A monoclonal antibody recognizes a human nuclear protein resembling *Xenopus* oocyte nucleoplasmin

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**Summary**

The monoclonal antibody 10BG2 (IgG3) was derived from a mouse immunized with human pre-B cells. In immunofluorescence studies the antibody revealed a human nuclear-associated determinant, which in interphase cells was entirely restricted to the nucleus. In metaphase cells 10BG2 antigen was detected throughout the cytoplasm with intensified staining at the periphery of chromosomes. 10BG2 antibody stained all human normal and transformed cells tested. In contrast, the antibody did not stain mouse 3T3 cells or Chinese hamster ovary (CHO) cells. Electrophoresis under denaturing and non-denaturing conditions revealed the 10BG2 antigen to be a 130 x 10³ to 140 x 10³ Mr protein with a subunit molecular weight of 29.5 x 10³. This suggests the protein is at least a tetramer. On two-dimensional gels the 10BG2 antigen had a streaked appearance and separated into two isoelectric variants (pH 5.2, 5.4). The protein was also shown to be phosphorylated and thermostable, and remained in solution at pH 3-6. 10BG2 antigen was highly soluble in aqueous buffers and co-migrated on non-denaturing gels with the nucleosome-assembly protein, nucleoplasmin, purified from *Xenopus* oocytes. The similarity of 10BG2 antigen to nucleoplasmin is discussed.

Key words: nucleoplasmin, nuclear protein, monoclonal antibody.

**Introduction**

The nucleosome represents the fundamental DNA packing unit in the eukaryotic chromosome. Consequently an understanding of nucleosome assembly and function is a prerequisite to a fuller understanding of gene regulation and function. Fractionation of a cell-free nucleosome assembly system from eggs of *Xenopus laevis* has led to the identification of a protein that facilitates nucleosome core assembly when histones and DNA are rapidly mixed *in vitro* (Laskey et al. 1978). The protein, which has been termed nucleoplasmin, represents approximately 10% of the total *Xenopus* oocyte nuclear protein and is present within the nucleus at similar concentrations to those at which nucleosomes assemble *in vitro* (Krohne & Franke, 1980a; Mills et al. 1980). Thus it is possible that nucleoplasmin also plays a role in nucleosome assembly *in vivo*. Nucleoplasmin purified from *Xenopus* oocytes has a native molecular weight of 145 x 10³ Mr and is composed of five 29 x 10³-30 x 10³ Mr subunits (Earnshaw et al. 1980) and is highly phosphorylated (Krohne & Franke, 1980a). A high content of potentially charged amino acids gives the protein an acidic isoelectric point (Mills et al. 1980). The protein is further characterized by being thermostable up to 80°C and by its extreme solubility in aqueous buffers, and it will remain in solution when the pH is lowered to 3-6 with acetic acid (Mills et al. 1980).

Biochemical and immunohistological studies using polyclonal antisera raised against *Xenopus* nucleoplasmin have revealed an entirely nuclear restriction of the protein (Krohne & Franke, 1980b). Polyclonal antisera have been used to demonstrate that nucleoplasmin is also present in *Xenopus*, chicken, rodent and human somatic cells (Krohne & Franke, 1980b). However, in these studies biochemical analyses were not used to demonstrate that the antigen recognized in these cells was nucleoplasmin or some antigenically similar protein. The availability of monoclonal antibodies reactive with nucleoplasmin would permit more precise study...
of its subcellular localization, its biochemistry, and its role in nucleosome assembly.

In this paper we describe the identification and characterization of the monoclonal antibody 10BG2, which recognizes a human nuclear protein with extensive biochemical similarities to nucleoplasmin. The 10BG2 antibody has been used to investigate the subcellular and tissue distribution of the nucleoplasmin-like protein.

Materials and methods

Cells and cultures

The following cell lines were maintained in RPMI 1640 medium (Gibco, Paisley, Scotland, UK) supplemented with 10% (v/v) foetal calf serum (FCS) (Gibco, Paisley, Scotland, UK), penicillin (100 units ml−1) and streptomycin (50 μg ml−1) (Gibco UK, Paisley, Scotland); the promyeloid cell lines HL60 (Collins et al. 1977) and ML1 (Takeda et al. 1982); the early erythroid cell line K562 (Lozzio & Lozzio, 1964); the B cell lines EB2 and ARH77 (Epstein et al. 1964; Burk et al. 1978); and the epithelial cell line H.Ep2 (Toolan, 1934). A simian virus 40 (SV40)-transformed human foetal keratinocyte cell line SV6-IBAM/HFK (provided by Dr P. H. Gallimore, Department of Cancer Studies, University of Birmingham) was maintained in Dulbecco’s modification of Eagle’s minimal essential medium (DME) supplemented with 10% (v/v) FCS (Sera Laboratories, Crawley Down, UK). The keratinocyte cell line SCC25 (provided by Dr E. K. Parkinson, Department of Cancer Studies, University of Birmingham) was maintained in DME supplemented with 20% (v/v) FCS and 0.4 μg ml−1 hydrocortisone. Details of culture conditions for primary cultures of fibroblasts (provided by Dr A. M. R. Taylor, Department of Cancer Studies, University of Birmingham), umbilical cord endothelial cells (provided by Ms C. Garner, Department of Investigative Pathology, University of Birmingham) and epidermal keratinocytes (provided by Dr E. K. Parkinson) have been described (Brown et al. 1985). Normal lymphocytes were prepared from peripheral blood using Ficoll-Trisol (Pharmacia AB, Uppsala, Sweden). Cells were washed three times in balanced buffered saline solution (BBSS) (Shortman et al. 1972) containing 0.1% bovine serum albumin (BSA) (Sigma, Poole, UK).

10BG2 monoclonal antibody

The monoclonal antibody 10BG2 (IgG3) was derived from a fusion experiment in which a Balb/c mouse was immunized with the pre-B cell line SMS/SB derived from a patient with acute lymphoblastic leukaemia. Mice were injected intraperitoneally with 1×107 cells and three weeks later boosted with 5×106 cells intraperitoneally and 1×107 cells intravenously. Three days later the spleen was removed for cell fusion. Hybridoma supernatants were screened by indirect immunofluorescence against air-dried acetone-fixed multipot preparations of the immunizing cell line. The 10BG2 antibody-stained nuclei of SMS/SB cells and was selected for detailed analysis. Procedures for cell fusion, cloning and characterization of hybridoma cultures have been described in detail (Brown et al. 1981).

Detection of 10BG2-reactive nuclear antigen

The presence of 10BG2-reactive antigen within various cell types was investigated by indirect immunofluorescence. Cyto-centrifuge (Shandon cytocentrifuge II, Runcorn, UK) preparations of cells were obtained by centrifugation at 500 revs min−1 for 3 min and either: (1) thoroughly air dried and fixed in acetone at room temperature for 3 min; or (2) fixed for 30 min at room temperature in 3:7% formaldehyde prepared in 0.1 M-phosphate buffered saline, pH 7.3 (PBS), and then rinsed with PBS; or (3) air dried and fixed in methanol for 5 min at −20°C. Frozen sections of tonsil and muscle, cut at 5 μm, were fixed in acetone for 10 min at room temperature prior to staining. 10BG2 antibody was applied as tissue culture supernatant and revealed using fluorescein-conjugated sheep antibody to mouse immunoglobulin. The staining procedure has been described in detail (Brown et al. 1985). Slides were mounted in buffered glycerol (pH 8.6) containing 25 g l−1 1,4-diazobicyclo[2.2.2]-octane (DABCO) to retard fading of fluorescence during microscopy (Johnson et al. 1982) and studied using a Leitz microscope equipped with a HBO50 mercury burner for incident illumination. Monoclonal antibodies that react with histone H2B (HBC-7 (Turner, 1982)), DNA (33; a gift from Drs A. Morgan and N. Staines, Department of Immunology, Kings College, London) and a nuclear envelope protein (AGF 2.3; Brown et al. 1983) were used as controls. Nuclei isolated from HL60 cells by using 2% Tween 40 to disrupt the cell membrane (Standing & Williams, 1978) were also incubated in suspension with 10BG2 antibody and the above control antibodies. Fluorescein-labelled sheep antibody to mouse immunoglobulin was used as second antibody. 10BG2 antibody binding to isolated HL60 nuclei, which had been fixed in 0.25% glutaraldehyde, was assessed using an indirect radioactive binding assay and 125I-labelled (Fab)’2 rabbit anti-mouse immunoglobulin (5 μCi μg−1) as the second antibody (Brown et al. 1986). The fixation procedure and binding assay as used with whole cells have been described in detail (Morris & Williams, 1975).

Immunoprecipitation, electrophoresis and immunostaining

HL60 cell proteins were internally radiolabelled with [35S]methionine as described (Brown et al. 1985). [35S]methionine-labelled extracts were also prepared from the cells of the marine phasmacytoma cell line NSI, which do not express the 10BG2 antigen. These extracts were used as controls in immunoprecipitation experiments using 10BG2 antibody. HL60 cells were also labelled with 32P to determine whether the 10BG2 antigen was phosphorylated. Cells were washed twice in PO4-free Locke’s solution supplemented with 5 mM-Hepes (pH 7.4), glucose (2 mg ml−1) and BSA (2 mg ml−1) and preincubated in this buffer at 3×106 ml−1 for 30 min at 37°C. 32PO4 (10 mCi ml−1) was added to the incubation flask to a final concentration of 200 μCi ml−1 and the incubation continued for 3 h. Cells were then washed twice in ice-cold PO4-free Locke’s solution containing 5 mM-Hepes (pH 7.4). The cell pellet was taken up in ice-cold 10BG2 extraction
buffer (20 mM-Tris-HCl, pH 8.0, containing 0.2 mM-EGTA, 5 mM-NaF, 1 mM-phenylmethylsulphonyl fluoride (PMSF) and 0.1% Triton X-100), pipetted vigorously and the supernatant was collected using a Beckman microfuge and stored at -20°C. The 32P-labelled proteins were analysed by non-denaturing gel electrophoresis and were also used in immunoprecipitation experiments. The procedure for immunoprecipitation of Triton-soluble proteins has been described in detail (Brown et al. 1985).

Triton-soluble extracts were also prepared from unlabelled cells as follows. Exponentially grown cells (1 X 10^7) were washed twice in cold BBSS and the pellet taken up in 100 µl of 10BG2 extraction buffer. Twenty five units of micrococcal nuclease (Sigma, Poole, UK) were added and the cells incubated for 5 min at 37°C, after which the sample was placed on ice and 400 µl of an appropriate electrophoresis sample buffer was added. For non-denaturing gel electrophoresis sample buffer contained 0.12 mM-Tris-HCl, pH 6.7, 20% glycerol and 0.0025% Bromophenol Blue (Bio-Rad, Watford, UK). For electrophoresis of samples under denaturing conditions 2.5% SDS and 10% β-mercaptoethanol (Bio-Rad, Watford, UK) were also added to the sample buffer. Extracts taken up in denaturing sample buffer were incubated in a boiling water bath for 5 min. Insoluble material was removed from all electrophoresis samples using a Beckman microfuge. Electrophoresis of cell extracts in gels containing 4-12% polyacrylamide was performed as described by Laemmli (1970) with the omission of SDS from both the gels and buffers in the case of non-denaturing electrophoresis. For immunoblotting proteins were electrophoretically transferred from non-denaturing gels onto nitrocellulose filters (BA 85, Schleicher and Schuell, Dassel, W. Germany) by the procedure of Towbin et al. (1979), but excluding methanol from the transfer buffer. Nitrocellulose filters were then immunostained for the presence of 10BG2 antigen using a 125I-labelled (Fab')2 rabbit anti-mouse immunoglobulin as the second antibody. Xenopus oocyte nucleoplasmin (kindly supplied by Dr M. A. Sheehan), which had been affinity purified using a specific monoclonal antibody (PA1C2), was electrophoresed on non-denaturing polyacrylamide gels in parallel with Triton extracts of HL60 cells. After blotting the proteins onto nitrocellulose filters, individual lanes were cut and immunostained with 10BG2 antibody when stained in suspension, but failed to stain air-dried and unfixed cytocentrifuged preparations of HL60 cells. Air-dried cytocentrifuged preparations of cells that had been pre-incubated with BBSS for 10 min at room temperature, prior to fixation in acetone, were unstained by 10BG2 antibody. HL60 nuclei isolated using Tween 40, were also unreactive with 10BG2 antibody when stained in suspension, but were intensely stained by monoclonal antibodies to DNA. In contrast to monoclonal antibodies to histone 2B, DNA and a nuclear envelope protein, 10BG2 antibody failed to stain air-dried and unfixed cytocentrifuged preparations of HL60 cells. Air-dried cytocentrifuged preparations of cells that had been pre-incubated with BBSS for 10 min at room temperature, prior to fixation in acetone, were unstained by 10BG2 antibody. HL60 nuclei isolated using Tween 40, were also unreactive with 10BG2 antibody when stained in suspension, but were intensely stained by monoclonal antibodies to DNA. In contrast, 10BG2 antibody bound well to isolated HL60 nuclei that had been fixed in 0-25% glutaraldehyde, giving 11 X 10^3 cts min^-1 per 10^6 nuclei in indirect radiobinding assays over a background of 1.5 X 10^3 cts min^-1 per 10^6 nuclei. Glutaraldehyde-fixed nuclei were also reactive with antibodies to DNA and histone 2B. These results suggest that the 10BG2 antigen can readily diffuse out of the nuclei unless immobilized by fixation. The possibility that 10BG2

Partial purification of 10BG2 antigen

In order to assess the stability of the 10BG2 antigen to heat and acid, the antigen was partially purified on a sucrose gradient. Nuclei prepared from 10^6 HL60 cells were extracted for 10BG2 antigen as described above. A 1 ml sample of the extract was loaded onto a 13 ml sucrose gradient (10% to 40%) and spun at 37500 revs min^-1 (MS665 ultracentrifuge, 6 X 14 ml swing-out rotor) for 16.5 h at 4°C. Fractions (0.5 ml) were collected from the sucrose gradient and were analysed for the 10BG2 antigen by inhibition of 10BG2 antibody binding to isolated nuclei as assessed by the indirect radioactive binding assay (Brown et al. 1986). Proteins of known molecular weight and sedimentation coefficient were applied to duplicate gradients in order to determine the sedimentation coefficient of the 10BG2 antigen. The proteins used were: haemoglobin, 4.6 S; urease, 8.5 S and 17 S; and catalase 11.2 S. All the gradient fractions were heated for 10 min at 80°C, following the addition of 0.025 vol. of PMSF (6 mg ml^-1 in ethanol), to assess thermostability. Acid stability was determined by lowering the pH of the gradient fraction to 3-6 with acetic acid. 10BG2 and control (RC2.43) immunoprecipitates of 32P-labelled HL60 proteins were treated in the same way. Precipitated material was removed after both treatments using a Beckman microfuge, the pH of the acid-treated fractions was returned to neutral with 3 M-Tris-HCl, pH 8.8. Treated and untreated extracts were analysed on SDS-polyacrylamide (10%) gels.

Results

Subcellular and tissue distribution of the 10BG2 antigen

Immunofluorescence studies using cytocentrifuge preparations of HL60 cells fixed with acetone, formaldehyde or methanol showed that in interphase cells 10BG2 antigen was located entirely within the nucleus. As shown in Fig. 1A, in which formaldehyde preparations were stained, the staining pattern was mottled in appearance and nucleoli were unstained. Gradual focusing adjustments using a ×95 objective lens revealed that the staining was distributed throughout the nucleus. Similar results were obtained with acetone and methanol-fixed cytocentrifuged cells. Metaphase cells showed intense diffuse cytoplasmic staining (Fig. 1B). The chromosomes of cells in metaphase were unstained though there was increased fluorescence at the periphery of individual chromosomes.

In contrast to monoclonal antibodies to histone 2B, DNA and a nuclear envelope protein, 10BG2 antibody failed to stain air-dried and unfixed cytocentrifuged preparations of HL60 cells. Air-dried cytocentrifuged preparations of cells that had been pre-incubated with BBSS for 10 min at room temperature, prior to fixation in acetone, were unstained by 10BG2 antibody. HL60 nuclei isolated using Tween 40, were also unreactive with 10BG2 antibody when stained in suspension, but were intensely stained by monoclonal antibodies to DNA. In contrast, 10BG2 antibody bound well to isolated HL60 nuclei that had been fixed in 0.25% glutaraldehyde, giving 11 X 10^3 cts min^-1 per 10^6 nuclei in indirect radiobinding assays over a background of 1.5 X 10^3 cts min^-1 per 10^6 nuclei. Glutaraldehyde-fixed nuclei were also reactive with antibodies to DNA and histone 2B. These results suggest that the 10BG2 antigen can readily diffuse out of the nuclei unless immobilized by fixation. The possibility that 10BG2

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antibody can only recognize a protein when altered by fixation is excluded by the negative result obtained when cytocentrifuged cells were preincubated with PBS and subsequently fixed in acetone prior to staining with 10BG2 antibody and also by precipitation studies, which showed that the antibody was able to recognize soluble antigen (see below). As shown in Table 1, all human cell lines tested and cells from primary cultures of fibroblasts, endothelial cells and keratinocytes were intensely stained by 10BG2 antibody. 10BG2 stained lymphocytes and monocytes in samples of blood mononuclear cells. All lymphoid areas of cryostat sections of tonsil were stained together with fibroblasts in areas of connective tissue and tonsil epithelial cells. The nuclei of muscle cells were strongly stained. In contrast, 10BG2 antibody did not stain Chinese hamster ovary cells (CHO-K1) and Balb/C 3T3 fibroblasts. Thus to date the 10BG2 antibody appears to recognize a human-specific determinant.

The biochemical nature of the 10BG2 antigen

The native molecular weight of the 10BG2 antigen was determined using Ferguson plots (Hedrick & Smith, 1968). For this analysis carbonic anhydrase ($30 \times 10^3$ $M_r$), ovalbumin ($45 \times 10^3$ $M_r$), bovine serum albumin ($66 \times 10^3$ $M_r$) and phosphorylase B ($185 \times 10^3$ $M_r$) were electrophoresed in non-denaturing gels containing 4, 6, 8, 10 and 12% polyacrylamide. The retardation coefficients ($K_r$) obtained for these proteins were plotted as a function of molecular weight (see Fig. 2A). The resultant line was then used to derive the molecular weight of the 10BG2 antigen using $K_r$ values obtained in parallel. In four independent determinations, one of which is shown in Fig. 2A, the 10BG2 antigen was

![Fig. 1. Expression of the 10BG2 antigen by cells during interphase and metaphase. Cytocentrifuge preparations of the human promyeloid cell line HL60 were fixed in formaldehyde and stained with 10BG2 antibody by indirect immunofluorescence as described in Materials and methods. The nuclear restriction of the 10BG2 antigen during interphase and its absence from nucleoli are clearly shown (A). 10BG2 antigen was expressed throughout the cytoplasm of cells in metaphase (B). In these cells chromosomes were not stained by 10BG2 antibody but the staining was intensified at their periphery. X950.](image)
29-5xl0 3iWr, which was not seen in control immunotreated with 10BG2 antibody (see Fig. 2B). Precipitates of antigen was determined using protein immunoprecipitation. Furthermore, the subunit molecular weight of the 10BG2 antigen was calculated to have a native molecular weight in the range from 130 x 10^3 to 140 x 10^3.

Precipitates prepared either with an irrelevant monoclonal antibody or with 10BG2 and an extract from HL60 cells gave a single sharp band of molecular weight range from 130 x 10^3 to 140 x 10^3.

It was noted that 10BG2-precipitated protein was estimated to be 9-2 S. The sucrose gradient fractions were also assessed for heat and acid stability and the 32x10^3 M_r protein band shown in Fig. 3 was stable to both treatments (data not shown). The value for molecular weight of the monomer here is slightly higher than that gained in earlier experiments and may reflect a change in the proteins used as standards. This is not an uncommon problem and has been commented on by other authors (Dingwall et al. 1982).

The isoelectric point of 10BG2 antigen was determined using the partially purified protein obtained from the sucrose gradient. All fractions containing 10BG2 activity were subjected to isoelectric focusing; Fig. 4 shows a two-dimensional gel of one of the peak fractions. The proteins indicated by arrows were the only ones found in each 10BG2 fraction and in addition were the only proteins stable to heat and acid treatments. Furthermore, a band containing 10BG2 activity, as identified by immunoblotting, was excised from a non-denaturing gel and the protein eluted from the gel was separated by two-dimensional electrophoresis. The gel contained proteins that electrophoresed in the same position as those indicated in Fig. 4. We are therefore sure that the 10BG2 antigen contains isoelectric variants, as indicated in Fig. 4, with pl values of 5-2 and 5-4. The streaked appearance of the 10BG2 antigen is very similar to that of nucleoplasmin and the presence of the pentameric form of the protein in the second dimension reflects a degree of resistance to the action of SDS. This resistance was also occasionally seen in denaturing gels of 10BG2 immunoprecipitates; Fig. 5 (lanes C and E) demonstrates the multiband pattern sometimes seen, which may represent the different polymeric forms of the 10BG2 antigen. In contrast Fig. 2B and Fig. 5, lane F, show that the monomer alone can be produced and imply that thorough treatment of samples with SDS is required to ensure complete denaturation of the protein to its monomeric form.

Table 1. Reactivity of 10BG2 antibody with various cell types

<table>
<thead>
<tr>
<th>Cell type</th>
<th>% Positive nuclei*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Human haemopoietic cells</strong></td>
<td></td>
</tr>
<tr>
<td>Normal cells:</td>
<td></td>
</tr>
<tr>
<td>Blood lymphocytes, monocytes</td>
<td>&gt;99</td>
</tr>
<tr>
<td>Blood erythrocytes</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Tonsil lymphocytes†</td>
<td>&gt;99</td>
</tr>
<tr>
<td>Cell lines:</td>
<td></td>
</tr>
<tr>
<td>HL60 (myeloid)</td>
<td>&gt;99</td>
</tr>
<tr>
<td>K562 (early erythroid)</td>
<td>&gt;99</td>
</tr>
<tr>
<td>EB2, ARH-77 (B cell)</td>
<td>&gt;99</td>
</tr>
<tr>
<td>SMS/SB (pre-B)</td>
<td>&gt;99</td>
</tr>
<tr>
<td><strong>Human non-haemopoietic cells</strong></td>
<td></td>
</tr>
<tr>
<td>Normal cells:</td>
<td></td>
</tr>
<tr>
<td>Tonsil epithelium†</td>
<td>&gt;99</td>
</tr>
<tr>
<td>Tonsil connective tissue†</td>
<td>&gt;99</td>
</tr>
<tr>
<td>Muscle cells†</td>
<td>&gt;99</td>
</tr>
<tr>
<td>Primary cell cultures:</td>
<td></td>
</tr>
<tr>
<td>Keratinocytes</td>
<td>&gt;99</td>
</tr>
<tr>
<td>Umbilical cord endothelial cells</td>
<td>&gt;99</td>
</tr>
<tr>
<td>Embryo lung fibroblasts</td>
<td>&gt;99</td>
</tr>
<tr>
<td>Cell lines:</td>
<td></td>
</tr>
<tr>
<td>H.Ep2 (carcinoma of larynx)</td>
<td>&gt;99</td>
</tr>
<tr>
<td>SCC25 (squamous cell carcinoma)</td>
<td>&gt;99</td>
</tr>
<tr>
<td>SV-40-IBAM/HFK (SV40-transformed foetal keratinocytes)</td>
<td>&gt;99</td>
</tr>
<tr>
<td><strong>Non-human cell lines</strong></td>
<td></td>
</tr>
<tr>
<td>CHO (Chinese hamster ovary)</td>
<td>&lt;1</td>
</tr>
<tr>
<td>3T3 fibroblasts</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

* Cyto centrifuged cell lines were fixed with acetone and stained using an indirect immunofluorescence.
† Studied using cryostat sections of tissue.

calculated to have a native molecular weight in the range from 130 x 10^3 to 140 x 10^3.

10BG2 antibody failed to bind to Triton X-100-soluble HL60 proteins when electrophoresed in denaturing gels and blotted onto nitrocellulose. Therefore, the subunit molecular weight of the 10BG2 antigen was determined using protein immunoprecipitated with 10BG2 antibody (see Fig. 2B). Precipitates from HL60 cells gave a single sharp band of 29.5 x 10^3 M_r, which was not seen in control immunoprecipitates prepared either with an irrelevant monoclonal antibody or with 10BG2 and an extract from mouse NSI cells, which do not express 10BG2 antigen. It was noted that 10BG2-precipitated protein accounted for approximately 1.7 % of the total 35S activity in extracts from whole HL60 cells.

Fig. 3 shows that the 10BG2 activity purified on the sucrose gradient corresponded to a major protein band on SDS–polyacrylamide gels of M_r, 32 x 10^7. From two determinations the sedimentation co-efficient of the protein was estimated to be 9.2 S. The sucrose gradient patterns were sometimes seen, which may represent the different polymeric forms of the 10BG2 antigen. In contrast Fig. 2B and Fig. 5, lane F, show that the monomer alone can be produced and imply that thorough treatment of samples with SDS is required to ensure complete denaturation of the protein to its monomeric form.

Fig. 5 shows that the 10BG2 antigen is phosphorylated as a phosphorylated protein from Triton X-100 extracts of 32P-labelled HL60 cells (Fig. 5, lane B) runs parallel, in a non-denaturing gel, to the protein revealed by immunoblotting with 10BG2 antibody (Fig. 5, lane A). Immunoprecipitates of Triton X-100 extracts from 35S-labelled HL60 cells were stable to heat (lane E) and acid (lane F) treatments. No labelled proteins were seen in immunoprecipitates produced by an irrelevant antibody to rotavirus (RC 2.43; Fig. 5, lane D).

The properties of the 10BG2 antigen bear a striking resemblance to those of the nucleosome-assembly protein nucleoplasmin. This similarity was confirmed by comparison of the electrophoretic mobility of the 10BG2 antigen with that of nucleoplasmin purified from *Xenopus* oocytes. As shown in Fig. 5 (lanes G and H), immunostaining of nitrocellulose blots for the...
presence of *Xenopus* nucleoplasmin and 10BG2 antigen revealed that both proteins had migrated the same distance on non-denaturing, 7.5% polyacrylamide gels. Similarly, nucleoplasmin and 10BG2 co-migrated in separate non-denaturing gels containing 6% and 10% polyacrylamide. These data confirm that 10BG2 antigen has a similar molecular weight to *Xenopus* nucleoplasmin and also reveal that the proteins carry a similar charge. 10BG2 antibody did not cross-react with *Xenopus* nucleoplasmin (data not shown) and similarly the PA3C5 and PB2E11 antibodies to *Xenopus* nucleoplasmin did not bind to proteins in Triton extracts of HL60 cells (data not shown). Furthermore, in immunofluorescence studies none of the anti-nucleoplasmin monoclonal antibodies was seen to bind to acetone-fixed HL60 cytocentrifuge preparations.

**Discussion**

Immunofluorescence studies showed that the 10BG2 monoclonal antibody recognized a protein that in interphase cells was entirely restricted to the nucleus. In these cells the 10BG2 staining was mottled in appearance and distributed throughout the nucleus, with the exception that nucleoli were completely unstained. In metaphase cells 10BG2 antigen was seen throughout the cytoplasm but was concentrated at the periphery of chromosomes, though the chromosomes themselves were not stained. Similar staining patterns were seen in all human cells tested. These included normal haemopoietic cells and cell lines, normal and transformed epithelial cells, fibroblasts and keratinocytes. The presence of 10BG2 antigen in the nuclei of such diverse cells suggests a key housekeeping function for this protein. In contrast, 10BG2 antibody failed to stain either mouse 3T3 fibroblasts or Chinese hamster ovary (CHO) cells.

Biochemical analyses of the 10BG2 antigen revealed extensive similarities with nucleoplasmin, a nuclear protein that was first identified in *Xenopus laevis* oocytes. Nucleoplasmin is a $145 \times 10^3 M_r$ pentamer composed of $29 \times 10^3$ to $30 \times 10^3 M_r$ subunits (Earnshaw et al. 1980). Ferguson plot analysis of the 10BG2 antigen revealed a native molecular weight in the range $130 \times 10^3$ to $140 \times 10^3$, while electrophoresis of 10BG2 immunoprecipitates from HL60 cells demonstrated a subunit of $29.5 \times 10^3 M_r$. Thus, the native and subunit sizes of nucleoplasmin and 10BG2 antigen are similar. It is of particular significance that the relative values of the native and subunit molecular weights of the 10BG2 antigen suggest that this protein shares with nucleoplasmin a highly unusual pentameric structure.

**Fig. 2.** Determination of native and subunit molecular weights for the 10BG2 antigen. A. The native molecular weight of the 10BG2 antigen was obtained by comparison of its retardation co-efficient ($K_r$) in non-denaturing gels (4–12% polyacrylamide), with those of carbonic anhydrase (●), ovalbumin (○), bovine serum albumin (▲) and phosphorylase B (Δ). In the experiment shown the $K_r$ of the 10BG2 antigen was found to be 14-7, corresponding to a relative molecular weight of $139.5 \times 10^3$. B. Triton X-100 extracts prepared from HL60 cells cultured in medium containing [35S]methionine were immunoprecipitated with 10BG2 antibody to determine the subunit molecular weight of the 10BG2 antigen (lanes 1 and 2) and also with an irrelevant monoclonal antibody to rotavirus (RC2.43) as a control (lanes 3 and 4). As shown in lanes 1 and 2, electrophoresis of 10BG2 immunoprecipitates in denaturing gels revealed a single band corresponding to a subunit molecular weight of $29.5 \times 10^3$ and no band was seen in control immunoprecipitates.
Fig. 3. Partial purification of 10BG2 antigen as a sucrose gradient. A 1 ml sample of a Triton X-100 extract of 10^6 HL60 nuclei was separated on a 13 ml sucrose gradient (10% to 40%) by centrifugation at 37 500 revs min^{-1} for 16.5 h at 4°C. Samples (0.5 ml) were removed and assayed for 10BG2 activity (Brown et al. 1986). Samples of each fraction were also applied to SDS–polyacrylamide (10%) gels, which were subsequently stained with Coomassie Blue. The peak of 10BG2 activity corresponded to a major protein band in the acrylamide gel; the gel strip is shown below the sucrose gradient profile. Numbers above the sucrose gradient indicate the position of marker proteins in a duplicate sucrose gradient. From these values the sedimentation coefficient of 10BG2 antigen was estimated.

Fig. 4. Separation of 10BG2 antigen from sucrose gradient by two-dimensional electrophoresis and estimation of isoelectric point. Peak fractions from the sucrose gradient were concentrated and 60 µl samples were loaded onto prepoured isoelectric focusing gels (LKB PAG Plates). Proteins were electrophoresed for 1.5 h at 1.5 kV, 50 mA and 30 W at 10°C on a multiphor II apparatus (LKB). Strips were cut out of the gel, soaked in SDS reducing buffer (62.5 mM Tris·HCl, pH 6.8, 5% β-mercaptoethanol, 3% SDS, 0.01% Bromophenol Blue) for 10 min at room temperature then applied to the second dimension gel, a SDS–polyacrylamide (10%) gel. The second-dimension gel was subsequently stained for proteins with Coomassie Blue. Although several proteins were seen in these gels, those that were stable to heat and acid (shown by arrows) had isoelectric points of 5.2 and 5.4.

Nucleoplasmin-like protein in human cells
Fig. 5. Biochemical characteristics of 10BG2 antigen. Lane A: Triton X-100 extract of HL60 cells electrophoresed on a 7.5% non-denaturing gel and immunoblotted with 10BG2 antibody. 10BG2 antibody bound to nitrocellulose filters was revealed by a $^{125}$I-labelled rabbit (Fab')$_2$ to mouse Ig. Lane B: Triton X-100 extract of HL60 cells, labelled with $^{32}$P, electrophoresed on a 7.5% non-denaturing gel and revealed by autoradiography. Lanes A and B are from the same gel and show that the 10BG2 antigen (lane A) runs parallel with a phosphorylated protein in the Triton X-100 extract (lane B). Lanes C–F: heat and acid stability was assessed using Triton X-100 extracts of HL60 cells labelled with $[^{35}$S]methionine, immunoprecipitated with 10BG2 antibody (lane C), and also with an irrelevant antibody to rotavirus (RC2.45) (lane D). 10BG2 immunoprecipitates were heated to 80°C for 10 min in the presence of 0.025 vol. of PMSF (6 mg ml$^{-1}$ in ethanol) to aid precipitation of proteins. Precipitated proteins were removed by centrifugation and proteins in solution were electrophoresed in SDS-polyacrylamide (10%) gel (lane E). 10BG2 immunoprecipitate were also treated with acetic acid to lower the pH to 3.6. Precipitated proteins were removed by centrifugation and the remaining supernatant was readjusted to neutral pH with Tris buffer prior to electrophoresis in a SDS-polyacrylamide (10%) gel (lane F). Lanes G, H: purified Xenopus nucleoplasmin and Triton X-100 extracts of HL60 were electrophoresed in adjacent lanes of a 7.5% polyacrylamide gel under conditions that would not denature the proteins (see Materials and methods). Lane G was loaded with 120μg of HL60 extract and lane H with 0.5μg of Xenopus nucleoplasmin. The proteins were electrophoretically transferred to nitrocellulose and incubated with 10BG2 antibody and antibodies to Xenopus nucleoplasmin (PA3C5 and PB2E11), respectively. Binding of antibodies to the filters was subsequently revealed using a $^{125}$I-labelled rabbit (Fab')$_2$ to mouse Ig.

Furthermore, since Xenopus nucleoplasmin and 10BG2 antigen co-migrated under electrophoresis in non-denaturing polyacrylamide gels the proteins must also carry a similar charge. In such gels both nucleoplasmin and 10BG2 gave broad diffuse bands. This may be due to the presence of several different phosphorylated forms of the proteins, which have already been described for Xenopus nucleoplasmin (Krohne & Franke, 1980b). We have shown that the 10BG2 antigen is phosphorylated and separates into two isoelectric variants with pI values of 5.2 and 5.4. This may explain the broad band routinely seen on non-denaturing gels of Triton X-100 extracts revealed by immunoblotting with 10BG2 antibody. In addition 10BG2 antigen gave two spots with a streaked appearance on two-dimensional gels as does nucleoplasmin. Other characteristics shared by 10BG2 antigen and nucleoplasmin are their stability to heat and acid
buffers (Krohne & Franke, 1980). In immunofluorescence studies, 10BG2 antibody failed to stain cytocentrifuge preparations of HL60 cells that had been preincubated in buffer prior to the staining procedure. Similarly, isolated HL60 nuclei were unreactive with 10BG2 antibody when stained in suspension. If, however, 10BG2 antigen was immobilized by fixation of nuclei immediately after their preparation then the binding of 10BG2 antibody was readily demonstrated using a radiobinding assay. In addition 10BG2 antigen was demonstrated, by electrophoresis and subsequent immunostaining, in the supernatant of isolated HL60 nuclei that had been incubated in buffer (data not shown). Thus, several approaches have shown that, like nucleoplasmin, 10BG2 antigen is readily extractable in aqueous buffers. Finally, the 10BG2 antigen shows a degree of resistance to SDS treatment, which can lead to the pentomeric and monomeric forms of the protein being detected on SDS-polyacrylamide gels of 10BG2 immunoprecipitates. Such a banding pattern has also been demonstrated for nucleoplasmin (Dingwall et al. 1982).

In conclusion, the 10BG2 antibody recognizes an apparently human-specific determinant expressed on a nuclear restricted protein. All the biochemical characteristics of this protein that have been investigated are similar to those of nucleoplasmin. Thus, it is likely that the protein recognized by 10BG2 antibody is human nucleoplasmin. It must, however, be emphasized that nucleoplasmin is defined by its ability to assemble histones and DNA into nucleosomes in vitro (Laskey et al. 1978). Consequently, to establish conclusively that the 10BG2 antigen represents a human nucleoplasmin analogue will require the demonstration of this activity. The 10BG2 antibody provides a means of purifying sufficient protein for sequence analyses and comparison with nucleoplasmin from Xenopus oocytes. If the 10BG2 antigen is nucleoplasmin, it is interesting to consider why the antibody does not stain nuclei. Several reports in the late 1970s demonstrated that unlike the majority of eukaryotic DNA, transcriptionally active nucleolar chromatin is not organized into nucleosome-like structures (see review by Franke et al. 1979). Thus it is possible that a nucleoplasmin-like protein is not required in the nucleolus.

To our knowledge 10BG2 is the first monoclonal antibody to a human nucleoplasmin-like protein. This antibody has already proved valuable in the biochemical characterization of the protein and will be used further to study its function in human nuclear organization.

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