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Drosophila ribosomal protein PO contains apurinic/apyrimidinic endonuclease activity

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ABSTRACT

***Drosophila* ribosomal protein PO was overexpressed in *Escherichia coli* to allow for its purification, biochemical characterization and to generate polyclonal antibodies for Western analysis. Biochemical tests were originally performed to see if overexpressed PO contained DNase activity similar to that recently reported for the apurinic/apyrimidinic (AP) lyase activity associated with *Drosophila* ribosomal protein S3. The overexpressed ribosomal protein was subsequently found to act on AP DNA, producing scissions that were in this case 5′ of a baseless site instead of 3′, as has been observed for S3. As a means of confirming that the source of AP endonuclease activity was in fact due to PO, glutathione S-transferase (GST) fusions containing a Factor Xa cleavage site between GST and PO were constructed, overexpressed in an *E.coli* strain defective for the major 5′-acting AP endonucleases and the fusions purified using glutathione–agarose affinity column chromatography. Isolated fractions containing purified GST–PO fusion proteins were subsequently found to have authentic AP endonuclease activity. Moreover, glutathione–agarose was able to deplete AP endonuclease activity from GST–PO fusion protein preparations, whereas the resin was ineffective in lowering DNA repair activity for PO that had been liberated from the fusion construct by Factor Xa cleavage. These results suggested that PO was a multifunctional protein with possible roles in DNA repair beyond its known participation in protein translation. In support of this notion, tests were performed that show that GST–PO, but not GST, was able to rescue an *E.coli* mutant lacking the major 5′-acting AP endonucleases from sensitivity to an alkylating agent. We furthermore show that GST–PO can be located in both the nucleus and ribosomes. Its nuclear location can be further traced to the nuclear matrix, thus placing PO in a subcellular location where it could act as a DNA repair protein. Other roles beyond DNA repair seem possible, however, since GST–PO also exhibited significant nuclease activity for both single- and double-stranded DNA.**

INTRODUCTION

DNA damage can have a number of deleterious consequences on the cell, not the least of which is formation of mutations that could lead to malignancy. Another outcome for some aberrant lesions in DNA is that they represent blocks to the progress of DNA replication; furthermore, they have an inhibitory effect on transcription (1). Thus, the preferential nucleotide excision repair of actively transcribed genes would seem to lessen this potential problem. In eukaryotic cells, a number of observations now support the notion that actively transcribed genes are indeed preferentially repaired by nucleotide excision when compared with silent genes (2).

Drosophila, unlike yeast and humans, apparently repair their DNA without regard to active versus silent genes (7), thus making transcription-coupled nucleotide excision repair in this organism unlikely. Instead, *Drosophila* may couple protein translation to DNA base excision repair. This suggestion is based on the recent discovery that ribosomal protein S3 contains an activity that acts on 8-oxoguanine residues in DNA (8). The ribosomal protein also contains an apurinic/apyrimidinic (AP) lyase activity, cleaving such sites by a β,δ elimination reaction (8,9).

Importantly, S3 is one of six human ribosomal genes whose expression is elevated in colorectal cancers (10). Another appears to be a tumor suppressor in *Drosophila*, namely S6 encoded by the *air*8 locus (*a*berrant *i*mmune *r*esponse) (11). Yet another of these ribosomal genes is PO, whose cDNA was originally cloned in *Drosophila* using an antibody prepared against the major AP endonuclease in human cells (12). This result, combined with the observations that the human PO gene is induced by antitumor agents and increased in human cells defective for the repair of O^6-methylguanine (Mer$^-$; 13), seems to suggest that PO may also represent a multifunctional protein with a role in DNA repair. Here we report that *Drosophila* PO acts on abasic DNA, a lesion known to block DNA replication and cause mutations in *Escherichia coli* (14). Notably, PO also contains vigorous DNase activity for both single- and double-stranded DNA. Finally, we show that PO can be found in the nucleus tightly associated with

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the nuclear matrix, where other DNA metabolic proteins have been found.

MATERIALS AND METHODS

Overexpression of PO

The *Drosophila* PO gene was cloned into a pGEX-3X vector (Pharmacia) resulting in PO being produced as a fusion with glutathione S-transferase (GST). The GST–PO fusion contained a Factor X cleavage site so that the two proteins could be proteolytically separated from one another. These fusions were overexpressed in an *E.coli* mutant (RPC501; 15) defective for the AP endonuclease activity associated with exonuclease III (*xth*) and endonuclease IV (*nfo*). The conditions for overexpression and purification of GST–PO were identical to those previously reported for the *Drosophila* GST–S3 fusion construct (8).

Biochemical analysis

A 37 bp 5′-^{32}P-end-labeled duplex DNA fragment [5′-CTTGGAC-TGGATGTCGGCACXAGCGGATACAGGAGCA-3′, where X at position 21 (labeled strand) was uracil] was used as a substrate for *E.coli* uracil-DNA glycosylase (2 U; Epicentre) to form an apyrimidinic site in the place of uracil (16). The resulting abasic site-containing oligonucleotide (AP 37mer) was incubated at 37°C for 30 min with GST–PO in 30 mM HEPES, pH 7.4, 100 mM NaCl, 5 mM $MgCl_2$ and 50 mM KCl. For *E.coli* endonuclease III and the major human AP endonuclease (GST–hAPE) incubations were as previously described (8). The DNA reaction products were then analyzed on a denaturing 16% polyacrylamide gel containing 7 M urea.

Activity on single-stranded DNA was monitored using 1 µg M13mp18 DNA (New England Biolabs) in a reaction that contained 30 mM NaCl, 50 mM KCl, 30 mM HEPES, pH 7.5, 1 mM $ZnSO_4$, 0.1 mM DTT and 0.05% Triton X-100. Incubations (20 min) were at 30°C and the products resolved by agarose (1%) gel electrophoresis.

Inhibition by glutathione–agarose

Purified GST–PO (20 µg) was dialyzed for 3 h at 4°C against 35 mM HEPES, pH 7.5, 50 mM NaCl and 0.05% Triton X-100 (buffer A) and a portion incubated overnight at 4°C with 1 µg Factor Xa (NEB) in a reaction that contained 20 mM Tris–HCl, pH 8.0, 200 mM NaCl and 2 mM $CaCl_2$. This mixture was then dialyzed against buffer A for 3 h at 4°C. GST–PO treated with Factor Xa or untreated GST–PO was then incubated with an equal volume of glutathione–agarose/1× PBS (50 µl) for 4 h at 4°C. The two mixtures were then spun for 5 min in a microcentrifuge and a fraction of the supernatant used to determine activity on the AP 37mer or single-stranded M13.

Complementation studies

To test for sensitivity to methyl methane sulphonate (MMS), wild-type *E.coli* AB1157, its derivative RPC501 [*nfo*-1::Kan-Δ(*xth*-pncA)90] and transformants of RPC501 containing the plasmids for GST–PO, GST and GST–hAPE were utilized. To determine the survival of these strains in MMS, overnight cultures grown at 37°C in LB medium were diluted to an OD_{595} = 0.1. When cultures reached an OD_{595} = 0.5, survival on MMS-containing gradient plates was determined by the method of Cunningham *et al.* (15) with the following modification: cells were grown to mid-log phase instead of stationary phase. The gradient of MMS was established by adding 25 ml molten LB agar containing MMS (1 mM) to a 9-cm square Petri dish elevated at one edge. After the agar hardened, the plate was placed on a flat surface and overlaid with molten LB agar (25 ml). To visualize bacterial growth, plates were stained with 1.5 ml acridine (0.2 mg/ml in ethanol). Afterwards, the plate was photographed under UV light.

Production and purification of antibodies

Anti-*Drosophila* PO antibodies were obtained by injection of rabbits with ~50 µg either native or denatured preparation of purified GST–PO fusion protein (17). Animals were injected with the antigen in an emulsion containing an equal volume of Hunter's TiterMax. Preimmune serum was collected at the time of the first injection using the ear bleed method. Red blood cells were removed from the serum by overnight incubation at 4°C and centrifugation at 3000 *g* for 10 min. Serum (the supernatant) was stored in aliquots at –20°C. Rabbits were anesthetized with ketamine (35 mg/kg) and rompun (5 mg/kg) prior to injections and bleeds. Animals were boosted every 3–5 weeks with an emulsion containing an equal volume of antigen and incomplete Freund's adjuvant.

For affinity purification of anti-PO, ~100 µg purified GST–PO fusion was electrophoresed on a 12% SDS–polyacrylamide gel and blotted onto a nitrocellulose membrane. The region of nitrocellulose which corresponded to the location of the fusion protein was cut into a strip and used to affinity purify the antibody as previously described (9).

Purification of post-nuclear and nuclear fractions

Following the procedure of Fisher *et al.* (18), flies were fractionated into post-nuclear supernatant (cytoplasm) and nuclear components as previously described (9). All steps were performed at 4°C. These fractions were analyzed by SDS–PAGE and Western blotting. For the developmental Western blots, only the post-nuclear supernatant (PNS) and the purified nuclei (N) were fractionated.

Subfractionation of *Drosophila* nuclei

Embryos were collected at 4–24 h, washed with distilled water and dechorionated by rinsing with 0.4% NaCl, 0.03% Triton X-100 for 1 min and with 50% Chlorox for 3 min. Embryos were thoroughly washed with distilled water, blotted dry and weighed. Samples were used immediately or stored at –70°C for several months. Purified nuclei were used for subnuclear fractionation by previously described procedures (9,18). Fractions collected included filtered crude homogenate (FCH), post-nuclear supernatant (PNS), purified nuclei, Triton X-100 wash of nuclei (TX), 2 M NaCl wash (supernatant from this wash; NaCl) and the purified nuclear matrix (NMX). These fractions were analyzed by immunoblotting techniques for the presence of the PO protein.

Nucleoli isolation from nuclei

Nucleoli were isolated using a modification of a previously described technique (19). Purified nuclei were sonicated in 10 vol 0.34 M sucrose/0.05 mM $MgCl_2$ for 1 min. This suspension was underlayed with 0.88 M sucrose/0.05 mM $MgCl_2$ (equal volumes) and centrifuged at 3000 *g* for 20 min. The supernatant contained

nucleoplasm and the pellet consisted of purified nucleoli. Equal amounts of protein from each fraction were run on SDS–PAGE and analyzed using antibody to PO.

Other methods

Drosophila melanogaster (Oregon R) stocks were maintained at 25°C and transferred to fresh medium every 2 or 3 weeks. The pH of all solutions was routinely measured at 50 mM and room temperature. Protein concentrations were determined by the BioRad protein assay. The chemical synthesis of the DNA fragment (37mer) used in this study was performed at the Indiana University School of Medicine.

RESULTS

AP endonuclease activity of PO

The original cloning of the PO gene in *Drosophila* revealed that it encoded a protein that cross-reacted with an antibody that was prepared against the major AP endonuclease present in humans (20). Once fusion constructs of PO were made and overexpressed in wild-type *E.coli*, we found that purified PO most likely acted 5′ of an AP site, as revealed by a Norit assay (21) that has been used in the past to demonstrate whether an existing nick adjacent to an AP site is either 5′ or 3′ of the lesion. In order to rule out the possibility of trace bacterial contamination as an explanation of our results, we subsequently constructed and overexpressed GST–PO fusion constructs in RPC501, which is defective for the 5′-acting AP endonucleases associated with *E.coli* exonuclease III (*xth*) and endonuclease IV (*nfo*). Tests for AP endonuclease activity were performed using a synthetic 5′-end-labeled oligonucleotide (AP 37mer) and the products of the reaction analyzed on a 16% polyacrylamide DNA sequencing gel. Purified GST–PO was found to specifically introduce scissions at the AP site in a protein-dependent manner (Fig. 1, lanes 9–11). The electrophoretic mobility of the cleavage products produced by GST–PO is similar to that produced by GST–hAPE (Fig. 1, lanes 1–3), which is a fusion construct of the major AP endonuclease in humans (22) and known to cleave DNA 5′ of an AP lesion via a hydrolytic mechanism (20). Conversely, the reaction products of GST–PO are unlike that produced by a fusion of *E.coli* endonuclease III (Fig. 1, lanes 5–7), which is a known β elimination catalyst (23).

The results presented in Figure 1 utilized soluble GST–PO fusion proteins purified from RPC501, thus ruling out trace bacterial contamination as an explanation for our results. Further confirmation through immunodepletion studies proved unsuccessful, since our antibody preparations proved to be non-inactivating. Moreover, we were unable to perform immunoprecipitation reactions since GST–PO and PO were extensively precipitated by a number of different commercially available preparations of protein A, a result not unlike that observed by Rich and Steitz for the human PO protein (24). However, we were able to verify the association of AP endonuclease activity with GST–PO in an assay that exploited both the Factor X cleavage site and that portion of the fusion construct containing GST. Briefly, we reasoned that GST–PO AP endonuclease activity, but not PO, should be depleted in the presence of glutathione–agarose. Both the fusion and non-fusion were incubated with glutathione–agarose, microcentrifuged and the supernatant

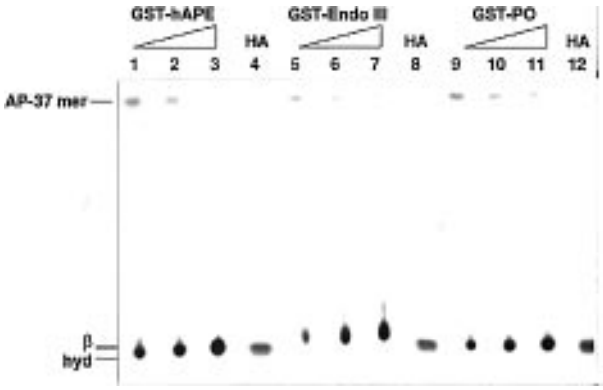

Figure 1. Activity on AP 37mer with different amounts of GST–PO, GST–Endo III and GST–hAPE. Reactions were for 30 min at 37°C and contained 1 pmol AP 37mer. The DNA reaction products were separated on a urea-containing 16% polyacrylaminde gel and analyzed by autoradiography. Lanes 1–3, incubations with 50, 100 and 200 pg GST–hAPE protein respectively. Lanes 4, 8 and 12, hot piperidine (HA) to generate a β,δ elimination reaction. Lanes 5–7, incubations with 100, 200 and 400 pg GST–Endo III protein respectively. Lanes 9–10, incubations with 0.5, 1 and 2 μg GST–PO protein respectively.

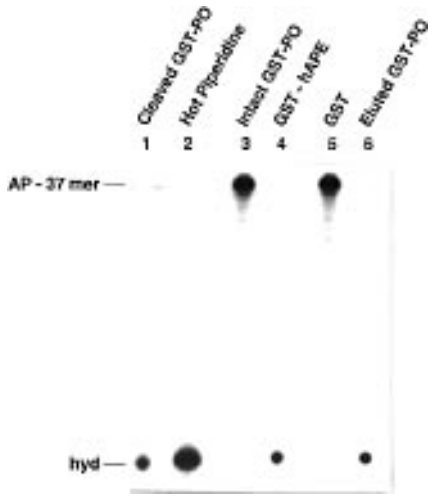

Figure 2. Depletion of GST–PO activity on AP 37mer. GST–PO was either cleaved with Factor X to liberate PO from the fusion (lane 1) or left intact (lane 3) prior to incubation with glutathione–agarose or purified and eluted as described in Materials and Methods either as a fusion (lane 6) or as GST alone (lane 5). For lanes 1, 3, 5 and 6, 1 μg protein was used to identify activity on the AP 37mer. Lane 2, hot piperidine (HA). Lane 4, incubation with 100 pg GST–hAPE protein.

removed and tested for AP endonuclease activity. As can be seen in Figure 2, activity was indeed depleted by glutathione–agarose for the intact GST–PO fusion (lane 3), whereas PO was able to completely process the AP 37mer to a product similar to that seen for the GST–PO fusion that had not been incubated with glutathione–agarose (lane 6). It should be noted that GST, purified under identical conditions as GST–PO, was totally inactive on the AP 37mer (lane 5), once again eliminating the possibility of trace bacterial contamination residing in our GST–PO protein preparations.

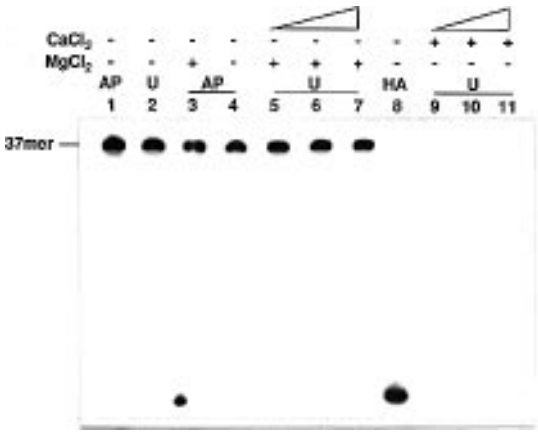

Figure 3. Effect of divalent cations on GST–PO activity. Incubations were for 30 min at 37°C and contained 1 pmol 37mer that was undamaged (U) or contained a single AP site (AP). Divalent cations in the form of $MgCl_2$ or $CaCl_2$ were at 5 mM. Lane 1, AP 37mer alone. Lane 2, undamaged 37mer alone. Lane 3, incubation with 0.2 µg GST–PO. Lane 4, as lane 3 but without $MgCl_2$. Lanes 5–7, incubations with 0.2, 1 and 2 µg GST–PO protein and undamaged DNA (U) respectively. Lane 8, hot piperidine (HA) treatment of AP 37mer. Lanes 9–11, incubations with undamaged DNA (U) in the presence of $CaCl_2$, with 200, 300 and 400 pg GST–PO protein respectively.

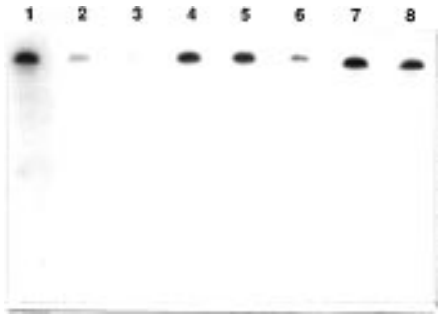

Figure 4. Effect of small molecules on GST–PO activity. Incubations contained 1 pmol undamaged 37mer. Lane 1, 37mer alone. Lane 2, incubations for 30 min with GST–PO (200 pg) and 5 mM $CaCl_2$ or 10 mM $CaCl_2$ (lane 3). Lanes 4–6, incubations with GST–PO (200 pg) containing 10 mM $CaCl_2$ for 5 (lane 4), 10 (lane 5) or 15 min (lane 6). Lanes 7 and 8, incubations for 30 min containing 200 pg GST–PO, 10 mM $CaCl_2$ and 10 mM EDTA (lane 7) or 10 mM EGTA (lane 8).

Figure 5. Activity on single-stranded M13 DNA. GST–PO (lane 2) was either cleaved with Factor X to generate PO (lane 3) and GST (lane 5) or left intact (lane 6) prior to incubation with glutathione–agarose or purified and eluted (lane 4). Lanes 1, 7 and 8, M13 alone. Incubations with M13 were at 30°C for 20 min and contained 10 ng protein (for results presented in lanes 2–5). cc, closed circular DNA; oc, open circles, linear single-stranded DNA.

Reaction conditions and other activities associated with GST–PO

The AP endonuclease activity of GST–PO is dependent upon the presence of $MgCl_2$ (Fig. 3, lanes 3 and 4), where the protein is totally specific for AP DNA under these conditions, failing to act on undamaged DNA (Fig. 3, lanes 5–7). However, a surprising result is obtained when $MgCl_2$ is replaced with $CaCl_2$, when GST–PO becomes completely non-specific (Fig. 3, lanes 9–11). Although not completely revealed in Figure 4, DNA degradation by GST–PO is more extensive in the presence of 10 mM $CaCl_2$ (lane 3) as compared with 5 mM $CaCl_2$ (lane 2). The same can be said for the time-dependent increase in DNA degradation as revealed by incubations (in the presence of 10 mM $CaCl_2$) for 15 min (lane 6) as compared with 5 and 10 min incubations (lanes 4 and 5 respectively). Both 10 mM EDTA and EGTA (lanes 7 and 8) inhibit $CaCl_2$-dependent degradation of the 37mer.

The surprising find that PO possessed a Ca^{2+}-dependent non-specific nuclease activity on a synthetic oligonucleotide prompted us to examine other DNA substrates. Supercoiled phage PM2 DNA was the first to be tested and the results were similar to that observed for the 37mer, $CaCl_2$ supported non-specific nuclease activity towards this substrate (not shown). The same phenomenon was observed using single-stranded M13 DNA. Moreover, $ZnCl_2$ was able to substitute for $CaCl_2$, where its presence led to massive degradation of M13.

To overcome our concerns that the observed non-specific nuclease activity was due to a bacterial contaminate, we repeated the glutathione–agarose depletion assay, described previously for AP endonuclease activity, using M13 as substrate in this case. As seen in Figure 5, supernatants recovered from incubations combining GST–PO and glutathione–agarose showed no activity on M13 (lane 6), whereas supernatants of Factor X-cleaved GST–PO resulted in almost complete degradation of the M13 substrate (lane 3).

Rescue of an *E.coli* mutant sensitive to MMS

Assays performed *in vitro* to show either AP endonuclease activity or non-specific nuclease activity show that far less GST–PO protein is needed to completely degrade a DNA substrate as opposed to producing a site-specific nick adjacent to an AP site. Therefore, a question arises as to whether the AP endonuclease activity of GST–PO represents a significant source of DNA repair activity *in vivo*. To address this, a mutant (RPC501; 15), deficient for the 5′-acting AP endonucleases in *E.coli*, namely exonuclease III and endonuclease IV, was transformed with GST, GST–PO or GST–hAPE and the resulting strains tested for sensitivity to MMS, an alkylating agent known to produce AP sites in DNA (15). As seen in Figure 6, gradient plates containing MMS revealed the ability of GST–PO (lane 3), but not GST (lane 5), to significantly reverse the sensitivity of RPC501 (lane 4) to MMS. It should be noted, however, that GST–PO was not as effective as GST–hAPE (lane 2) in rescuing the sensitivity of RPC501 to MMS.

Production of anti-PO antibodies and developmental Western blot analysis

The foregoing tests suggested that, if indeed PO acted in some DNA repair capacity, its location in the cell would be distributed in places other than ribosomes. As a means of testing this, immunoblot analysis was conducted on subcellular fractions of various developmental stages of *Drosophila*.

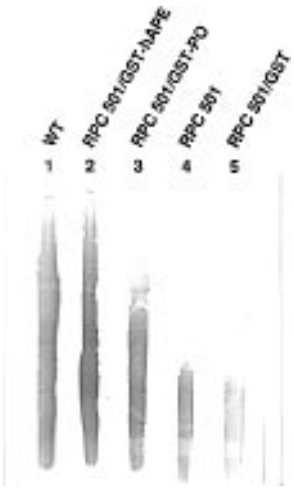

Figure 6. Sensitivity of RPC501 to MMS as revealed by gradient plates. The length of bacterial growth from bottom (LB agar overlay) to top (LB agar containing MMS) is a measure of the strain's resistance to MMS.

Figure 7 depicts typical developmental Western blots showing that PO is present in both the cytoplasm (PNS) and nuclei throughout development. The cytoplasmic fraction was further fractionated into ribosomes, mitochondria and the remaining cytosol. PO was detected associated with ribosomes and in the cytosol, but not mitochondria (data not shown). Furthermore, upon subnuclear fractionation, PO was found tightly associated with the nuclear matrix and the nucleoplasm and only very slightly with isolated nucleoli (Fig. 8). Thus, the presence of PO in the nucleus is not an artifact of nucleoli contamination.

DISCUSSION

Previous studies have shown that PO is associated with the large ribosome and can also be cross-linked to some small subunit proteins, as well as to elongation factor EF2 (25–27). While these observations suggest a role in protein translation, it is noteworthy that PO also appears to exchange on and off the ribosome. Furthermore, the degree of association with ribosomes seems to depend on the level of phosphorylation, where it has been observed that non-ribosomal P proteins are less phosphorylated than those present on the ribosome. This has led to the suggestion that phosphorylation may provide a mechanism for increasing the affinity of PO for ribosomes (28).

As shown here, PO can be serologically found in both the nucleus and in the cytoplasm associated with ribosomes. The protein can be further traced to the nuclear matrix, where a number of DNA metabolic proteins can be found (18). These findings alone support the idea that PO is carrying out more than one function in the cell.

Based upon our findings, it appears that PO could act in a number of different capacities associated with DNA metabolism. One of these could be DNA repair, where it could function as an AP endonuclease. In support of this notion is the ability of PO to significantly restore the survival of RPC501 exposed to MMS. On the other hand, our *in vitro* experiments with the AP 37mer suggest that the AP endonuclease activity of PO is extremely modest, but nevertheless totally specific for AP lesions in the presence of $MgCl_2$.

In an attempt to see if Ca^{2+} could substitute for Mg^{2+} in reactions using the AP 37mer, we discovered that PO no longer behaved as an AP endonuclease, but instead acted non-specifically on both the AP 37mer and the undamaged 37mer. Moreover, only picogram amounts of PO were needed to reveal nuclease activity, whereas much greater amounts of PO were needed to observe strand scissions on the AP 37mer (1 pmol).

We also observed that divalent catons such as Ca^{2+} or Zn^{2+} supported non-specific nuclease activity on double- and single-stranded DNA. The DNase activity of GST–PO is especially evident on M13 DNA, where it appears that the substrate is degraded, therefore suggesting that PO possesses both endo and exonuclease activities.

Another AP endonuclease that contains exonuclease activity is *E.coli* exonuclease III. Interestingly, Ca^{2+} in this case inhibits exonuclease activity (29) and also lowers the k_{cat} for AP sites (5 mM Ca^{2+}) when compared with reactions conducted in the presence of 5 mM Mg^{2+} (30).

It is difficult to propose a biochemical model that would encompass all the activities thus far observed for PO. It is, however, possible that PO acts in a combined divalent cation-dependent AP endonuclease/exonuclease fashion at AP sites that is so rapid in the presence of Ca^{2+} or Zn^{2+} as to make it difficult to resolve the intermediates of the reaction. Under this scenario, it could be concluded that Mg^{2+} only poorly supports the AP endonuclease activity of PO. The ability of PO to rescue the MMS sensitivity

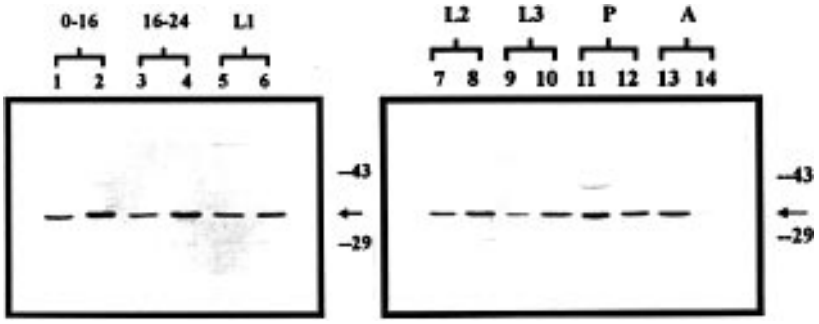

Figure 7. Developmental Western blot analysis of *Drosophila* PO in post-nuclear supernatant and nuclei. Twenty micrograms of protein from either post-nuclear supernatant (lanes 1, 3, 5, 7, 9, 11 and 13) and nuclei (2, 4, 6, 8, 10, 12 and 14) from 0–16 h old embryos (lanes 1 and 2), 16–24 h embryos (lanes 3 and 4), first instar larvae (lanes 5 and 6), second instar larvae (lanes 7 and 8), third instar larvae (lanes 9 and 10), pupae (lanes 11 and 12) and adult males and females (lanes 13 and 14). Samples were electrophoresed, blotted to nitrocellulose and assayed using affinity-purified anti-PO antibody and detected using ^{125}I-labeled anti-rabbit IgG secondary antibody. Arrows point to the 35 kDa PO protein.

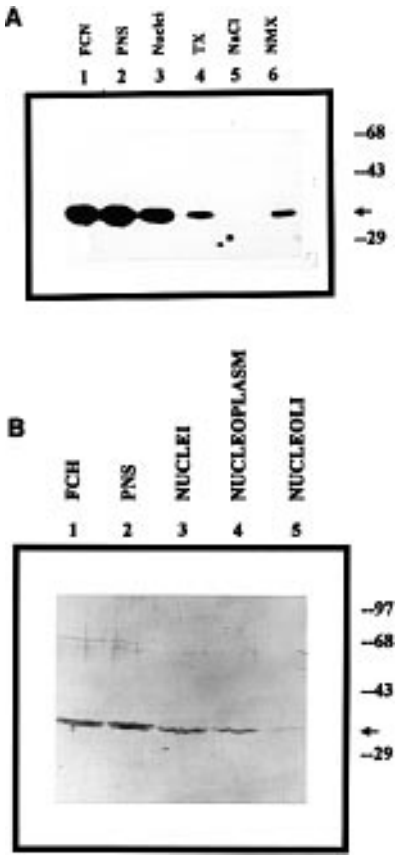

Figure 8. Western blot analysis of nuclear matrix and nucleoli preparations for subnuclear localization of the *Drosophila* PO gene product. (**A**) Embryos (4–24 h) were fractionated into filtered crude homogenate (FCH, lane 1), post-nuclear supernatant (PNS, lane 2), purified nuclei (Nuclei, lane 3), Triton X-100 wash (supernatant after wash, TX, lane 4), 2 M NaCl wash of the nuclei (NaCl, lane 5) and the purified nuclear matrix (NMX, lane 6). Nuclear matrix purification was verified using antibody to topoisomerase II (8). Twenty micrograms of protein were loaded for each fraction. (**B**) Embryos (4–24 h) were fractionated into filtered crude homogenate (FCH, lane 1), post-nuclear supernatant (PNS, lane 2), purified nuclei (lane 3), nucleoplasm (lane 4) and purified nucleoli (lane 5). For both (A) and (B), samples were detected using an affinity-purified PO antibody as described in Materials and Methods and Figure 7. Arrow points to the 35 kDa PO protein.

of RPC501 indicates that the ribosomal protein can function as a DNA repair protein. Another possibility, however, is that the non-specific nuclease activity of PO induces the SOS response, in which case the lesions produced by MMS would be repaired by a pathway other than that initiated by an AP endonuclease.

It may also be possible that sequestering PO to the ribosome would prevent what would otherwise be viewed as a detrimental nuclease activity, as has been suggested for *E.coli* ribosomal protein S16, which also possesses nuclease activity (31). Or, as noted elsewhere (9), it may be that the ribosome frees certain protective proteins as a consequence of stalled mRNA transcription at sites of DNA damage, which would therefore result in lowered ribosomal activity for the release to occur.

ACKNOWLEDGEMENTS

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REFERENCES

1 Sauerbier,W. and Hercules,K. (1978) *Annu. Rev. Genet.*, **12**, 329–363.
2 Drapkin,R., Sancar,A. and Reinberg,D. (1994) *Cell*, **77**, 9–12.
3 Sayre,M.H., Tschochner,H. and Kornberg,R.D. (1992) *J. Biol. Chem.*, **267**, 23376–23382.
4 Feaver,W.J., Svejstrup,J., Bardwell,L., Bardwell,A.J., Buratowski,S., Gulyas,K.D., Donahue,T.F., Friedberg,E.C. and Kornberg,R.D. (1993) *Cell*, **75**, 1379–1387.
5 Guzder,S.N., Qui,H., Sommers,C.H., Sung,P., Prakash,L. and Prakash,S. (1994) *Nature*, **36**, 91–94.
6 Wang,Z., Svejstrup,J.Q., Feaver,W.J., Wu,X., Kornberg,R.D. and Friedberg,E.C. (1994) *Nature*, **368**, 74–75.
7 deCock,J.G.R., Klink,E.C., Ferro,W., Lohman,P.H.M. and Eecken,J.C.J. (1992) *Mutat. Res.*, **293**, 11–20.
8 Yacoub,A., Auglri,L., Kelley,M.R., Doetsch,P.W. and Deutsch,W.A. (1996) *EMBO J.*, **15**, 2306–2312.
9 Wilson,D., Deutsch,W.A. and Kelley,M.R. (1994) *J. Biol. Chem.*, **269**, 25359–25364.
10 Pogue-Geile,K., Geiser,J.R., Shu,M., Miller,C., Wool,I.G., Meisler,A.I. and Pipas,J.M. (1991) *Mol. Cell. Biol.*, **11**, 3842–3849.
11 Watson,K.L., Konrad,K.D., Woods,D.F. and Bryant.,P.J. (1992) *Proc. Natl. Acad. Sci. USA*, **89**, 11302–11306.
12 Kelley,M.R., Venugopal,S., Harless,J. and Deutsch,W.A. (1989) *Mol. Cell. Biol.*, **9**, 965–973.
13 Grabowski,D.T., Pieper,R.O., Futscher,B.W., Deutsch,W.A., Erickson,L.C. and Kelley,M.R. (1992) *Carcinogenesis*, **13**, 259–263.
14 Loeb,L.A. and Preston,B.D. (1986) *Annu. Rev. Genet.*, **20**, 201–230.
15 Cunningham,R.P., Saporito,S.M., Spitzer,S.G. and Weiss,B. (1986) *J. Bacteriol.*, **168**, 1120–1127.
16 Lindahl,T. (1980) *Methods Enzymol.*, **65**, 284–295.
17 Harlow,E. and Lane,D. (1988) *Antibodies: A Laboratory Manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
18 Fisher,P.A., Berrios,M. and Blobel,G. (1982) *J. Cell Biol.*, **92**, 674–686.
19 Chan,P.K., Aldrich,M. and Busch,H. (1985) *Exp. Cell Res.*, **161**, 101–110.
20 Kane,C.M. and Linn,S. (1981) *J. Biol. Chem.*, **256**, 3405–3414.
21 Levin,J.D. and Demple,B. (1990) *Nucleic Acids Res.*, **18**, 5069–5075.
22 Demple,B., Herman,T. and Chen,D.S. (1991) *Proc. Natl. Acad. Sci. USA*, **88**, 11450–11454.
23 Bailly,V. and Verly,W.G. (1987) *Biochem. J.*, 242, 565–572.
24 Rich,B. and Steitz,J. (1987) *Mol. Cell. Biol.*, **7**, 4065–4074.
25 Uchiumi,T., Kikuchi,M., Terao,K. and Ogata,K. (1985) *J. Biol. Chem.*, **260**, 5675–5682.
26 Uchiumi,T., Kikuchi,M. and Ogata,K. (1986) *J. Biol. Chem.*, **261**, 9663–9667.
27 Uchiumi,T., Kikuchi,M., Terao,K., Iwasaki,K. and Ogata,K. (1986) *Eur. J. Biochem.*, **156**, 37–48.
28 Sanchez-Madrid,F., Vidales,F.J. and Ballesta,J.P.G. (1981) *Eur. J. Biochem.*, **114**, 609–613.
29 Rogers,S.G. and Weiss,B. (1980) *Methods Enzymol.*, **65**, 201–211.
30 Takeuchi,M., Lillis,R., Demble,B. and Takeshita,M. (1994) *J. Biol. Chem.*, **269**, 21907–21914.
31 Oberto,J., Bonnefoy,E., Mouray,E., Pellegrini,O., Wilkstrom,P.M. and Rouviere-Yaniv,J. (1996) *Mol. Microbiol.*, **19**, 1319–1330.