K1065R alleles of *rpoB* as silent and dominant negative, respectively. Yet, in spite of its toxicity, the K1065R polypeptide accumulates in substantial amounts when induced with IPTG in liquid culture and is deposited in the insoluble fraction of the cell, as does the wild-type β (*11, 12*).

For the biochemical analysis of the mutants, RNA polymerase holoenzymes containing the K1051R and K1065R β subunits were reconstituted in vitro from individually overexpressed subunits (*13*) and tested in a standard in vitro transcriptional assay with T4 DNA as the template. The specific activity of K1051R polymerase was indistinguishable from that of the intact wild-type polymerase, whereas the K1065R enzyme showed no detectable activity (Fig. 2A). This result correlates with the mutants' phenotypes described above.

We next addressed the question whether the K1065R polymerase would interfere with the functioning of active enzyme in the in vitro system. For this purpose, standard reactions were set up with a limiting amount of T4 DNA template, to which increasing amounts of the mutant polymerase were added together with a fixed amount of normal enzyme. The K1065R enzyme strongly inhibited the normal reaction (Fig. 2B). At a 1:1 ratio of the mutant to the wild-type enzyme, this inhibition was almost fivefold and reproducible. The inhibition was not observed when transcription is performed at DNA excess, suggesting that competition for a DNA target is the basis for the inhibition.

To find out whether the K1065R enzyme forms specific promoter complexes, we used the DNA gel retardation assay (*14, 15*) with a 130-bp DNA fragment carrying the A1 promoter of bacteriophage T7 (*15*). The

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