Specific Binding of 8-Oxoguanine-Containing RNA to Polynucleotide Phosphorylase Protein†

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ABSTRACT: 8-Oxoguanine, an oxidized form of guanine, has the potential to pair with both cytosine and adenine, and thus, the persistence of this base in messenger RNA would cause translational errors. To prevent such an outcome, organisms probably have a mechanism for recognizing RNA molecules carrying 8-oxoguanine and prevent them from entering into the cellular translational machinery. We now report that the *Escherichia coli* cell possesses proteins that bind specifically to RNA carrying 8-oxoguanine. On incubation with a cell-free extract, 8-oxoguanine-containing RNA is stable while normal RNA is degraded by cellular nucleases. The RNase protection assay and gel shift assay revealed that some proteins bind specifically to 8-oxoguanine-containing RNA, hence preventing nuclease attacks. Among the complexes that were detected, one with a 77 kDa protein exhibits tight binding between RNA and protein components. This protein was identified as polynucleotide phosphorylase, encoded by the *pnp* gene. *pnp*$^-$ mutants are hyperresistant to paraquat, a drug that induces oxidative stress in the cell. Binding of Pnp protein to 8-oxoguanine-containing RNA would inhibit cell growth, probably due to withdrawal of such RNA from the translational machinery. The Pnp protein may, therefore, discriminate between an oxidized RNA molecule and a normal one, thus contributing a high fidelity of translation.

Oxygen radicals produced through cellular metabolism damage biologically important substances. More than 20 different types of oxidatively altered purine and pyrimidine bases have been detected in nucleic acids (*1, 2*). Among them, an oxidized form of a guanine base, 8-oxoguanine[1] (8-oxo-7,8-dihydroguanine), appears to be most important since it causes mispairing during DNA synthesis (*3*−*5*). This oxidized base can pair with cytosine and with adenine at almost equal efficiencies, and as a result, G·C to T·A and T·A to G·C transversion mutations are induced (*6*−*9*).

Organisms are equipped with elaborate mechanisms for counteracting such deleterious effects of 8-oxoguanine. In *Escherichia coli*, two glycosylases encoded by the *mutM* and *mutY* genes function to prevent mutation caused by 8-oxoguanine in DNA. The MutM protein removes 8-oxoguanine paired with cytosine, while the MutY protein removes adenine paired with 8-oxoguanine (*10*−*13*). Oxidation of guanine also proceeds in the form of free nucleotide, and an oxidized form of dGTP, 8-oxo-dGTP, is a potent mutagenic substrate for DNA synthesis. The MutT protein of *E. coli* hydrolyzes 8-oxo-dGTP to 8-oxo-dGMP, thereby preventing misincorporation of 8-oxoguanine into DNA (*14*). The concerted actions of the MutM, MutY, and MutT proteins result in a high fidelity of DNA replication against the threat of oxygen radicals (*13*).

Part of this error avoidance mechanism could function in reducing the error rate in RNA synthesis. *E. coli* RNA polymerase can utilize 8-oxoGTP as a substrate, thus leading to misincorporation of 8-oxoguanine into RNA. This could be prevented by the action of the MutT protein, since it has the potential to hydrolyze 8-oxoGTP as efficiently as 8-oxo-dGTP does (*15*). However, when guanine residues in RNA are oxidized in situ, this mechanism would not function. Correction of errors by repair proteins such as MutM and MutY depends on the double-stranded nature of DNA and is not applicable to RNA molecules, which are single-stranded in most domains. Persistence of oxidized guanine residues in RNA may lead to formation of a large amount of error-containing proteins, which would be hazardous to the cell. One way to avoid such a catastrophe is to discriminate 8-oxoguanine-containing RNA from normal RNA and prevent the former from entering into the cellular translational machinery. We, therefore, initiated this study to explore such a possibility. Here we report that the *E. coli* cell possesses a protein that specifically binds to 8-oxoguanine-containng RNA and that mutants defective in the protein are hyperresistant to a drug that induces oxidative stress.

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[1] Abbreviations: 8-oxoguanine, 8-oxo-7,8-dihydroguanine; 8-oxo-GTP, 8-oxo-7,8-dihydroguanosine 5′-triphosphate; 8-oxo-dGTP, 8-oxo-7,8-dihydro-2′-deoxyguanosine 5′-triphosphate; 8-oxo-dGTPase, 8-oxo-7,8-dihydrodeoxyguanosine triphosphatase; 8-oxoGMP, 8-oxo-7,8-dihydroguanosine 5′-monophosphate; 8-oxo-dGMP, 8-oxo-7,8-dihydro-2′-deoxyguanosine 5′-monophosphate; 8-oxoGDP, 8-oxo-7,8-dihydroguanosine 5′-diphosphate; 8-oxo-dGDP, 8-oxo-7,8-dihydro-2′-deoxyguanosine 5′-diphosphate; DTT, dithiothreitol; TLC, thin-layer chromatography; SDS, sodium lauryl sulfate; PAGE, polyacrylamide gel electrophoresis.

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EXPERIMENTAL PROCEDURES

Chemicals and Enzymes. 8-OxoGTP was prepared as described previously (*16*). *E. coli* RNA polymerase, RNase A, yeast tRNA, polyG, and polynucleotide phosphorylase were purchased from Sigma. 8-Oxoguanine-containing deoxyribo oligomer 5′-ggggAATTCCg*GAggggCCCC-3′, in which *G stands for 8-oxoguanine, was purchased from NIPPON Flour Mills Co., Ltd. Cell-free extracts from *E. coli* strains were prepared as described previously (*17*).

[^{32}P]-8-OxoGMP-labeled poly(8-oxoG·A) and [^{32}P]UMP-labeled poly(U·A) were prepared, as described previously (*15*). Briefly, the reaction was carried out with *E. coli* RNA polymerase (0.12 unit/μL) in 0.1 M Tris-HCl (pH 7.8), 2.5 mM $MnCl_2$, 0.2 mM DTT, 0.1 mM poly(dA-dT), 0.2 mM ATP, and 0.05 mM [α-^{32}P]rUTP or [α-^{32}P]-8-oxo-rGTP. After incubation at 37 °C for 60 min, RNase-free DNase I (0.5 unit/μL) was added and the mixture was incubated for a further 10 min. A portion of the reaction mixture (0.1 mL) was diluted with 0.5 mL of 10 mM Tris-HCl (pH 7.5) and 0.1 M NaCl and applied on a small column containing 20 μL of AG 50W-X8 (Bio-Rad) and 100 μL of DE52 (Whatman). The column was washed with 1 mL of 10 mM Tris-HCl (pH 7.5) and 0.1 M NaCl and then with 1 mL of 10 mM Tris-HCl (pH 7.5) and 0.3 M NaCl. RNAs were eluted with 1 mL of 10 mM Tris-HCl (pH 7.5) and 0.6 M NaCl and precipitated with 2 volumes of ethanol. Poly(U·A) was partially digested with RNase A to adjust the molecular size to that of poly(8-oxoG·A). The concentrations of the RNAs were determined by using the specific activity and were represented as nucleotides.

Bacterial Strains and Plasmids. E. coli strains defective in various combinations of the RNase genes, including *pnp* (for polynucleotide phosphorylase), *rne* (for RNase E), and *rnb* (for RNase II), were provided by S. Kushner. Plasmid pB15-6, carrying the *pnp* gene, was obtained from P. Regnier.

RNase Protection Assay. Ten picomoles of [^{32}P]UMP-labeled poly(U·A) and [^{32}P]-8-oxoGMP-labeled poly(8-oxoG·A) were preincubated with or without a crude extract of *E. coli* TK1001 (8 mg of protein/mL) in a reaction mixture containing 0.1 M Tris-HCI (pH 7.4), 5 mM $MgCl_2$, and 0.4 mM DTT at 0 °C for 10 min. After preincubation, 2 μL of RNase A (0.4 mg/mL) was added to 10 μL of the reaction mixture, and the mixture was further incubated at 30 °C for 10, 20, and 40 min. At the indicated times, 0.5 μL of the reaction mixture was spotted onto a TLC plate, PEI-cellulose F (Merck), and the plate was developed in 1 M LiCl. Radioactive spots were monitored using a Fuji BAS2000 image analyzer.

Gel Shift Assay. As a standard assay, 10 pmol of ^{32}P-labeled polynucleotides was preincubated with various amounts of crude extract in 5 μL of a reaction mixture containing 0.1 M Tris-HCl (pH 7.4), 5 mM $MgCl_2$, and 0.4 mM DTT for 10 min on ice, and then incubated with or without 0.1 mg/mL RNase A at 30 °C for 10 min. A solution (2 μL) containing 1% bromophenol blue, 25% glycerol, and 50 mM EDTA was added to the reaction mixture, and the samples were analyzed via native 5% PAGE. Electrophoresis was performed at 10 V/cm for 2 h in 90 mM Tris-borate (pH 8.0)/2 mM EDTA buffer. Gels were dried, and radioactive bands were monitored, as described above.

Two-Dimensional Gel Analysis. Eighty picomoles of ^{32}P-labeled poly(8-oxoG·A) was incubated with 480 μg of crude extract protein from an overnight culture of *E. coli* TK1001 in 20 μL of the standard reaction mixture at 0 °C for 10 min. After incubation, the mixture was treated with 0.1 mg/mL RNase A for 60 min at 30 °C. The sample was then irradiated with UV light (at a distance of 10 cm from the UV lamp for 45 min on ice) to cross-link the RNA and the protein components. The first isoelectric focusing with an immobilized pI gradient (pI 4−7) was carried out according to the manufacturer's instruction manual (Amersham Pharmacia Biotec Inc.). After denaturation, the immobilized DryStrip was overlaid onto a 1% SDS−8% PAGE gel and covered by 0.3% agarose. The second electrophoresis was performed at 15 V/cm for 4 h, and the gels were stained with 0.25% Coomassie Blue (R-250), 5% methanol, and 7.5% acetic acid. The radioactive spots were analyzed using a BAS2000 image analyzer. To identify the binding protein, a small radioactive piece of gel was cut out and marked with ^{32}P-labeled ink. The protein was located by comparing the autoradiogram and the stained gels.

Other Procedures. Protein concentrations were determined using Bio-Rad protein assay kits with bovine serum albumin as a standard (*18*). Other procedures were described previously (*16*).

RESULTS

Binding of 8-Oxoguanine-Containing RNA to Cellular Proteins. For the RNase protection assay, RNase A was used since this enzyme cleaves poly(8-oxoG·A) as well as poly(U·A). In 8-oxoguanine-containing polynucleotides, most of 8-oxoguanine residues adopt the syn conformation, and it has been shown that a purine base in the syn conformation can be accommodated in the active site of RNase A (*19*). When poly(8-oxoG·A), in which the 8-oxoGMP moiety was labeled with ^{32}P, was incubated with an excess amount of RNase A, all radioactive materials were efficiently converted to nucleotides (Figure 1A). However, when the labeled poly(8-oxoG·A) was preincubated with an *E. coli* extract and then exposed to the same amount of RNase, about half of the 8-oxoguanine-containing polyribonucleotide remained as a polymer, even after prolonged incubation (Figure 1A). On the other hand, poly(U·A) was completely digested under the same conditions. Protection of 8-oxoguanine-containing RNA from nuclease digestion may be associated with binding of RNA to protein factors present in the extract.

To detect such RNA-protein complexes, a gel shift assay was carried out. [^{32}P]-8-OxoGMP-labeled poly(8-oxoG·A) and [^{32}P]UMP-labeled poly(U·A) were incubated with a crude extract of *E. coli*, and then the mixtures were subjected to gel electrophoresis (Figure 1B). Two distinct bands were detected in the poly(8-oxoG·A) sample (lane 6), whereas no such band was detected in the case of poly(U·A) (lane 3). These bands may represent specific complexes of 8-oxoguanine-containing RNA with some cellular proteins. It should be noted that the bands remained even after treatment with large amounts of RNase A, which could completely degrade free 8-oxoguanine-containing polyribonucleotide to mononucleotide (lane 5). Various compounds were added to the reaction mixture to examine their effects on complex

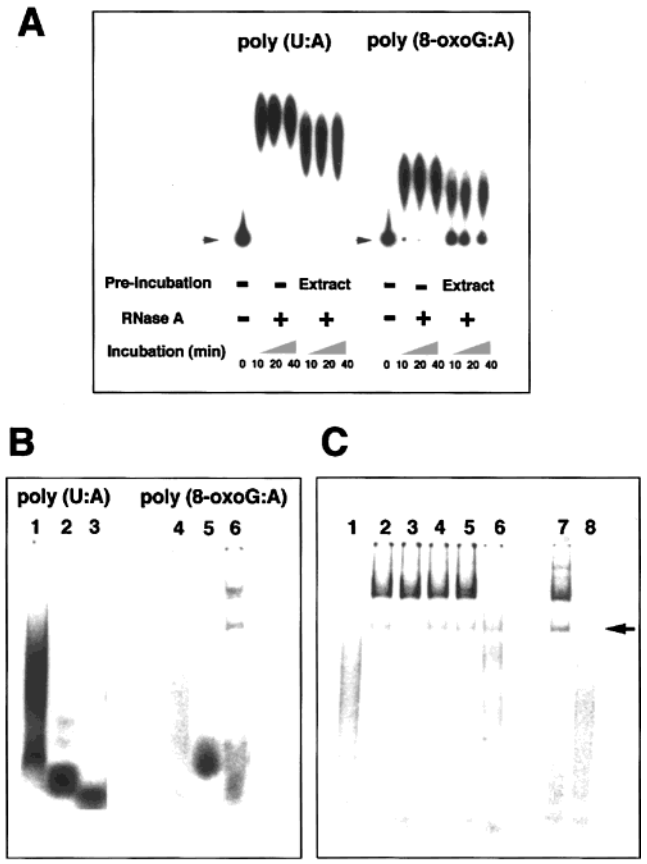

FIGURE 1: Analyses of oxidized RNA-binding proteins in *E. coli* cell-free extracts. (A) RNase protection assay for 8-oxoguanine-containing polyribonucleotide. The reaction mixtures were analyzed with TLC. Arrows denote the origin, where the polynucleotides remain. (B) Gel shift assay for detection of the binding complexes. [^{32}P]UMP-labeled poly(U·A) and [^{32}P]-8-oxoGMP-labeled poly-(8-oxoG·A) were subjected to the following treatments, and the materials were applied to gel electrophoresis: lanes 1 and 4, incubated in a buffer containing 0.1 M Tris-HCl (pH 7.4), 5 mM $MgCl_2$, and 0.4 mM DTT at 0 °C for 10 min and then at 30 °C for 10 min; lanes 2 and 5, treated with RNase A (0.4 mg/mL) in the buffer, under the same conditions; and lanes 3 and 6, incubated in the same buffer containing a crude extract (8 mg of protein/mL) at 0 °C for 10 min and then treated with RNase A (0.4 mg/mL) at 30 °C for 10 min. (C) Competition for binding to 8-oxoguanine-containing polyribonucleotide. Ten picomoles of [^{32}P]-8-oxoGMP-labeled poly(8-oxoG·A) was preincubated with 20 μg of crude extract protein of *E. coli* TK1001 in a reaction mixture (5 μL), with or without competitors for 10 min on ice, and then incubated at 30 °C for 10 min without RNase A. An arrow denotes the lower complex: lane 1, without the cell-free extract; lanes 2 and 7, without the competitor; lane 3, with 5 nmol of yeast tRNA; lane 4, with 5 nmol of 8-oxoguanine-containing deoxyribooligomer; lane 5, with 5 nmol of 8-oxoGTP; lane 6, with 5 nmol of polyG; and lane 8, with 50 pmol of 8-oxoguanine-containing polyribonucleotide.

formation (Figure 1C). When a 5-fold excess of nonlabeled 8-oxoguanine-containing RNA was added to the reaction mixture, complex formation was almost completely abolished (lane 8). However, addition of large amounts of yeast tRNA, 8-oxoguanine-containing deoxyribooligonucleotide (chain length, 21), and 8-oxoGTP had no appreciable effect on complex formation (lanes 3−5, respectively). When 5 nmol of polyG, which amounts to 500 times the amount of labeled 8-oxoguanine-containing poly(8-oxoG·A), was added to the mixture, complex formation was partially blocked and only a lower band appeared (lane 6). To determine if this band represents a protein that binds tightly to 8-oxoguanine-

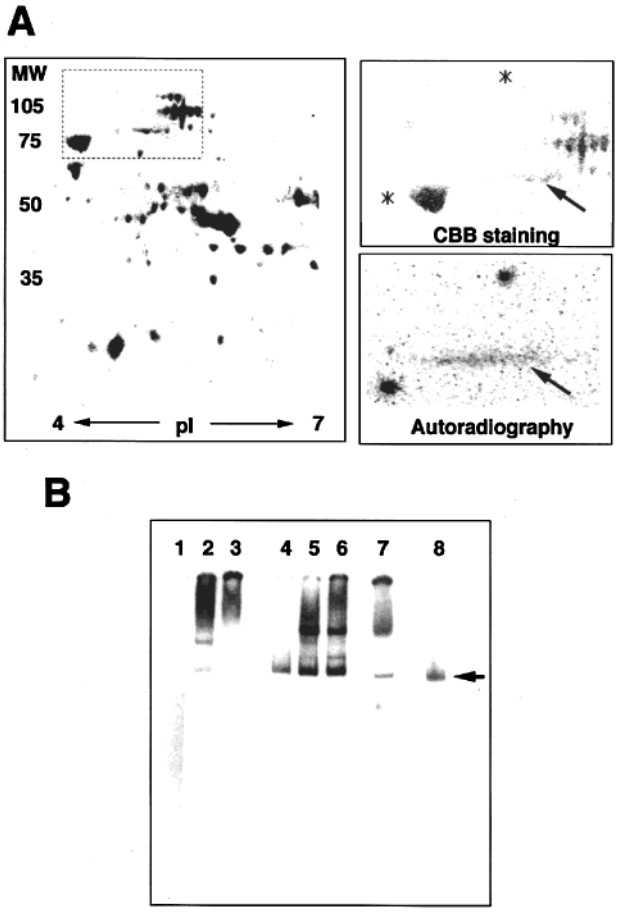

FIGURE 2: Identification of an oxidized RNA-binding protein. (A) Two-dimensional gel analysis of the binding complexes. The large left panel represents a wide area of the gel, and the two right panels represent the focused area, the top being a Coomassie Blue-stained gel and the bottom an autoradiogram of the gel. Asterisks denote ^{32}P-labeled maker ink, and arrows denote the binding protein. (B) Binding of 8-oxoguanine-containing ribopolymer to Pnp protein. Five picomoles of [^{32}P]-8-oxoGMP-labeled poly(8-oxoG·A) was preincubated with crude extracts of *E. coli* or purified enzyme in a reaction mixture (5 μL) for 10 min on ice, and then incubated at 30 °C for 10 min without RNase A. An arrow denotes the complex: lane 1, without enzyme; lanes 2 & 7, with 6 μg of crude extract protein of *E. coli* MG1693 (wild type); lane 3, with 6 μg of crude extract protein of *E. coli* SK5691 (*pnp*-7); lanes 4−6, with 0.06, 0.6, and 6 μg of crude extract protein, respectively, of *E. coli* SK5691 harboring plasmid pB15-6; and lane 8, 0.018 unit of the purified polynucleotide phosphorylase protein.

containing RNA, further analyses of this protein were carried out.

Identification of the Oxidized RNA-Binding Protein as Polynucleotide Phosphorylase. To identify the binding protein, two-dimensional gel electrophoresis was performed. The complex was formed by incubating ^{32}P-labeled poly(8-oxoG·A) with an *E. coli* extract and treated with an excess amount of RNase A to remove free polyribonucleotide. The materials were then irradiated with ultraviolet light to form cross-links between the RNA and the protein moieties of the complex and spread on the gels. As shown in Figure 2A, a labeled protein was located by comparing the autoradiogram and the Coomassie Blue-stained gel. Using a database (http://expasy.nhri.org.tw/ch2dothergifs/publi/ecoli-high.gif), it was revealed that the radioactive spot represents polynucleotide phosphorylase protein. From the mobility on SDS−PAGE, the molecular mass of protein was estimated

to be 77 kDa, which corresponds to the value for polynucleotide phosphorylase (77 101 Da) (*20*).

This finding was supported by a gel shift analysis of *E. coli* mutants defective in the *pnp* gene (Figure 2B). It has been shown that the *pnp* gene encodes polynucleotide phosphorylase (*21*). The band for a protein to which radioactive poly(8-oxoG·A) is bound was present in wild-type samples (lanes 2 and 7) but absent from the mutant one (lane 3). When plasmid pB15-6, which carries the *pnp* gene, was introduced into the mutant cell, complex formation was restored (lanes 4−6). A purified preparation of polynucleotide phosphorylase was capable of binding poly(8-oxoG·A) to form a complex (lane 8), which is indistinguishable from that produced in the wild-type extract.

To obtain further support for the notion that Pnp protein binds tightly to 8-oxoguanine-containing RNA, additional experiments were performed. When a purified preparation of polynucleotide phosphorylase was incubated with poly(8-oxoG·A) and with poly(U·A), several bands were detected in the gel shift assay (Figure 3A). When, however, the samples were treated with RNase A, only one distinct bind was detected in the poly(8-oxoG·A) sample whereas no band was found in the case of poly(U·A). Various types of RNAs were then added to the reaction mixture to examine their effects on competition for complex formation (Figure 3B). An addition of a 5-fold excess of nonlabeled poly(8-oxoG·A) efficiently prevented formation of the labeled complex. On the other hand, much larger amounts of polyG and tRNA are required to prevent complex formation.

Effect of Paraquat on Wild-Type and pnp⁻ Mutant Cells. Paraquat is known to induce oxidative stress in metabolically active cells (*22*). We examined the effect of this drug on the growth of two types of *E. coli* cells, one carrying the normal *pnp* gene and the other having a mutated gene. In the presence of 0.6 mM paraquat, growth of wild-type cells was almost completely inhibited, whereas the *pnp*⁻ mutant cells grow under the same conditions (Figure 4A). Introduction of a plasmid carrying the *pnp* gene made the *pnp*⁻ mutant susceptible to paraquat. It should be noted that this effect of paraquat is bacteriostatic but not bactericidal; on removal of paraquat, even wild-type cells grew to produce a colony (data not shown).

The Pnp protein has been defined as a component of the RNA degradosome, which is involved in mRNA turnover in *E. coli* (*23*, *24*). We then examined the effects of mutations of various RNase genes, whose products are involved in RNA metabolism. When mutants defective in various combinations of the *pnp* (for polynucleotide phosphorylase), the *rne* (for RNase E), and the *rnb* (for RNase II) gene were examined for their sensitivity to paraquat, the result shown in Figure 4B was obtained. Only cells defective in the *pnp* gene exhibited hyperresistance to the agent.

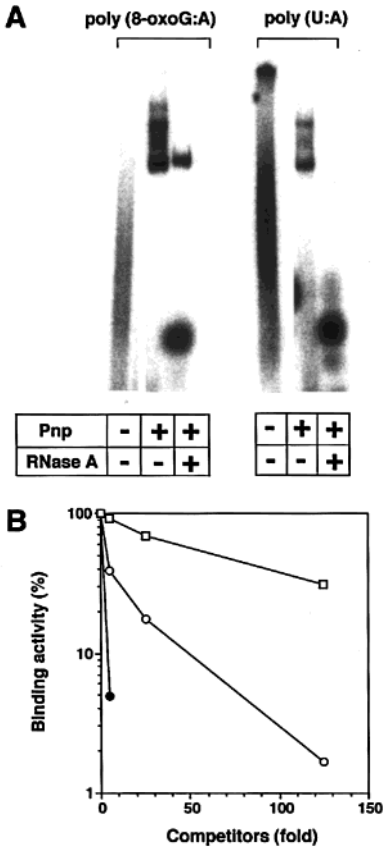

FIGURE 3: Characterization of oxidized RNA-binding protein. (A) Effect of RNase digestion on complexes. Ten picomoles of [^{32}P]-UMP-labeled poly(U·A) and [^{32}P]-8-oxoGMP-labeled poly(8-oxoG·A) were incubated with or without 0.009 unit of the purified Pnp protein in a reaction mixture (5 μL) for 10 min on ice, and then the reaction mixtures were further incubated with or without an excess amount of RNase A (0.4 mg/mL) at 30 °C for 10 min. (B) Competition for binding to 8-oxoguanine-containing polyribonucleotide. Labeled poly(8-oxoG·A) was incubated with 0.009 unit of the purified Pnp protein in a reaction mixture containing various amount of RNAs, including poly(8-oxoG·A), tRNA, and polyG. The levels of radioactivity of the complexes that formed were monitored using a Fuji BAS2000 image analyzer. Relative binding activities were determined by dividing the levels of radioactivity in the presence of competitor RNAs by those without the competitor: (●) poly(8-oxoG·A), (○) tRNA, and (□) polyG.

DISCUSSION

We have shown in a previous study that *E. coli* RNA polymerase can utilize 8-oxoGTP as a substrate, and as a result, 8-oxoguanine was incorporated into RNA at a rate that was 10% of the rate of guanine (*15*). The MutT protein of *E. coli* prevents this misincorporation, by degrading 8-oxoGTP to 8-oxoGMP, a form that cannot be utilized for RNA synthesis. Thus, under normal conditions, misincorporation of 8-oxoguanine into RNA may be limited to a very low level. There is, however, another possible route for yielding 8-oxoguanine in RNA. Guanine residues of nucleic acids may be attacked by active oxygen species, formed through normal cellular metabolism, and this in situ oxidation would occur more frequently in RNA molecules, which are mostly single-stranded, as compared with double-stranded DNA, in which the bases are protected by their hydrogen bonding. Moreover, once the guanine bases in RNA are oxidized, they cannot be repaired. This is in contrast to the case of DNA, in which most of the damaged bases could be excised and replaced through the work of the efficient DNA repair machinery.

Persistence of oxidized forms of guanine in messenger RNA would lead to error in translation. When these situations are considered, it may be important for organisms to be equipped with a certain mechanism that recognizes RNA molecules carrying 8-oxoguanine and eliminates them from the cellular translation machinery. In the study presented here, we found that the *E. coli* cell possesses proteins that

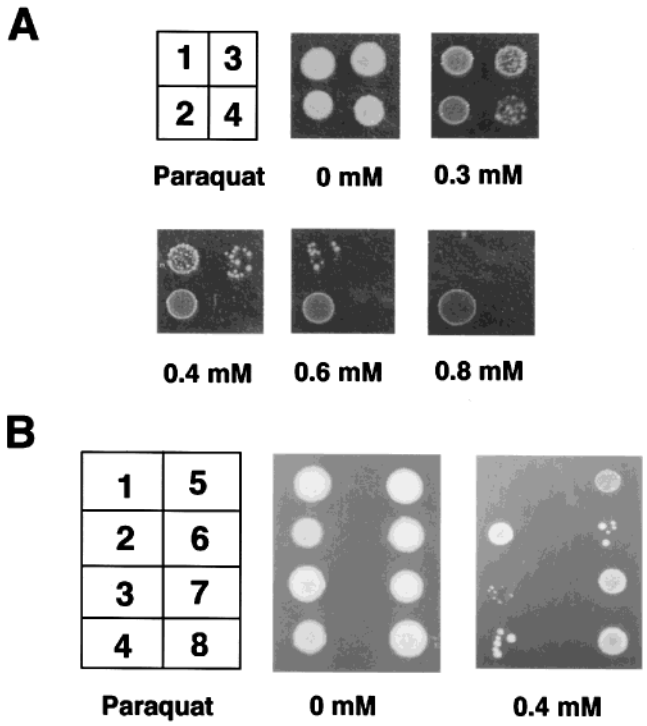

FIGURE 4: Effect of paraquat on growth of cells with or without Pnp protein. Overnight cultures of *E. coli* were diluted 1:1000 with M-salt medium, and 2 μL of dilutes was spotted on L plates containing 10 mg of thymine/L and various amounts of paraquat. (A) Growth of cells with or without *pnp*$^+$ plasmid pB15-6 in the presence of various concentrations of paraquat. The plates were incubated at 37 °C overnight: region 1, MG1693 (wild type); region 2, SK5691 (*pnp*-7); region 3, MG1693 with plasmid pB15-6; and region 4, SK5691 with plasmid pB15-6. (B) Growth of mutant cells defective in combinations of RNase genes in the absence and presence of 0.4 mM paraquat. The plates were incubated at 30 °C for 2 days, because some of them contained ts mutant: region 1, MG1693 (wild type); region 2, SK5691 (*pnp*-7); region 3, SK5665 (*rne*-1); region 4, SK5689 (*rnb*-500); region 5, SK5671 (*pnp*-7, *rne*-1); region 6, SK5715 (*rnb*-500, *rne*-1); region 7, SK5726 (*pnp*-7, *rnb*-500/pDK39[Cm^r *rnb*-500]); and region 8, SK5704 (*pnp*-7, *rne*-1, *rnb*-500).

bind specifically to RNA carrying 8-oxoguanine. On incubation with a cell-free extract, normal RNA is degraded efficiently while 8-oxoguanine-containing RNA is stable under the same conditions. The RNase protection assay revealed that 8-oxoguanine-containing RNA molecules are tightly bound to protein moieties so that RNAs are protected from nuclease attack. In the gel shift assay, two distinct complexes were detected, which exhibit different molecular masses and also different extents of exclusion with a high concentration of polyG. Among them, a 77 kDa protein that binds tightly to 8-oxoguanine-containing RNA was analyzed in detail in this study.

On two-dimensional gel analysis, in conjunction with the gel shift assay using the mutants, we identified it as the Pnp protein. The protein carries polynucleotide phosphorylase activity that catalyzes conversion of polyribonucleotide to ribonucleoside diphosphate on consumption of inorganic phosphate (*25*, *26*). With use of high initial nucleoside diphosphate concentrations, it can produce polynucleotides with random base compositions, according to given amounts of substrate nucleotides. This is the first enzyme found to form a polynucleotide, and has been used extensively in various studies, especially in those related to elucidating the genetic code. Yet the exact physiological function of this enzyme remains to be elucidated.

Polynucleotide phosphorylase has been defined as a component of the RNA degradosome which is involved in mRNA turnover in *E. coli* (*23*, *24*). It has been assumed that mRNA turnover in *E. coli* is initiated by a series of endonucleolytic cleavages catalyzed primarily by RNase E following processive 3′→5′ exonucleolytic degradation by polynucleotide phosphorylase and/or RNase II. The findings that Pnp protein specifically binds to 8-oxoguanine-containing RNA and that *pnp* mutants are hyperresistant to paraquat imply the unique involvement of Pnp protein in cellular oxidative processes. These results can be taken as proof that, even within cell, the Pnp protein is capable of discriminating 8-oxoguanine-containing RNA from normal RNA. Binding of Pnp protein to 8-oxoguanine-containing RNA would inhibit growth, probably due to withdrawal of RNA from the translational machinery. On the other hand, in the *pnp* mutants, the oxidized RNA can enter into the translation process, yielding error-containing proteins. The finding that *pnp* mutants are hyperresistant to paraquat is the first indication that this protein is involved in the cellular oxidative process. At present, how closely the catalytic phosphorylase activity and the binding capacity for 8-oxoguanine-containing RNA are associated is uncertain. These two capacities might be a function of two separate domains of a single protein.

Oxidative damage to RNA is a prominent feature of vulnerable neurons in mammals. In in situ immunostaining analysis, a large amount of 8-oxoguanine was found in the cytoplasm of neurons of Alzheimer's disease patients but not of normal subjects (*27*). The oxidized base appears to be predominantly associated with RNA, because the extents of immunoreactions were diminished by preincubation with RNase but not with DNase. It has also been reported that oxidative damage in cytoplasmic RNA as well as in DNA is associated with the neurodegeneration found in subjects with Parkinson's disease (*28*). Whether mammalian tissues, particularly those related to neurons, contain a protein(s) capable of binding to 8-oxoguanine-containing RNA is the subject of our investigations.

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REFERENCES

1. Gajewski, E., Rao, G., Nackerdien, Z., and Dizdaroglu, M. (1990) *Biochemistry 29*, 7876−7882.
2. Demple, B., and Harrison, L. (1994) *Annu. Rev. Biochem. 63*, 915−948.
3. Kasai, H., Crain, P. F., Kuchino, Y., Nishimura, S., Ootsuyama, A., and Tanooka, H. (1986) *Carcinogenesis 7*, 1849−1851.
4. Ames, B. N., and Gold, L. S. (1991) *Mutat. Res. 250*, 3−16.
5. Bessho, T., Tano, K., Kasai, H., and Nishimura, S. (1992) *Biochem. Biophys. Res. Commun. 188*, 372−378.
6. Wood, M. L., Dizdaroglu, M., Gajewski, E., and Essigmann, J. M. (1990) *Biochemistry 29*, 7024−7032.
7. Moriya, M., Ou, C., Bodepudi, V., Johnson, F., Takeshita, M., and Grollman, A. P. (1991) *Mutat. Res. 254*, 281−288.
8. Shibutani, S., Takeshita, M., and Grollman, A. P. (1991) *Nature 349*, 431−434.
9. Cheng, K. C., Cahill, D. S., Kasai, H., Nishimura, S., and Loeb, L. A. (1992) *J. Biol. Chem. 267*, 166−172.

10. Tchou, J., Kasai, H., Shibutani, S., Chung, M.-H., Laval, J., Grollman, A. P., and Nishimura, S. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 4690−4694.
11. Michaels, M. L., Cruz, C., Grollman, A. P., and Miller, J. H. (1992) *Proc. Natl. Acad. Sci. U.S.A. 89*, 7022−7025.
12. Michaels, M. L., and Miller, J. H. (1992) *J. Bacteriol. 174*, 6321−6325.
13. Tajiri, T., Maki, H., and Sekiguchi, M. (1995) *Mutat. Res. 336*, 257−267.
14. Maki, H., and Sekiguchi, M. (1992) *Nature 355*, 273−275.
15. Taddei, F., Hayakawa, H., Bouton, M.-F., Cirinesi, A.-M., Matic, I., Sekiguchi, M., and Radman, M. (1997) *Science 278*, 128−130.
16. Hayakawa, H., Hofer, A., Thelander, L., Kitajima, S., Cai, Y., Oshiro, S., Yakushiji, H., Nakabeppu, Y., Kuwano, M., and Sekiguchi, M. (1999) *Biochemistry 38*, 3610−3614.
17. Fuller, R. S., Kaguni, J. M., and Kornberg, A. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 7370−7374.
18. Bradford, M. M. (1976) *Anal. Biochem. 72*, 248−254.
19. Kelemen, B. R., Schultz, L. W., Sweeney, R. Y., and Raines, R. T. (2000) *Biochemistry 39*, 14487−14494.
20. Regnier, P., Grunberg-Manago, M., and Portier, C. (1987) *J. Biol. Chem. 262*, 63−68.
21. Reiner, A. M. (1969) *J. Bacteriol. 97*, 1437−1443.
22. Corasaniti, M. T., Strongoli, M. C., Rotiroti, D., Bagetta, G., and Nistico, G. (1998) *Pharmacol. Toxicol. 83*, 1−7.
23. Arraiano, C. M., Yancey, S. D., and Kushner, S. R. (1988) *J. Bacteriol. 170*, 4625−4633.
24. Carpousis, A. J., Vanzo, N. F., and Raynal, L. C. (1999) *Trends Genet. 15*, 24−28.
25. Grunberg-Manago, M., and Ochoa, S. (1955) *J. Am. Chem. Soc. 77*, 3165−3166.
26. Kimhi, Y., and Littauer, U. Z. (1968) *J. Biol. Chem. 243*, 231−240.
27. Nunomura, A., Perry, G., Pappolla, M. A., Wade, R., Hirai, K., Chiba, S., and Smith, M. A. (1999) *J. Neurosci. 19*, 1959−1964.
28. Zhang, J., Perry, G., Smith, M. A., Robertson, D., Olson, S. J., Graham, D. G., and Montine, T. J. (1999) *Am. J. Pathol. 154*, 1423−1429.

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