Oxidative Thymine Dimer Repair in the DNA Helix

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The metallointercalator $Rh(phi)_2DMB^{3+}$ (phi, 9,10-phenanthrenequinone diimine; DMB, 4,4′-dimethyl-2,2′-bipyridine) catalyzed the repair of a thymine dimer incorporated site-specifically in a 16–base pair DNA duplex by means of visible light. This repair could be accomplished with rhodium noncovalently bound to the duplex and at long range (16 to 26 angstroms), with the rhodium intercalator tethered to either end of the duplex assembly. This long-range repair was mediated by the DNA helix. Repair efficiency did not decrease with increasing distance between intercalated rhodium and the thymine dimer, but it diminished with disruption of the intervening π-stack.

Solar ultraviolet radiation damages our genetic material and leads to mutations and cancer. The most thoroughly studied light-induced DNA lesion is the cyclobutyl thymine dimer that results from a [2+2] photocycloaddition between adjacent thymine bases on the same polynucleotide strand (*1*). Eukaryotic cells eliminate the thymine dimer by excision, whereas bacteria such as *Escherichia coli* use photolyase enzymes (*2*, *3*) to repair the lesion photochemically without excision. In the latter process, visible light (350 to 450 nm) induces a one-electron photoreduction of the thymine dimer substrate by the enzyme, initiating its repair. In model systems, photoexcited tryptophan has served as a reductant with irradiation at ≤300 nm (*4*). Work with pyrimidine dimer model compounds indicated that repair of the lesion could also be initiated by photoinduced oxidation with ultraviolet light (≤300 nm) (*5*). Thus, repair may proceed through both radical cation and radical anion intermediates (*6*).

We recognized that intercalating complexes of rhodium(III), which bind tightly to DNA and are also strong photo-oxidants, might directly promote the repair of thymine dimers incorporated in duplex DNA substrates. The complex $Rh(phi)_2DMB^{3+}$ binds in the major groove of double-stranded DNA with an affinity of $>10^6$ M^{-1} by intercalation of the phi ligand (*7*). Excitation of the complex at 400 nm, where DNA itself does not absorb light, yields an oxidizing excited state that decays on the nanosecond time scale (*8*).

$Rh(phi)_2DMB^{3+}$ was found to catalyze the repair of a thymine dimer incorporated site-specifically (*9*) in the center of a synthetic 16–base pair (bp) oligonucleotide duplex. Excitation of the rhodium complex at 400 nm in the presence of dimer-containing duplex resulted in the disappearance of the dimer-containing strand in a first-order kinetic process, with concomitant appearance of the repaired oligonucleotide (Fig. 1). Quantitative repair has been observed with concentrations of rhodium complex as low as 500 nM (*10*) and a >10-fold excess of DNA duplex. This synthetic repair system is remarkable in that the metal complex is activated by visible light. Repair of dimer-containing oligonucleotide substrates can be accomplished with catalytic amounts of metal complex and sunlight.

Because the DNA π-stack has been shown to mediate long-range electron transfer reactions, we considered whether the DNA helix might similarly facilitate long-range oxidative repair. We previously reported that the π-stacked array of heterocyclic, aromatic bases of DNA facilitates long-range photoinduced electron transfer between donors and acceptors bound by intercalation (*11*–*13*). Moreover, oxidative damage to guanine 5′-GG-3′ doublets in DNA by photoinduced charge transfer at long range (≥34 Å) from a rhodium intercalator covalently tethered to the DNA duplex has been demonstrated (*14*). To explore the remote repair of thymine dimer lesions mediated by DNA, we prepared dimer-containing 16-bp duplexes with a Rh(III) intercalator covalently tethered to one end (*15*) and irradiated these assemblies with 400-nm light (Fig. 2). High-performance liquid chromatography (HPLC) analysis (under denaturing conditions) of the reaction mixture showed the steady disappearance of the dimer-containing strand and the corresponding appearance of the repaired strand as a function of irradiation at 400 nm (*16*).

Photocleavage of DNA by phi complexes of rhodium with irradiation at higher energies (313 nm) has been useful in mark-

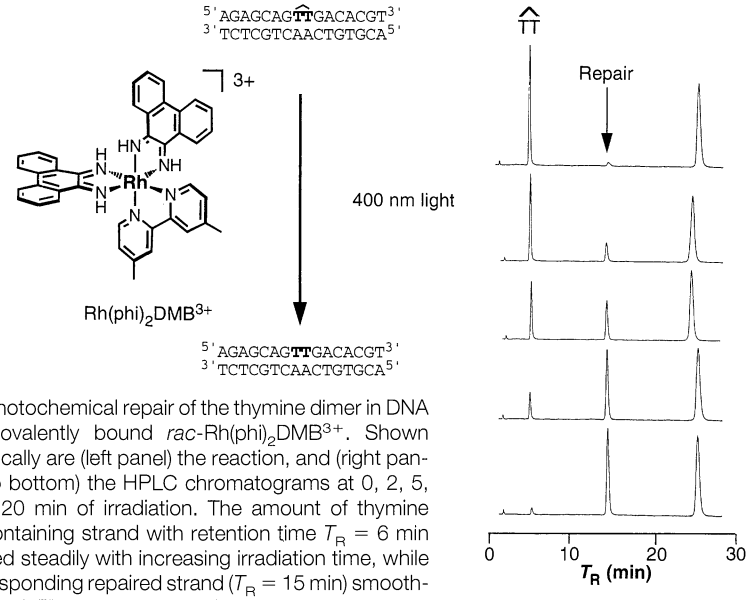

Fig. 1. Photochemical repair of the thymine dimer in DNA by noncovalently bound *rac*-$Rh(phi)_2DMB^{3+}$. Shown schematically are (left panel) the reaction, and (right panel, top to bottom) the HPLC chromatograms at 0, 2, 5, 10, and 20 min of irradiation. The amount of thymine dimer–containing strand with retention time T_R = 6 min decreased steadily with increasing irradiation time, while the corresponding repaired strand (T_R = 15 min) smoothly appeared. The constant signal at T_R = 26 min corresponds to the complementary strand. Solutions containing thymine dimer–modified 16-bp duplex and one equivalent of *rac*-$Rh(phi)_2DMB^{3+}$ were irradiated with 400-nm light to effect DNA repair. The quantum yield for thymine dimer repair measured for 20 min of irradiation was 0.0001 mol per Einstein with stoichiometric rhodium. The light source was a Hanovia Hg-Xe arc lamp equipped with a monochromator. The samples (40 μl) containing 8 μM DNA duplex, 8 μM *rac*-$Rh(phi)_2DMB^{3+}$, 50 mM NaCl, and 5 mM tris (pH 8.50) were irradiated at ambient temperature. Product analysis was accomplished by high-temperature reversed-phase HPLC on a Microsorb MV C_{18} analytical column (Rainin) maintained at 80°C (flow rate = 0.8 ml min^{-1}; we used a linear gradient of 20 mM ammonium acetate containing 2 to 3% CH_3CN for 10 min, then 3 to 4% CH_3CN for 30 min); under these conditions, each oligonucleotide elutes with a characteristic retention time. The identity of the individual compounds was confirmed by coinjection with authentic samples. Electrospray ionization mass spectrometric analysis of the repaired strand also showed the expected mass.

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ing sites of intercalation (*7*) and permits the determination of distances (*11*, *14*) over which DNA-mediated electron transfer reactions can occur. Photocleavage of 5′-modified and 3′-modified duplexes at 313 nm indicated intercalation up to four base steps from the end of the duplex (Fig. 2). Therefore, on the basis of the photocleavage results, tethering of rhodium to the duplex precludes direct contact of the metal center and thymine dimer within a duplex.

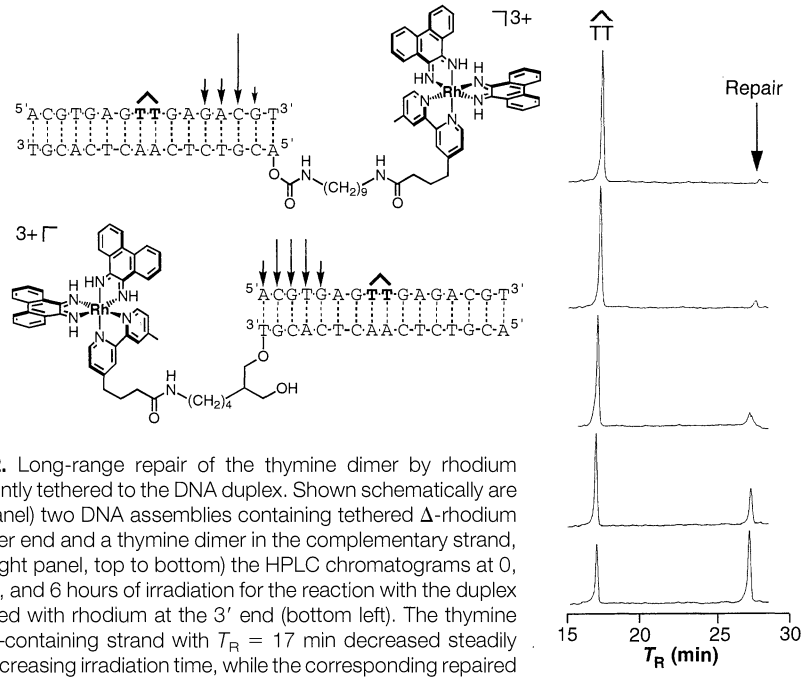

Fig. 2. Long-range repair of the thymine dimer by rhodium covalently tethered to the DNA duplex. Shown schematically are (left panel) two DNA assemblies containing tethered Δ-rhodium at either end and a thymine dimer in the complementary strand, and (right panel, top to bottom) the HPLC chromatograms at 0, 1, 2, 4, and 6 hours of irradiation for the reaction with the duplex modified with rhodium at the 3′ end (bottom left). The thymine dimer–containing strand with T_R = 17 min decreased steadily with increasing irradiation time, while the corresponding repaired strand (T_R = 26 min) smoothly appeared. The metallated oligonucleotide eluted at T_R = 45 min and is not shown here. Reaction conditions were identical to those in Fig. 1 except for the HPLC gradient: linear gradient of 20 mM ammonium acetate containing 2 to 3% CH_3CN over 10 min, to 4.5% CH_3CN over 30 min, and to 20% over 10 min. Analysis of the visible absorption spectrum showed that the metal complex did not degrade during the course of the reaction, consistent with a catalytic repair process. The quantum yield for thymine dimer repair calculated for 6 hours of irradiation was 2×10^{-6} mol per Einstein. The arrows (left panel) indicate relative intensities for the direct photocleavage (*14*) with 313 nm light. The distribution of intensities indicates that the most probable site for intercalation of rhodium is in the 5′-CG-3′ step for the 5′-Rh–modified oligomer and equally in the 5′-CG-3′ step and 5′-GT-3′ step for the 3′-Rh–modified duplex. With these intercalation sites, the distance between the catalyst and the cyclobutane ring of the dimer is 19 to 22 Å.

To test whether the repair of Rh-modified DNA duplexes proceeded in an interduplex reaction, we examined dimer repair for an Rh-modified duplex over a concentration range of 2 to 32 μM. The repair efficiency of rhodium noncovalently bound to the duplex appears to be 30 times that in Rh-modified DNA duplexes, and we considered whether an interduplex reaction might account for this difference. In the Rh-modified DNA duplexes, we found that the repair efficiency varied by only 2% between 2 and 16 μM, with a 6% increase in efficiency between 16 and 32 μM. If the repair were primarily intermolecular, we would have expected an increase in repair efficiency of at least 500% over this concentration range. These data therefore indicate that at 8 μM, where we performed most of the repair experiments, duplex dimerization is not significant (*17*). We also tested the intermolecularity of the reaction at 8 μM directly in an experiment in which a 16-bp duplex containing a thymine dimer was irradiated in the presence of a separate, undamaged duplex bearing a pendant rhodium complex; we observed no detectable repair. Lastly, interduplex intercalation is inconsistent with DNA photocleavage experiments measured as a function of concentration with Rh-modified duplexes. These experiments, consistent with the repair reactions, also support an interduplex dimerization constant of $\leq 10^3$ M^{-1} (*18*). Hence, repair in the Rh-modified duplexes occurs in an intraduplex reaction and is initiated from a remote position 16 to 26 Å

Table 1 (left). Long-range thymine dimer repair in duplexes with tethered rhodium. Repair is expressed as the percentage of thymine dimer repaired after 6 hours of irradiation. Repair efficiencies are given for assemblies containing either Δ- or Λ-Rh. Combined random error in sample preparation, irradiation, and HPLC analysis, ≤5%. Separation distances between catalyst and thymine dimer are based on intercalation at the sites shown schematically. Melting temperatures (T_m) were determined with 2 μM solutions of modified duplex. Reaction conditions for repair were identical to those in Fig. 1. **Table 2 (right).** Long-range thymine dimer repair in duplexes with tethered rhodium without and with intervening bulges. Repair is expressed as the percentage of thymine dimer repaired after 6 hours of irradiation. Combined random error in sample preparation, irradiation, and HPLC analysis, ≤5%. Reaction conditions for repair were identical to those in Fig. 1.

Duplex	Distance (Å)	T_m (°C) +TT	T_m (°C) -TT	Repair (%) Δ-Rh	Repair (%) Λ-Rh
5′A-C-G-T-G-A-G-T-T-G-A-G-A-C-G-T 5′ T-G-C-A-C-T-C-A-A-C-T-C-T-G-C-A Rh	19			67	59
5′A-C-G-T-G-A-G-T-T-G-A-G-A-C-G-T 5′ T-G-C-A-C-T-C-A-A-C-T-C-T-Rh-G-C-A	19	59	65	79	47
5′A-C-G-T-G-A-T-G-T-T-G-T-A-G-A-C-G-T 5′ T-G-C-A-C-T-A-C-A-A-C-A-T-C-T-Rh-G-C-A	22	61	65	91	87
5′A-C-G-T-G-C-A-T-G-T-T-G-T-A-C-G-A-C-G-T 5′ T-G-C-A-C-G-T-A-C-A-A-C-A-T-G-C-T-Rh-G-C-A	26	63	68	100	62

Duplex	T_m (°C) +TT	Repair (%) Δ-Rh
5′A-C-G-T-G-C-A-T-G-T-T-G-T-A-C-G-A-C-G-T 5′ T-G-C-A-C-G-T-A-C-A-A-C-A-T-G-C-T-Rh-G-C-A	63	100
5′A-C-G-T-G-C-A-T-G-T-T-G-T-A-C-G-A-C-G-T T-G-C-A-C-T-A-C-A-A-C-A-T-C-T-Rh-G-C-A 5′	45	80
5′A-C-G-T-G-C-A-T-G-T-T-G-T-A-C-G-A-C-G-T T-G-C-A-C-T-A-C-A-A-C-A-T-C-T-Rh-G-C-A 5′	45	47

away through the DNA base stack (*19*). The DNA helix, which facilitates long-range electron transfer, also mediates the long-range repair of the thymine dimer.

The lower repair efficiency observed with rhodium tethered to a remote site relative to that seen with rhodium noncovalently bound could be a consequence of a decrease in repair efficiency with increasing rhodium-dimer separation, the destacking of intervening base pairs, or both. We therefore prepared a series of duplexes, each containing a thymine dimer and a covalently bound rhodium intercalator, to test the sensitivity of the reaction to both distance and destacking (Tables 1 and 2). In all the assemblies, the 4-bp (5′-ACGT-3′) intercalation sites at either end of the duplex and the GAG sequences flanking the T<>T lesion were identical. We systematically varied the duplex length, the position of modification by rhodium (3′ or 5′ terminus), and the configuration about the Rh(III) center (Δ or Λ). We also hybridized the 5′-Δ-Rh–modified 16-nucleotide (nt) strand and 18-nt strand to the 20-nt thymine dimer strand, creating two assemblies with internal bulges between the rhodium intercalator and the thymine dimer.

Correlations between structure and repair efficiency can be made from our results. First, repair is sensitive to stacking of both the donor and the acceptor into the helix. This is evident in the consistently greater repair efficiency with Δ-isomers relative to that with Λ-isomers (*20*). As found in previous studies of DNA-mediated electron transfer (*12*, *13*), this result reflects the deeper stacking of the right-handed metal complex into the right-handed DNA helix (*21*). Second, we see comparable repair in reactions with rhodium tethered to the 3′ or 5′ side of the dimer. From this observation, we infer that stacking on the 3′ and 5′ sides of the thymine dimer within the helix is not substantially different (*22*). We can account for the differences in repair efficiencies in these constructs on the basis of the differential stacking of the rhodium intercalator tethered to either end of the duplex (*23*). As seen in other electron transfer assemblies (*11*–*14*), we find that repair efficiencies do not diminish with increased separation between the thymine dimer lesion and tethered rhodium. Indeed, the overall increase in repair efficiency observed clearly for the Δ-Rh diastereomer over this range of distances likely reflects the increased helical stabilization with increased duplex length. These data also argue against a mechanism in which fraying of the ends of the DNA duplex might allow the tethered rhodium complex to contact the thymine dimer and initiate repair at close range.

Although the efficiency of these DNA-mediated repair reactions does not appear to diminish with increasing distance between the dimer and the oxidative catalyst, the reactions are sensitive to stacking of the intervening bases. The decrease in repair efficiency caused by intervening bulges in the DNA illustrates this sensitivity (*24*) (Table 2). Single- and double-base bulges do not completely destack a DNA duplex, but they do introduce structural perturbations (*25*); we have now shown that such defects in the intervening π-stack interfere with the long-range repair process. This result confirms that the DNA helix mediates this long-range oxidative repair reaction.

The difference in the repair efficiency of rhodium covalently and noncovalently bound to DNA may reflect features of the structure of the thymine dimer within the duplex. Our results show that increasing the distance between intercalated rhodium and the thymine dimer does not lead to a reduction in repair efficiency; therefore, the disparity likely results from differences in π-stacking. A likely possibility is that the rhodium tether restricts optimal overlap of the intercalator with the helical π-stack. The lower repair efficiency in Rh-modified duplexes may also indicate that the thymine dimer is not fully stacked in the helix to either its 3′ or 5′ side. It is possible that repair by $Rh(phi)_2DMB^{3+}$ noncovalently bound to the duplex is more efficient because the complex can stack directly against the thymine dimer (*26*). Alternatively, binding of $Rh(phi)_2DMB^{3+}$ in the center of the sequence may serve to stabilize the duplex containing the thymine dimer in a stacked conformation where DNA-mediated oxidation is more favorable (*27*). Although we have shown that long-range oxidative repair through DNA is therefore possible, the decreased efficiency of reactions with tethered rhodium, which are mediated by the helix, suggests that it may be advantageous in an enzymatic repair to project the thymine dimer fully out of the helix for efficient reaction (*28*), as has been proposed (*3*) on the basis of the crystal structure of DNA photolyase.

This work illustrates how a synthetic catalyst may be applied in the repair of thymine dimers in DNA with visible light. The repair process functions at both short and long range through the DNA bases over a distance of at least 26 Å. This repair process represents an illustration of long-range DNA-mediated electron transfer chemistry and underscores the sensitivity of such reactions to π-stacking but not to distance. These electron transfer reactions therefore provide a sensitive probe of intervening DNA helical structure. Although our results do not imply that DNA-mediated repair is operative in enzymatic photoreactivation, they do provide chemical evidence for repair from a remote position mediated by the DNA helix. We suggest that these phenomena are important to consider in the context of physiologically relevant charge-transfer reactions involving double-helical DNA.

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10. Plots of repair activity as a function of rhodium concentration are sigmoidal, consistent with catalysis that depends on binding of $Rh(phi)_2DMB^{3+}$ to the DNA duplex.
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15. Rhodium-modified oligonucleotides were prepared by coupling the complex, $Rh(phi)_2bpy'^{3+}$ (bpy′, 4-butyric acid 4′-methyl-2,2′-bipyridine), activated as the *N*-succinimidyl ester, to alkylamino functionalized oligonucleotides attached to controlled pore glass (CPG). The 3′-alkylamino DNA was prepared

by standard phosphoramidite synthesis starting from C7-amino-link 500 Å CPG (Glen Research) and selective deprotection of the R-N(H)-Fmoc by treatment with 20% piperidine in DMF (v/v) at ambient temperature for 30 min. The 5′-alkylamino DNA was prepared from dT 2,000 Å CPG (Glen Research), followed by standard phosphoramidite synthesis and functionalization with a nonamethylene aminoalkyl linker [L. Wachter, J. A. Jablonski, K. L. Ramachandran, *Nucleic Acids Res.* **14**, 7985 (1986)]. Activation of racemic metal complex, coupling to DNA, and HPLC purification of the diastereomeric Δ-Rh and Λ-Rh conjugates was identical for 3′- and 5′-functionalized oligonucleotides [see (*14*)].
16. After 6 hours of irradiation at 400 nm, 5% repair of the thymine dimer was found in control samples lacking rhodium (light control) and represents the background repair by light in our experiments.
17. To account for the repair efficiency observed in Rh-modified duplexes based on an intermolecular reaction, the duplex dimerization constant would be $\sim 10^4$ M^{-1}. On the basis of the observed concentration dependence in repair and the comparison with the limiting results with noncovalently bound rhodium, we estimate the duplex dimerization constant instead to be $\leq 10^3$ M^{-1}.
18. Photocleavage reactions at 313 nm, measured by phosphoimagery, sensitively detect strand cleavage at several orders of magnitude lower intensity than required to detect rhodium reaction near the tethered end of the duplex in Rh-modified oligomers. Photocleavage reactions as a function of concentration of Rh-modified duplexes also indicate that interduplex reaction is negligible at duplex concentrations of ≤25 μM.
19. Assuming a 3.4 Å centroid-to-centroid distance between stacked base pairs, the separation between the intercalated phi ligand of the rhodium complex and the center of the thymine dimer is 19 Å for both the 5′-Rh– and 3′-Rh–tethered assemblies when the complex is intercalated between the third and fourth base pair from the end of the duplex. On the basis of the photocleavage pattern (Fig. 2), the closest distance between intercalator and thymine dimer along the helical axis appears to be 16 Å for both assemblies with intercalation in the fourth base step.
20. For both diastereomers, direct photocleavage experiments (313 nm) show the metal complex to be fully bound by intercalation at these concentrations.
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23. To evaluate the stacking interaction of tethered rhodium into the DNA duplex, we measured the steady-state luminescence quenching of a stoichiometric amount of *rac*-Ru(phen)$_2$dppz^{2+} (phen, 1,10-phenanthroline; dppz, dipyrido[3,2-a:2′,3′-c]phenazine) noncovalently bound to the duplex by intercalation of the dppz ligand for the individual assemblies. The amount of quenching provides a measure of the efficiency of electron transfer from electronically excited ruthenium to ground-state rhodium through the DNA helix and can be assumed, within an individual DNA sequence, to reflect the ability of rhodium to intercalate into the duplex (*12*). For the 3′-Rh–tethered 16-bp duplex (Table 1, top), luminescence quenching was 49% and 39% for Δ-Rh and Λ-Rh, respectively, versus luminescence of *rac*-Ru(phen)$_2$dppz^{2+} intercalated into the unmodified duplex (without thymine dimer). For the corresponding 5′-tethered rhodium duplex, luminescence quenching was 58% and 52% for Δ-Rh and Λ-Rh, respectively. The higher repair yields therefore correlate with higher quenching of ruthenium luminescence.
24. An analogous sensitivity in long-range oxidation of guanine doublets to intervening bulges in DNA has been observed in our laboratory (D. Hall and J. K. Barton, unpublished results).
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26. Direct photocleavage studies with Rh(phi)$_2$DMB^{3+} on the duplex containing a thymine dimer do not suggest preferential binding of the rhodium adjacent to the dimer.
27. The T_m for unmodified dimer-containing duplexes increased by 2°C in the presence of 1 equivalent of Rh(phi)$_2$DMB^{3+} and by 5°C with Δ-Rh tethered to the 5′-terminus.
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29. Supported by NIH grant GM49216, a postdoctoral fellowship from the Cancer Research Fund of the Damon Runyon–Walter Winchell Foundation (P.J.D.), and an NSF predoctoral fellowship (R.E.H.). We thank N. J. Turro and E. D. A. Stemp for helpful discussions.

24 July 1996; accepted 23 January 1997

Requirement of the DEAD-Box Protein Ded1p for Messenger RNA Translation

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The *DED1* gene, which encodes a putative RNA helicase, has been implicated in nuclear pre–messenger RNA splicing in the yeast *Saccharomyces cerevisiae*. It is shown here by genetic and biochemical analysis that translation, rather than splicing, is severely impaired in two newly isolated *ded1* conditional mutants. Preliminary evidence suggests that the protein Ded1p may be required for the initiation step of translation, as is the distinct DEAD-box protein, eukaryotic initiation factor 4A (eIF4A). The *DED1* gene could be functionally replaced by a mouse homolog, *PL10*, which suggests that the function of Ded1p in translation is evolutionarily conserved.

Eukaryotic translation initiation requires many factors to promote the binding of the 80*S* ribosome to the initiation codon in the mRNA (*1*, *2*). Binding of the 43*S* preinitiation complex, which is derived from the 40*S* subunit of the 80*S* ribosome, to mRNA is a rate-limiting step that requires three eukaryotic initiation factors: 4A (eIF4A), 4B, and 4F (consisting of three subunits, eIF4A, eIF4E, and eIF4G). It has been proposed (*3*) that eIF4B and eIF4F together form an RNA-helicase complex that binds to the 5′ cap of the mRNA through eIF4E. This complex then unwinds duplex structures in the 5′ untranslated region, thereby permitting the 43*S* complex to scan the mRNA until the first AUG codon is selected. eIF4A is thought to play a major role in this unwinding process, because it can unwind short RNA duplexes in vitro in conjunction with eIF4B (*4*). eIF4A belongs to the evolutionarily conserved DEAD-box protein family (*5*), whose members share nine highly conserved amino acid regions, including the distinct Asp-Glu-Ala-Asp (DEAD) sequence. Here, we report that translation in yeast requires another DEAD-box protein, Ded1p, whose function appears to be conserved in evolution.

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The *DED1* gene was originally identified as an essential open reading frame adjacent to *HIS3* (*6*). Ded1p was hypothesized to function in nuclear pre-mRNA splicing because *spp81-1*, a mutant allele of *DED1*, suppresses the growth and splicing defects caused by the *prp8-1* mutation (*7*). More recently, it was reported that overexpression of *DED1* can suppress the growth defect of an RNA polymerase III (Pol III) mutant, which suggests that Ded1p can also influence Pol III transcription, although it may not normally participate in this process (*8*). To investigate the function of Ded1p, we isolated two *ded1* cold-sensitive mutants (*9*). The *ded1-120* and *ded1-199* alleles yield mutant forms of Ded1p with predicted amino acid substitutions of $Gly^{108} \rightarrow$ Asp and $Gly^{494} \rightarrow$ Asp (*ded1-120*) and $Gly^{368} \rightarrow$ Asp (*ded1-199*). At 25°C both mutants grew substantially slower than did the wild-type strain, and at 15°C they did not grow.

We first examined the *ded1-120* and *ded1-199* mutants for splicing defects by Northern (RNA) blotting. No splicing defects were detected in either *ded1* mutant after shifting cultures to 15°C for 2 hours. This observation was in sharp contrast to the aberrant rise in *ACT1* pre-mRNA levels and decline in mature *CRY1* mRNA levels in control strains *prp2* and *prp11*, which harbor splicing mutations (*10*).

Instead, we found that, at 15°C, the incorporation of [^{35}S]methionine into acid-precipitable peptides in each *ded1* mutant strain was ~10% of that incorporated in the isogenic wild-type strain. This inhibition of protein synthesis seemed to be gen-