Nonhistone Protein BA Is a Glutathione S-Transferase Localized to Interchromatinic Regions of the Cell Nucleus

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Abstract. A DNA-binding nonhistone protein, protein BA, was previously demonstrated to co-localize with U-snRNPs within discrete nuclear domains (Bennett, F. C., and L. C. Yeoman, 1985, Exp. Cell Res., 157:379–386). To further define the association of protein BA and U-snRNPs within these discrete nuclear domains, cells were fractionated in situ and the localization of the antigens determined by double-labeled immunofluorescence. Protein BA was extracted from the nucleus with the 2.0 M NaCl soluble chromatin fraction, while U-snRNPs were only partially extracted from the 2.0 M NaCl-resistant nuclear structures. U-snRNPs were extracted from the residual nuclear material by combined DNase I/RNase A digestions. Using an indirect immunoperoxidase technique and electron microscopy, protein BA was localized to interchromatinic regions of the cell nucleus.

Protein BA was noted to share a number of chemical and physical properties with a family of cytoplasmic enzymes, the glutathione S-transferases. Comparison of the published amino acid composition of protein BA and glutathione S-transferases showed marked similarities. Nonhistone protein BA isolated from saline-EDTA nuclear extracts exhibited glutathione S-transferase activity with a variety of substrates. Substrate specificity and subunit analysis by SDS polyacrylamide gel electrophoresis revealed that it was a mixture of several glutathione S-transferase isoenzymes. Protein BA isolated from rat liver chromatin was shown by immunoblotting and peptide mapping techniques to be two glutathione S-transferase isoenzymes composed of the Yb and Yb' subunits.

Glutathione S-transferase Yb subunits were demonstrated to be both nuclear and cytoplasmic proteins by indirect immunolocalization on rat liver cryosections. The identification of protein BA as glutathione S-transferase suggests that this family of multifunctional enzymes may play an important role in those nuclear domains containing U-snRNPs.
identified as glutathione S-transferase, a family of enzymes generally believed to be involved in xenobiotic detoxification (20).

**Materials and Methods**

**Materials**

1-Chloro-2,4-dinitrobenzene and 1,2-dichloro-4-nitrobenzene were purchased from Eastman Kodak Co. (Rochester, NY). 1-Chloro-2,4-dinitrobenzene was further purified by recrystallization from ethanol. 1,2-Epoxy-3-(p-nitrophen- oxy)-propane, p-nitrophenyl acetate, cumene hydroperoxide, ethacrynic acid, p-nitrophenyl chloride, and N-chlorosuccinimide were purchased from Sigma Chemical Co. (St. Louis, MO). 125I-Nal was purchased from Amersham Corp. (Arlington Heights, IL). N-ethyl-l-phenylalanine chloromethyl ketone-treated trypsin was purchased from Worthington Biochemical Corp. (Freehold, NJ).

**Isolation of Proteins**

Protein BA\textsubscript{normal} was isolated from rat liver chromatin as described by Catino et al. (9). Protein BA\textsubscript{normal} was isolated from 0.075 M NaCl/0.025 M EDTA, pH 8.0 extracts of rat liver nuclei as previously described (3). Individual glutathione S-transferase isoenzymes were prepared from rat liver cytosol as described by Hubig et al. (17). Phenylmethylsulfonyl fluoride (1 mM) and leupeptin (0.1 mM) were added to all cellular extracts to inhibit proteolysis. Total rat liver glutathione S-transferases were purchased from Sigma Chemical Co. Glutathione S-transferase activity towards various substrates was determined by spectrophotometric assays (16). Protein concentrations were determined by the method of Lowry et al. (27).

**Immunoaassay**

Antibodies to protein BA were prepared by immunizing a New Zealand White male rabbit with 50 μg of protein BA\textsubscript{normal} as previously described (2). Immunoblots (44) were performed using primary antibody dilutions of 1:250. Immunoreactivity was detected with 125I-protein A, followed by autoradiography (32). Hybridoma cells secreting monoclonal Sm antibody (24) were a gift from Dr. Joan A. Steitz.

**Peptide Mapping**

Partial N-chlorosuccinimide peptide maps were performed using the method of Lieschwe and Ochs (25) with 5.0 μg of gel purified proteins. Peptides were detected by silver staining (46). For tryptic digests, purified protein BA\textsubscript{normal} and protein BA\textsubscript{normal} (20-50 μg) were labeled with 1 μCi of [35S] by the chloromethane-T procedure to an approximate specific activity of 5 x 10^6 cpm/μg protein (19). Individual subunits were separated on a 10% SDS polyacrylamide gel (23), visualized by brief staining with 0.25% Coomassie Brilliant Blue R, and extracted from a gel slice with 0.1% SDS/0.025 M Tris/0.19 M glycine/1% β-mercaptoethanol, pH 8.3. Extracted protein was precipitated with aceton, redissolved in 200 μl of 0.5 M Tris/100 mM EDTA/8 μm urea/8 μM β-mercaptoethanol, pH 8.5 and carboxy methylated according to the method of Gracy (15). Protein was dialyzed against 200 mM N(H2)CO3, pH 8.0 and digested with three separate additions of 1 μg of N-tosyl-L-phenylalanine chloromethyl ketone-treated trypsin at 0, 3, and 6 h. At 24 h digestion was terminated by freezing and lyophilization. Labeled peptides were dissolved in 0.05 ml of 20% acetic acid and injected onto a Waters C18 μBondapak column (Waters Associates, Milford Corp., Milford, MA). A linear gradient of 0.1% trifluoroacetic acid to 60% acetonitrile containing 0.05% trifluoroacetic acid was developed over 2 h at a flow rate of 2.0 ml/min (14, 29) on a Varian Model 3000 Liquid Chromatograph (Varian Associates, Inc., Palo Alto, CA). Trial runs had shown that no peptides eluted after 60% acetonitrile. Fractions of 2.0 ml were collected and counted in a Beckman gamma 4000 counter (Beckman Instruments, Inc., Palo Alto, CA).

**Immunofluorescence**

Fresh rat liver cryosections (10 μm) were fixed in 2.0% paraformaldehyde diluted in 10 mM sodium phosphate/150 mM NaCl, pH 7.4 (PBS) for 10 min at 25°C. Tissue sections were washed in PBS, permeabilized in aceton for 4 min at -20°C, washed again in PBS for 45 min, then in PBS containing 10% goat serum (Gibco, Grand Island, NY) for 30 min at 37°C. Primary antibodies were diluted in 10% goat serum and incubated with the samples for 1 h at 37°C in a humidified chamber. Samples were washed for several changes of PBS for 1 h before incubation for 45 min at 37°C with 5.0 μg/ml biotinylated goat anti-rabbit IgG diluted in 10% goat serum. Cryosections were again washed in several changes of PBS for 1 h, then incubated with 5.0 μg/ml fluorescein isothiocyanate-conjugated streptavidin (Bethesda Research Laboratories, Gaithersburg, MD) for 45 min at 37°C. Samples were washed four times with PBS, 15 min each, at 25°C, then overnight at 4°C. Samples were mounted in glycerol/PBS and viewed and photographed on a Zeiss epifluorescence microscope (Carl Zeiss, Inc., Thornwood, NY) equipped with a Nikon UFX automatic camera (Nikon Inc., Garden City, NY).

Normal rat liver cells, clone 9, were purchased from the American Type Culture Collection (Rockville, MD). Cells were grown on 22 x 22-mm coverslips in Williams’s medium E (Gibco) supplemented with 10% fetal calf serum and 5 U insulin/liter. Indirect immunofluorescence was performed as previously described (2, 32); polyclonal rabbit antibody against protein BA was detected using a 1:100 dilution of fluorescein-conjugated goat anti-rabbit IgG; monoclonal antibody Y2 against Sm antigen (a gift from Dr. Joan A. Steitz) was detected using a 1:50 dilution of rhodamine-conjugated goat anti-mouse IgG (Cappel Laboratories, Cochranville, PA). In situ nuclear matrices were prepared using the procedure of Staufenbiel and Deppert (42). After each extraction cells were fixed in 2.0% paraformaldehyde and processed for indirect immunofluorescence.

**Immunoelectron Microscopy**

Immunoelectron microscopy (41) was performed on the normal rat liver cells grown in 35-mm petri dishes (Permuno tissue culture dish; Lab-Tek Division, Miles Laboratories, Inc., Naperville, IL). Samples were processed for immunoelectron microscopy according to the following procedure. Cells were fixed in 2% paraformaldehyde in PBS/pH 7.3, made fresh before use, for 1 h at 4°C. Cells were washed in four changes, 15 min each, of PBS/pH 7.3 and incubated in PBS/0.2% Triton X-100/0.5% normal goat serum for 5 min at 4°C. Samples were washed for 30 min in several changes of PBS/pH 7.3, containing 0.5% normal goat serum and incubated with antibody (1:20) or preimmune sera (1:20) for 1 h at 37°C. Cells were washed in several changes of PBS/pH 7.3, the last wash extended to 16 h.

Samples were incubated in peroxidase-conjugated goat anti-rabbit IgG (Cappel Laboratories) at a dilution of 1:20 for 1 h at 37°C and washed for an additional hour in PBS/pH 7.3. Cells were fixed in 2% glutaraldehyde in PBS/pH 7.3 for 30 min, washed in 0.3 M glycine for 15 min, PBS/pH 7.3 for 15 min, and 0.5 M Tris-HCl for 30 min at 4°C. Samples were incubated in 0.05% 3,3'-diaminobenzidine in 0.05 M Tris-HCl/pH 7.6 for 25 min at room temperature and suspended in 0.05% 3,3'-diaminobenzidine supplemented with 0.01% H2O2 for 4 min. Cells were washed in 0.05 M Tris-HCl, pH 7.6 for 30 min and fixed in 2% OsO4 in 0.1 M sodium cacodylate buffer pH 7.3 for 1 h. Samples were rinsed in distilled water, dehydrated in a graded ethanol series followed by 100% propylene oxide, infiltrated, and embedded in Epon 812 (41). Polymerization was carried out at 60°C for 48 h.

Sections were cut on a Sorvall MT-2 ultra micromtome using a SAG Internaional diamond knife and were examined in a JEOL 100 transmission electron microscope (JEOL USA, Peabody, MA) operated at 60 kV.

**Results**

**Co-localization of Protein BA with U-snRNPs**

It was demonstrated in previous studies that protein BA co-localizes with the nuclear domains (2). Co-localization of U2 (3), U1 (2), U4 (2), U5 (2), and U6 snRNAs. Protein BA antibodies produced both a nuclear and a cytoplasmic fluorescence in a normal rat liver cell line fixed with paraformaldehyde and permeabilized with aceton (Fig. 1a). The nuclear staining was inhibited by preabsorption of the antibodies with highly purified protein BA (2). In contrast, the Sm antigens were localized exclusively within the nucleus (Fig. 1 b) as previously demonstrated (2, 24, 31, 41). By using double label immunofluorescence it was possible to determine that protein BA and U-snRNPs were
both concentrated in the same nuclear regions, corresponding to the speckled immunostaining domains (Fig. 1, a and b). Extraction of the cells with 1% Nonidet P-40 before fixation did not result in a change in the number or the location of those regions that contain protein BA (Fig. 1c) or the Sm antigen (Fig. 1d). Some reduction in fluorescence intensity was observed with the Sm antibody, while only a slight reduction in fluorescence intensity was detected with anti-BA
antibody. Extraction of the cells with 1% Nonidet P-40 followed by digestion with DNase I also failed to produce a change in the distribution or the fluorescence intensity resulting from each of the antigens (Fig. 1, e and f). However, protein BA was extracted from the nucleus and cytoplasm with the 2.0 M NaCl soluble chromatin fraction (Fig. 1g), whereas a portion of the Sm antigen remained associated with the high salt resistant nuclear residue (Fig. 1h) as previously demonstrated (30, 41, 50). The Sm antigen was extracted from the high salt resistant nuclear structures by combined DNase I/RNase A digestion (Fig. 1j). Protein BA antibodies stained a small cytoplasmic structure in cells extracted with 2.0 M NaCl (Fig. 1g) and in cells digested with DNase I and RNase A (Fig. 1i), which may correspond to a microtubule organizing center. These results demonstrate that protein BA and U-snRNPs (detectable with the Sm antibody) share common areas of localization in the cell nucleus, but their association with these nuclear domains is mediated by different types of molecular interactions. Protein BA was extracted in the 2.0 M NaCl soluble chromatin fraction, while at least a portion of the U-snRNPs was more tightly associated with the nuclear residue fraction.

**Immunoelectron Microscopy**

Immunoelectron microscopy was used to sublocalize protein BA within the nucleus of the normal rat liver cell line.

Immunoreactivity was detected with peroxidase-labeled goat anti-rabbit antibodies. Protein BA antibodies stained discrete domains within the nucleus, which corresponded to interchromatinic regions (Fig. 2a). Regions of condensed chromatin (c) were not immunostained with protein BA antibodies (Fig. 2a). There was no specific immunoreaction product observed in normal rat liver cells labeled with preimmune immunoglobulins (Fig. 2b). Previous studies have demonstrated that the Sm antibody labels interchromatinic regions of the nucleus (11, 41), thus both protein BA and U-snRNPs are concentrated within interchromatinic nuclear domains.

**Identification of Protein BA as Glutathione S-Transferase**

Comparison of the physical and chemical properties of protein BA with other proteins reported in the literature revealed a marked similarity between protein BA and the glutathione S-transferases (EC 2.5.1.18). The glutathione S-transferases are a family of enzymes, which in addition to their transferase activity, exhibit selenium-independent peroxidase activity (35), steroid isomerase activity (4), and the ability to bind various ligands (20, 26). All glutathione S-transferase isoenzymes are dimeric proteins, forming either homo- or heterodimers from at least six different subunits that range in molecular weight from 25,000 to 29,000 (21). The major glutathione S-transferase isoenzymes found in rat liver are

**Figure 2.** Electron microscopic immunolocalization of protein BA. Normal rat liver cells were incubated with protein BA antibodies (a) or preimmune immunoglobulins (b) followed by peroxidase-conjugated goat anti-rabbit antibodies. Numerous interchromatinic regions appear immunostained (Fig. 2a, arrowheads); however, regions containing condensed chromatin (C in Fig. 2a) are not immunostained with protein BA antibodies. No post-staining. Bars, 1 μm.
Glutathione S-Transferase

**Table 1. Amino Acid Composition of Protein BA and Glutathione S-Transferase**

| Amino acid | Mole percent |
|------------|--------------|
|            | BA\textsuperscript{a} | BA\textsuperscript{b} | Transf B\textsuperscript{a} | Transf B\textsuperscript{b} | Transf C\textsuperscript{a} |
| Ile        | 8.2          | 9.7          | 8.8          | 9.2          | 9.2          |
| His        | 2.8          | 2.2          | 1.6          | 1.5          | 1.6          |
| Arg        | 6.5          | 5.8          | 5.5          | 5.6          | 5.5          |
| Asp        | 9.5          | 11.2         | 11.7         | 9.4          | 11.6         |
| Thr        | 4.3          | 3.9          | 3.4          | 2.8          | 3.2          |
| Glu        | 11.4         | 12.2         | 10.9         | 11.7         | 10.8         |
| Pro        | 6.0          | 5.4          | 5.7          | 5.1          | 5.8          |
| Gly        | 8.8          | 6.0          | 4.9          | 5.4          | 5.5          |
| Ala        | 5.4          | 7.1          | 5.2          | 7.9          | 5.3          |
| Cys        | ND           | ND           | 1.6          | 1.0          | 1.6          |
| Val        | 6.5          | 6.0          | 2.9          | 6.4          | 3.2          |
| Met        | 2.2          | 1.5          | 2.6          | 2.0          | 2.6          |
| Ile        | 5.0          | 5.2          | 5.7          | 4.6          | 5.0          |
| Leu        | 10.0         | 11.6         | 11.7         | 12.8         | 11.8         |
| Tyr        | 3.4          | 3.5          | 6.0          | 3.3          | 5.8          |
| Phe        | 4.1          | 3.9          | 5.2          | 4.3          | 5.8          |
| Trp        | 0.5          | ND           | 1.6          | 2.3          | 1.6          |

\textsuperscript{a}Mole percent determined.
\textsuperscript{b}Values from reference 3.
\textsuperscript{c}Values from reference 17.

Transfase AA (Yc homodimer), transfase A (Yb homodimer), transfase B (Ya, Yc heterodimer), transfase C (Yb,Yb' heterodimer), and transfase D (Yb' homodimer).

The published amino acid composition of both the bound and the free forms of protein BA (3) and glutathione S-transferase isoenzymes A, B, and C (17) are presented in Table 1. There was a marked similarity between the reported amino acid compositions of protein BA\textsubscript{free} and glutathione S-transferase B. A statistical analysis of the values (28) suggested that all of the proteins in Table 1 were closely related, i.e., S values comparing the compositions of BA\textsubscript{bound} with transfases A, B, and C were 51, 39, and 48, respectively. Values comparing the compositions of BA\textsubscript{free} with transfases A, B, and C were 27, 9, and 25, respectively. Values lower than 0.1 indicate relatedness, whereas a value of unity indicates identity (28).

To ascertain whether protein BA was in fact a glutathione S-transferase, the reactivity of protein BA\textsubscript{free} towards various transfase substrates was determined (Table II). The specific activity of protein BA\textsubscript{free} towards these substrates was close to values reported in the literature for glutathione S-transfases (16), thus ruling out the possibility that the enzyme activity was due to contaminating glutathione S-transferase. Furthermore, protein BA\textsubscript{free} was selectively isolated from saline-EDTA nuclear extracts using a glutathione-affinity column (unpublished data). Simons and Vander Jagt (40) have previously reported that glutathione S-transfases selectively bind to glutathione-affinity columns, while other enzymes involved in glutathione metabolism do not bind. These data strongly implicate protein BA\textsubscript{free} as being glutathione S-transferase. Analysis of the substrate specificity of protein BA\textsubscript{free} suggested that it was actually a mixture of several glutathione S-transferase isoenzymes. This was further substantiated by SDS polyacrylamide gel electrophoresis analyses. Protein BA\textsubscript{free} resolved into three distinct polypeptides corresponding to BA\textsubscript{free} \textsuperscript{A}, BA\textsubscript{free} \textsuperscript{B}, and BA\textsubscript{free} \textsuperscript{C} subunits that co-migrate with glutathione S-transferase subunits Ya, Yb, and Yc, respectively (Fig. 3\textit{a}, lane D).

Protein BA isolated from chromatin, protein BA\textsubscript{bound}, does not exhibit enzymatic activity. This was anticipated after considering the rather harsh conditions required to isolate protein BA\textsubscript{bound} from chromatin, which include extraction of chromatin with 0.4 N H\textsubscript{2}SO\textsubscript{4}, denaturation in urea, and preparative gel electrophoresis (9).

**Immunological Analysis**

To identify which glutathione S-transferase subunits correspond to protein BA\textsubscript{bound}, immunoblots were performed. An antibody produced against protein BA\textsubscript{free}, which was known to also recognize protein BA\textsubscript{bound} (Fig. 3\textit{b}, lane C), was used to probe the individual glutathione S-transferase subunits. Protein BA\textsubscript{bound}, as isolated from chromatin, migrates on SDS polyacrylamide gels as a single band between the Yb and Yc subunits (Fig. 3\textit{a}, lane A). Total liver proteins (Fig. 3\textit{a}, lane A), probed with the antibodies that recognize protein BA\textsubscript{bound}, produced an immunoreaction product with a 27,000-mol-wt protein (Fig. 3\textit{b}, lane A). The antibody also reacted with a 27,000-mol-wt protein in protein BA\textsubscript{free} preparations and weakly with a 25,500-mol-wt protein (Fig. 3\textit{b}, lane D). It was determined by exciting the individual protein BA\textsubscript{free} subunits from a polyacrylamide gel and re-electrophoresis on an SDS polyacrylamide gel (Fig. 3\textit{a}, lanes E–G), followed by immunoblotting, that the major immunoreactive polypeptide corresponded in migration to the BA\textsubscript{free} subunits (Fig. 3\textit{b}, lane F). This was further supported by the strong immunoreaction product obtained with glutathione S-transferase A purified from rat liver cytosol, which is a homodimer of Yb subunits (Fig. 3\textit{b}, lane B). The BA\textsubscript{free} subunit produced a very weak immunoreaction product (Fig. 3\textit{b}, lane G).

The greater molecular weight observed for protein BA\textsubscript{bound} when compared to the Yb subunits was probably caused by the purification procedure, since total liver proteins probed with the antibody produced only one band on immunoblots. This band corresponded in migration to the Yb subunits (Fig. 3\textit{b}, lane A). The antibody reacts equally well with glutathione S-transferase Yb subunits purified either from nuclei or cytoplasm (Fig. 3\textit{b}, lanes B and D). These data suggest that protein BA\textsubscript{bound} corresponds to the Yb subunits of glutathione S-transferase, and that Yb subunits isolated from rat liver cytosol are immunologically related to BA\textsubscript{free} subunits isolated from nuclei.

**Table II. Substrate Specificity of Protein BA\textsubscript{free}**

| Substrate                      | Specific activity (\textmu mol/min per mg) |
|-------------------------------|------------------------------------------|
| 1-Chloro 2,4-dinitrobenzene   | 11.05                                    |
| 1,2-Dichloro 4-nitrobenzene   | 0.64                                     |
| 1,2-Epoxy 3-(p-nitrophenoxy)-propane | ND                                         |
| p-Nitrophenyl acetate         | 0.26                                     |
| Cumene hydroperoxide          | 4.13                                     |
| Ethacrynic acid               | 0.55                                     |
| p-Nitrobenzyl chloride        | 0.86                                     |

Substrate specificity of nuclear glutathione S-transferase. The specific activity of glutathione S-transferase isolated from saline-EDTA rat liver nuclear extracts was determined as described in Materials and Methods. ND, no detectable activity under conditions used.
Figure 3. Immunoblot analysis of glutathione S-transferase subunits. Proteins were separated on a 12.5% SDS polyacrylamide gel (23) and either stained with Coomassie Brilliant Blue R (a) or transferred to nitrocellulose paper (44) and reacted with antibodies directed against protein BA (b). Lanes: (A) 150 μg total rat liver proteins; (B) 5 μg glutathione S-transferase A (Yb homodimer); (C) 5 μg protein BA_{bound}; (D) 10 μg protein BA_{free}; (E) BA_{free} subunits; (F) BA_{free} subunits; (G) BA_{free} subunits.

Peptide Mapping

To further show that protein BA_{bound} was homologous to the Yb subunits of glutathione S-transferase, peptide fragments provided by two different methods were compared. Partial peptide maps of cytosolic Yb subunits (Fig. 4, lane A), protein BA_{free} subunits (Fig. 4, lane B), glutathione S-transferase A (Fig. 4, lane C), and protein BA_{bound} (Fig. 4, lane D) were obtained using the N-chlorosuccinimide procedure (25), which cleaves tryptophanyl peptide bonds (39). The Yb sub-
Units were isolated from cytoplasmic glutathione S-transferase (Fig. 4, lane A) and protein BA\textsubscript{free} (Fig. 4, lane B) by excising the bands from an SDS polyacrylamide gel (23). BA\textsubscript{free} subunits isolated from nuclei and Yb subunits isolated from cytoplasm gave identical peptide fragments (Fig. 4, lanes A and B). N-Chlorosuccinimide cleavage of glutathione S-transferase A (Yb homodimer) yielded four peptides with molecular weights of 21,000, 17,000, 11,000, and 6,300. These data suggest that there are at least two tryptophan residues in the Yb subunits. Protein BA\textsubscript{bound} gave a very similar partial peptide fragment pattern to that obtained for glutathione S-transferase A. The BA\textsubscript{free} subunits from nuclear and Yb subunits from cytoplasmic glutathione S-transferases produced very similar partial peptide patterns to those obtained for glutathione S-transferase A and protein BA\textsubscript{bound}. The peptides near uncleaved Yb and BA\textsubscript{free} subunits (Fig. 4, lanes A and B) represent contamination by BA\textsubscript{free} subunits when the BA\textsubscript{free} subunits were excised from the gel.

Further demonstration that protein BA\textsubscript{bound} corresponded to the Yb subunits of glutathione S-transferase was provided by high performance liquid chromatography analysis of \textsuperscript{125}I-tryptic peptides. The individual subunits of protein BA\textsubscript{free} (BA\textsubscript{free}, BA\textsubscript{free}, and BA\textsubscript{free}) share some homologous peptides (Fig. 5, h, c, and d); however, upon close examination it was evident that there are differences between them. These data agree with other reports suggesting that the individual glutathione S-transferase subunits are related but distinct polypeptides (5, 13, 45). The \textsuperscript{125}I-tryptic peptide pattern obtained for protein BA\textsubscript{bound} (Fig. 5A) was very similar to that obtained for BA\textsubscript{free} subunits (Fig. 5C) and distinct from BA\textsubscript{free} subunits (Fig. 5B) and BA\textsubscript{free} subunits (Fig. 5D), providing further evidence that protein BA\textsubscript{bound} isolated from chromatin is most similar in peptide composition to the Yb subunits of glutathione S-transferase. The \textsuperscript{125}I-tryptic peptide patterns obtained for subunits BA\textsubscript{free} and BA\textsubscript{free} isolated from nuclei were virtually identical to the patterns obtained for cytoplasmic glutathione S-transferase subunits Ya and Yc (data not shown).

**Immunolocalization of Glutathione S-Transferase Yb subunits**

The subcellular localization of the glutathione S-transferases has been controversial (1, 6, 12, 37). The differences in results may be due to a different subcellular localization for each isoenzyme, or subunit (37). To confirm that glutathione S-transferase Yb subunits (protein BA\textsubscript{free} and BA\textsubscript{bound}) were in fact nuclear proteins in rat liver and not contaminants produced by the nuclear isolation procedure or growth of cells in culture, we localized the Yb subunits in rat liver cryosections. Antibodies reactive against protein BA\textsubscript{free} and BA\textsubscript{bound} (Fig. 3b, lanes C and F) produced both a mottled nuclear fluorescence and a generalized cytoplasmic fluorescence (Fig. 6, a and b). Preimmune immunoglobulins produced a weak cytoplasmic fluorescence (Fig. 6, c and d). Thus, glutathione S-transferases are found both in the nucleus and in the cytoplasm of rat liver and in an established rat liver cell line. The more intense and generalized fluorescence is thought to result from nuclear localization.

![Figure 4](https://example.com/figure4.png)

**Figure 4.** N-Chlorosuccinimide partial peptide cleavage of Yb subunits. The Yb subunits of cytoplasmic rat liver glutathione S-transferase (a mixture of AA, A, B, C, D, and E) (A), protein BA\textsubscript{free} (B), glutathione S-transferase A (C), and protein BA\textsubscript{bound} (D) were excised from a gel and treated with N-chlorosuccinimide as described in Materials and Methods. Peptides were detected by silver staining (46).

![Figure 5](https://example.com/figure5.png)

**Figure 5.** \textsuperscript{125}I-Tryptic peptides of nuclear glutathione S-transferase subunits. Proteins were iodinated, separated by SDS polyacrylamide gel electrophoresis (23), eluted from gel slices, and digested with trypsin as described in Materials and Methods. \textsuperscript{125}I-Tryptic peptides were separated by reverse phase high performance liquid chromatography on a C18 μBondapak column. (A) Protein BA\textsubscript{bound}; (B) BA\textsubscript{free} subunits; (C) BA\textsubscript{free} subunits; (D) BA\textsubscript{free} subunits.
largely from the 40-fold greater amount of transferase in liver than in cultured hepatocytes (unpublished data).

Discussion

There have been numerous nonhistone proteins that have been identified in the literature (reviewed in reference 7). However, relatively few of these proteins, including such widely studied proteins as the HMG proteins, have been identified with an enzyme activity or assigned a specific function. In this report we have identified nonhistone protein BA (3, 9) as glutathione S-transferase. This conclusion was based on homologies in amino acid composition, immunological identification, peptide maps, and enzymatic activity. Using the criterion mentioned above, we have determined that protein BA isolated from the saline-EDTA nuclear extract (protein BA\text{free}) was a mixture of several glutathione S-transferase isoenzymes, composed of BA\text{a}^{\text{free}}, BA\text{b}^{\text{free}}, and
BA\textsubscript{free} subunits, that co-migrate with cytoplasmic Ya, Yb, and Yc subunits, respectively. Protein BA isolated from rat liver chromatin (protein BA\textsubscript{bound}) was determined to be composed of glutathione S-transferase Yb subunits. Two-dimensional, non-equilibrium, isoelectric focusing/SDS polyacrylamide gel analysis has demonstrated that protein BA\textsubscript{bound} exhibits two major pl variants, with the majority of the protein present in the more basic form (3). Thus, protein BA\textsubscript{bound} is probably a mixture of subunits corresponding to Yb and Yb' cytoplasmic glutathione S-transferase subunits, with the more basic Yb subunit (13) comprising the majority of the protein.

Antibodies that recognize glutathione S-transferase Yb subunits stained discrete regions of the cell nucleus that were also shown to contain U-snRNPs (2), which have been implicated in RNA splicing (33, reviewed in reference 36). Thus, we have identified an enzyme generally thought to be involved in detoxification (20) in the same interchromatinic domains where RNA processing occurs. The associations of BA\textsuperscript{8} subunits and U-snRNPs to these regions are mediated by different types of molecular interactions. The BA\textsuperscript{8} subunits were associated with the 2.0 M NaCl soluble chromatin fraction, while a portion of the U-snRNPs remained associated with the nuclear residue.

The nuclear domains that contain both the BA\textsuperscript{8,free} subunits, BA\textsuperscript{bound}, and U-snRNPs (11, 41) were shown by immunoelectron microscopy to be the interchromatinic regions of the nucleus. Previous studies demonstrated that protein BA\textsuperscript{bound} was localized to areas of condensed chromatin in isolated rat liver nuclei (8). This variance is probably the result of antigen redistribution during nuclear isolation or differences in epitope specificity between this antibody and the one used in previous studies. Our studies have shown that when rat liver is homogenized in aqueous buffers (pH 7–8) most, but not all, of the immunological and enzymatic activity are recovered in the soluble fraction (2, unpublished results). Thus, amounts of protein BA\textsubscript{free} and BA\textsubscript{bound} are thought to diffuse out of the nucleus during cellular fractionation.

The precise function or functions of the glutathione S-transferases in the cell nucleus are not known. The possibility that would seem to be the most obvious is the biotransformation of electrophilic compounds that have escaped detoxification in the cytoplasm thus preventing their interaction with DNA or other sensitive macromolecules. Many chemical carcinogens are known to form conjugates with glutathione, and in many cases, this reaction is catalyzed by the glutathione S-transferases (10). Glutathione S-transferases inhibit the formation of covalent adducts between benzo(a)pyrene and DNA in vitro and in vivo (18, 22). Furthermore, administration of anticarcinogenic antioxidants results in an increased synthesis of glutathione S-transferases, which is one proposed mechanism for their protective action (34).

A second possibility is that they may play a regulatory role in nuclear function. The discrete subnuclear localization of the Yb subunits to areas that contain U-snRNPs (2) suggests that these subunits function in or adjacent to regions of the nucleus where transcription or processing of RNA takes place. The Yb subunits have also been shown to associate with DNA in vitro (3, 9) and with DNA-containing structures in vivo (2, and Fig. 1). Granted, the amounts of glutathione S-transferase in the cell (0.1 mM in rat liver, reference 43) would seem to argue against a role involving specific gene regulation, however, this does not rule out the possibility that glutathione S-transferases play some, as yet undefined, role in modulating gene expression. It has recently been reported that actin, a major cellular protein, is involved in the transcription of chromosome loops in Pleurodeles oocytes (38), suggesting that abundant cellular proteins may play a role in the transcriptional process.

Although a specific role for the glutathione S-transferases in modulating gene function has not been demonstrated, there is some evidence to suggest that they may do so. Further experiments addressing this question are needed. The identification of nonhistone protein BA as glutathione S-transferase indicates an enzymatic activity for a previously described nonhistone protein, while the identification of glutathione S-transferase as a DNA-binding nonhistone protein opens up the possibility of nuclear interactions for this group of multifunctional enzymes.

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