Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 7552–7556, November 1987
Cell Biology

Nuclear pore complex contains a family of glycoproteins that includes p62: Glycosylation through a previously unidentified cellular pathway

(nuclear envelope/nuclear antigens/*N*-acetylglucosamine/wheat germ agglutinin)

LAURA I. DAVIS AND GÜNTER BLOBEL

Laboratory of Cell Biology, Howard Hughes Medical Institute, The Rockefeller University, New York, NY 10021

Contributed by Günter Blobel, July 1, 1987

ABSTRACT **Using a monoclonal antibody (mAb 414), we previously identified a protein of 62 kDa (p62) that was localized to the nuclear pore complex by immunoelectron microscopy. We also showed that p62 binds specifically to wheat germ agglutinin. Therefore, we proposed that this nuclear pore complex protein might be a member of a recently characterized family of glycoproteins that are labeled by *in vitro* galactosylation of rat liver nuclei and contain O-linked monosaccharidic GlcNAc residues. In support of this, we now show that incubation with *N*-acetylglucosaminidase reduces the molecular mass of p62 by ≈3 kDa because of the removal of terminal GlcNAc residues. Moreover, p62 can be galactosylated *in vitro* by using UDP-[^{3}H]galactose and galactosyltransferase. We also show that most of the GlcNAc residues are added within 5 min of synthesis, when p62 is soluble and cytosolic. Thus, the addition of GlcNAc is carried out in the cytoplasm and is clearly distinct from the N- and O-linked glycosylation pathways of the endoplasmic reticulum and Golgi complex. Using another mAb with a broad specificity for nuclear GlcNAc-containing proteins, we show by immunofluorescence and protein blotting of subnuclear fractions that some of these proteins are in the interior of the nucleus, and others are most likely located in the pore complex.**

Using a monoclonal antibody (mAb 414), we recently identified a nuclear pore complex protein of 62 kDa (designated p62) (1). p62 was localized to the pore by immunoferritin electron microscopy of isolated rat liver nuclei.

We previously showed that p62 affinity-purified from rat liver nuclei reacted with wheat germ agglutinin (WGA) but not with Con A (1). These data suggested that p62 contains terminal GlcNAc but no mannose residues. Therefore, these GlcNAc residues are unlikely to be part of an asparagine-linked oligosaccharide, since the latter also contain mannose. Thus, we suggested that p62 might be a member of a family of glycoproteins that have been shown to contain O-linked GlcNAc residues (2, 3). Interestingly, we found that newly synthesized p62 was present as a smaller protein in the cytosolic fraction and not, as would be expected for proteins destined to be glycosylated, in the membrane fraction. The data in the present paper provide evidence that p62 does indeed contain terminal "O-linked GlcNAcs" and that it is not glycosylated within the lumen of the endoplasmic reticulum or Golgi apparatus. Moreover, using another antinuclear mAb, we show that several of the other proteins containing such sugars are located inside the nucleus, whereas some fractionate with the nuclear envelope, as previously shown (2).

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MATERIALS AND METHODS

Metabolic Labeling of Tissue Culture Cells. Labeling of Buffalo rat liver (BRL) cells with [^{35}S]methionine (≈1000 Ci/mmol, New England Nuclear–DuPont; 1 Ci = 37 GBq) was carried out as described (1) with 40 μCi of isotope per ml in all experiments except pulse–chase analysis, in which a concentration of 100 μCi/ml was used.

***In Vitro* Galactosylation of Nuclear Proteins.** Galactosyltransferase-mediated galactosylation of terminal GlcNAc residues was carried out essentially as described (3). Sixty equivalents of rat liver nuclei (≈1.8×10^8 nuclei) prepared as described (1, 4) were resuspended in 60 μl of buffer S [250 mM sucrose/10 mM triethanolamine hydrochloride, pH 7.4/25 mM KCl/1.5 mM $MgCl_2$/0.3 mM phenylmethylsulfonyl fluoride ($PhMeSO_2F$)]. Ten microliters of 100 mM galactose, 2 μl of 125 mM 5′-AMP, 2 μl of 250 mM $MnCl_2$, and 4 μl of galactosyltransferase were then added in that order. To start the reaction, 20 μl of uridine diphospho-[6-^{3}H]galactose (UDP-[^{3}H]Gal; 5–15 Ci/mmol, Amersham) were added, and the sample was incubated at 37°C for 30 min. After incubation, 1 ml of ice-cold buffer S was added, and the sample was divided into three equal aliquots, which were centrifuged at 15,000 × g_{avg} at 4°C for 10 min. Two of the resulting pellets were each sonicated briefly in 250 μl of 0.4% $NaDodSO_4$/50 mM triethanolamine hydrochloride, pH 7.4/100 mM NaCl/2 mM EDTA/0.3 mM $PhMeSO_2F$. After incubation in a boiling-water bath for 5 min, samples were centrifuged at 15,000 × g_{avg} for 10 min to clear them of particulate matter. The supernatants were brought to a final volume of 500 μl and to final concentrations of 0.2% $NaDodSO_4$, 1% Triton X-100, 50 mM triethanolamine hydrochloride (pH 7.4), 100 mM NaCl, 2 mM EDTA, 2% bovine serum albumin, and 0.3 mM $PhMeSO_2F$. These samples were immunoprecipitated as described (1) with either mAb 414 or mAb 457. In the latter case, the affinity resin was prepared by first incubating 10 μl of protein A-Sepharose (Pharmacia) with 40 μg of affinity-purified rabbit anti-mouse IgG (Cooper Biomedicals, Malvern, PA), before adding 1 ml of culture supernatant containing mAb 457. The other pellet was treated in the same manner, except that the EDTA was replaced by 1 mM $MgCl_2$ and no bovine serum albumin was included. This sample was precipitated with 10 μl of WGA-Sepharose (Pharmacia) and washed with the same buffer that was used for precipitation.

Glycosidase Digestion of Immunoprecipitates. For digestion with *N*-acetyl-D-glucosaminidase (GlcNAcase), 50 μl of 50 mM citrate buffer (pH 4.5) containing 2 mM EDTA, 6 μg each

Abbreviations: WGA, wheat germ agglutinin; BRL, Buffalo rat liver; $PhMeSO_2F$, phenylmethylsulfonyl fluoride; mAb, monoclonal antibody; GlcNAcase, *N*-acetyl-D-glucosaminidase; UDP-Gal, uridine 5′-diphosphogalactose.

of leupeptin (Boehringer Mannheim) and pepstatin (Sigma) per ml, 200 trypsin-inhibitor units of Trasylol (Mobay Chemical, New York) per ml, and 0.5 mM $PhMeSO_2F$ with or without 2 mg of GlcNAcase (Boehringer Mannheim) per ml and 400 mM *N*-acetylglucosamine (Aldrich) were added to 10 μl of protein A-Sepharose beads containing immunoadsorbed p62 (1). Samples were incubated 15 hr at 37°C, after which the supernatant was removed and precipitated with 10% CCl_3COOH. Pellets from CCl_3COOH treatment were dissolved in $NaDodSO_4$/PAGE buffer, and the contents were transferred back to the corresponding Sepharose pellet to elute any p62 that may have remained bound to the Sepharose. The samples were boiled for 5 min and subjected to $NaDodSO_4$/PAGE.

For digestion with endoglycosidase H, 25 μl of 160 mM citrate buffer (pH 5.5) containing 0.3% $NaDodSO_4$, 0.4 mM $PhMeSO_2F$, and 0.05 unit of endoglycosidase H (Boehringer Mannheim) were added to 10 μl of protein A-Sepharose containing immunoadsorbed p62. The samples were incubated and processed as described for those digested with GlcNAcase.

Peptide Mapping of Galactosylated Proteins. An immunoprecipitate from [^{3}H]galactosylated rat liver nuclei (60 equivalents of nuclei) with mAb 414 was prepared exactly as described above. The sample was electrophoresed on a 1-mm-thick 7% $NaDodSO_4$/polyacrylamide gel, after which the lane containing the sample was excised from the gel. The excised gel strip was laid horizontally across the top of the stacking gel of a 1.2-mm-thick 10–15% gradient $NaDodSO_4$/polyacrylamide gel. Proteolytic digestion with V8 protease was carried out essentially as described (5). The gel strip was overlaid with 250 mM Tris base/0.1% $NaDodSO_4$/1 mM EDTA/10% (wt/vol) glycerol/20 mM dithiothreitol/2 μg of *Staphylococcus aureus* V8 protease (Cooper Biomedicals). Electrophoresis was interrupted for a period of 30 min after stacking had been achieved to allow digestion.

RESULTS

Digestion of p62 with Glycosidases. To investigate whether p62 is, as previously suggested (1), a member of a family of glycoproteins that contain O-linked GlcNAc, we labeled BRL cells with [^{35}S]methionine for 16 hr before lysis by homogenization in hypotonic buffer. The homogenate was subjected to one differential centrifugation to prepare the pellet and postmitochondrial supernatant fractions. Both fractions were solubilized by the addition of $NaDodSO_4$ and immunoprecipitated with mAb 414. The immunoprecipitates were then digested with either of two different glycosidases (Fig. 1). We found that the molecular mass of the pellet fraction was reduced by ≈3 kDa (Fig. 1, lane 4). Moreover, the p62 in the supernatant was also reduced in mass by ≈2.5 kDa, migrating with a molecular mass identical to that of p62 in the pellet fraction. The mobility difference between the treated and untreated forms of p62 was more clearly evident when the two were mixed prior to $NaDodSO_4$/PAGE (Fig. 1, lanes 7 and 8). That the shift in mobility was due to the removal of GlcNAc by the enzyme and not, for example, to proteolytic cleavage by a contaminating protease was shown by inclusion of 0.5 M GlcNAc in the digestion mixture to inhibit the GlcNAcase activity (Fig. 1, lanes 5 and 6). Therefore, we conclude that both the supernatant (which represents the soluble form) and the pellet fraction (presumably representing the pore complex form) contain p62 that is modified by GlcNAc residues, the pellet form containing more GlcNAc than the supernatant form.

We also carried out digestion of p62 from both fractions with endoglycosidase H [which cleaves between GlcNAc residues of high-mannose-containing core oligosaccharides (6)]. As expected from its lack of reactivity with Con A

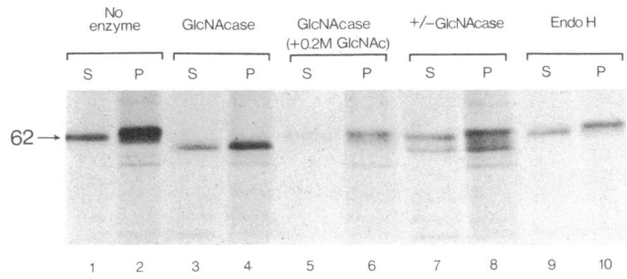

FIG. 1. Digestion of immunoprecipitates with glycosidases. Cultured BRL cells were labeled with [^{35}S]methionine for 12 hr, fractionated into postmitochondrial supernatant (lanes S) and pellet (lanes P) fractions, and immunoprecipitated with mAb 414. The washed immunoprecipitates were then left untreated (lanes 1 and 2; the arrow indicates the position of undigested p62) or digested with GlcNAcase in the absence (lanes 3 and 4) or presence (lanes 5 and 6) of 250 mM GlcNAc or digested with endoglycosidase H (endo H; lanes 9 and 10). In lanes 7 and 8, samples treated with GlcNAcase were electrophoresed in the same lane as untreated samples to show the difference in molecular weight more clearly.

(unpublished results), the mobility of p62 was not affected by endoglycosidase H (Fig. 1, lanes 9 and 10); thus p62 does not appear to contain N-linked high-mannose oligosaccharides. This conclusion is supported by the fact that the addition of tunicamycin, an antibiotic that inhibits the first step in synthesis of the lipid-linked high-mannose oligosaccharide precursor (7–9), had no effect on the level of glycosylation of p62 (data not shown).

Timing of Sugar Addition to p62. To establish the time of addition of the bulk of GlcNAc to p62, we pulse-labeled cultured BRL cells for 5 min with [^{35}S]methionine and chased with unlabeled methionine for various periods of time (Fig. 2). In contrast to previous experiments, the initial homogenate was centrifuged to sediment microsomes. p62 present in the pellet and supernatant fractions was immunoprecipitated with mAb 414 (Fig. 2*a*). Half of the immunoprecipitate was treated with GlcNAcase, and the treated and untreated samples were coelectrophoresed (Fig. 2*b*) to display the mobility difference and to estimate the amount of GlcNAc that had been added to p62 at each time point. Even at the earliest time point (0 chase), p62 appeared already to have received the bulk of its GlcNAc residues (Fig. 2*b*, lane 3). There was no detectable unglycosylated p62 even at this time (Fig. 2*a*, lanes 1 and 2). However, at the 0- and 5-min time points (Fig. 2*a*, lanes 1 and 3), the bands corresponding to p62 were lighter and more diffuse than those at later points, indicating that glycosylation was probably not yet complete. Thus, we conclude that the bulk of the GlcNAc residues is added within 5 min from the start of translation and perhaps even cotranslationally. The fact that all of the newly synthesized p62 at early time points was found in the postmicrosomal supernatant (Fig. 2*a*) suggested that p62 is synthesized as a soluble cytosolic protein that at no time is associated with microsomal or Golgi membranes and receives the bulk of its sugar complement while it is in this form. However, a small amount appears to be added much later, after incorporation of p62 into the pore complex (ref. 1; Fig. 2*a*, lanes 15 and 16).

***In Vitro* Galactosylation of Rat Liver Nuclei.** Galactosyltransferase can transfer galactose from UDP-Gal to terminal GlcNAc residues *in vitro* (10, 11). Using UDP-[^{3}H]Gal and characterizing the labeled saccharide after β-elimination, Hart and coworkers (2, 3) have identified a family of glycoproteins that contain O-linked, monosaccharidic GlcNAc residues. To determine whether p62 is a member of this family of glycoproteins, we incubated rat liver nuclei with UDP-[^{3}H]Gal and galactosyltransferase, solubilized the nuclei with $NaDodSO_4$, and then precipitated proteins with

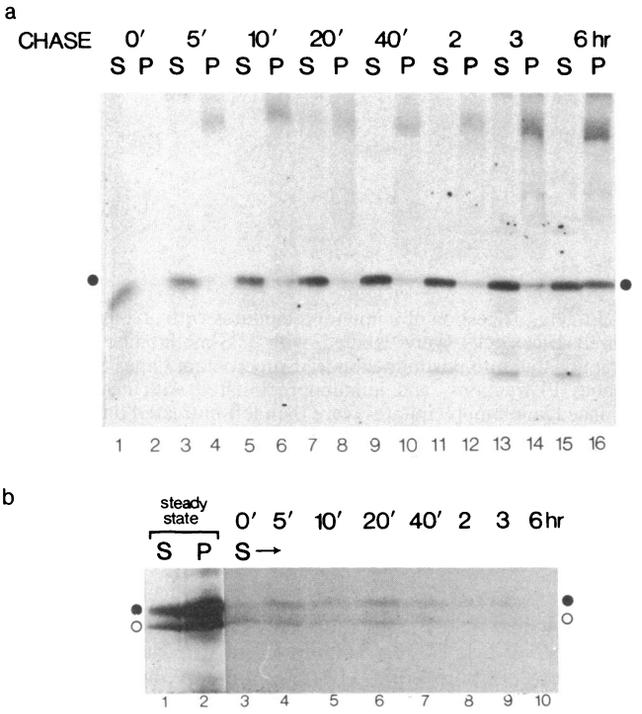

FIG. 2. Pulse–chase labeling of cultured cells. (*a*) Cultured BRL cells were labeled for 5 min with [^{35}S]methionine and chased for the indicated periods of time with medium containing nonradioactive methionine. The cells were then fractionated into postmicrosomal supernatant (lanes S) and pellet (lanes P) fractions and were immunoprecipitated with mAb 414. (*b*) Half of the immunoprecipitate from each supernatant fraction was treated with GlcNAcase (○) and recombined with the corresponding untreated sample (●) prior to electrophoresis (lanes 3–11). For comparison, immunoprecipitation was also performed on supernatant (lane 1) and pellet (lane 2) fractions of cells labeled for 12 hr.

various probes (Fig. 3). p62 was in fact one of the major galactosylated proteins immunoprecipitated by mAb 414 (Fig. 3, lane 1). Taken together with the results shown in Figs. 1 and 2, these data provide further evidence that p62 contains O-linked GlcNAc residues.

Surprisingly, mAb 414 also immunoprecipitated two other galactosylated proteins of about 175 and 270 kDa. The 175-kDa polypeptide had appeared previously, both in immunoprecipitations from [^{35}S]methionine-labeled tissue culture cells and on protein blots probed with mAb 414 (1), but the amount was always low when compared to p62. Most likely, these high molecular weight proteins are not as efficiently transferred to nitrocellulose. It is also possible that labeling of these two species with [^{3}H]galactose was more efficient than with [^{35}S]methionine.

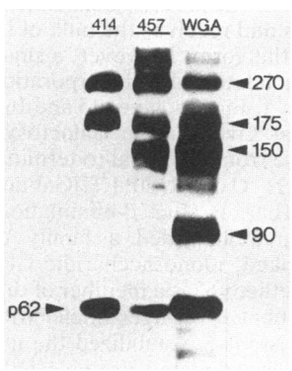

FIG. 3. *In vitro* [^{3}H]galactosylation of nuclear proteins. Isolated rat liver nuclei (60 equivalents) were incubated with UDP-[^{3}H]Gal in the presence of galactosyltransferase. After incubating 30 min at 37°C, nuclei were washed and boiled in buffer containing 0.4% $NaDodSO_4$ before precipitating with mAb 414 (lane 1) or mAb 457 (lane 2) or WGA (lane 3). After $NaDodSO_4$/PAGE, the gel was impregnated with 20% 2,5-diphenyloxazole in dimethyl sulfoxide, dried, and fluorographed for 24 hr.

These three polypeptides were among the galactosylated proteins that were precipitated by two other probes, mAb 457 (Fig. 3, lane 2), and WGA (Fig. 3, lane 3). mAb 457 is a monoclonal antibody that was generated in the same fusion that gave rise to mAb 414. In addition to the three polypeptides precipitated by mAb 414, this antibody precipitated most of the [^{3}H]galactose-labeled polypeptides recognized by WGA (Fig. 3, lane 3), albeit not always with the same efficiency. In the case of the 90-kDa polypeptide, galactosylation apparently resulted in greatly diminished reactivity with mAb 457 (Fig. 3, lane 2), as the nongalactosylated species clearly reacted with mAb 457 when this antibody was used to probe protein blots of nuclei (see Fig. 5).

Immunofluorescence Microscopy with mAb 457. *In vitro* galactosylation of nuclei vs. nuclear envelopes has suggested that only a subset of nuclear proteins containing "O-linked GlcNAc" fractionate with the nuclear envelope (2). Since mAb 457 provided us with a probe specific for the entire set of nuclear GlcNAc-containing proteins, we could investigate the *in situ* distribution of these polypeptides using immunofluorescence microscopy. Whereas mAb 414 yielded the previously observed (1) characteristic rim-staining pattern (Fig. 4*a*), which could be seen to be finely punctate upon focusing at the top of the nucleus (Fig. 4*b*), mAb 457 gave a granular intranuclear staining with a striking exclusion of staining from the nucleolar regions (Fig. 4*c*). The expected punctate rim pattern was largely obscured in the latter case by intranuclear staining in the interphase nucleus (Fig. 4*c*) but became clearly discernible in prophase cells as the intranuclear antigen became gradually dispersed (not shown).

Protein Blots of Subnuclear Fractions Probed with mAb 457 and WGA. To investigate which of the mAb 457- (and WGA) reactive proteins are located in the nucleoplasm, rat liver nuclei were subfractionated as described (1, 12), and supernatant and pellet fractions resulting from each step were subjected to $NaDodSO_4$/PAGE. The proteins were transferred to nitrocellulose sheets, which were then probed with either mAb 457 or WGA (Fig. 5). Both the mAb 457 and the WGA profiles show that a discrete set of proteins was released during DNase digestion (open arrows), whereas another set was associated with the crude pore complex–lamina fraction (closed arrows). Members of the latter set were extracted in the presence of high salt to various extents, as is p62. It is most likely that the set released upon DNase digestion is responsible for the intranuclear staining pattern detected by mAb 457. It is also likely that the other set, which includes the pore complex proteins recognized by mAb 414, are all in the nuclear pore, since they fractionate with the crude pore complex–lamina. Of course, proof for this will only be obtained when monospecific antibodies can be produced against each polypeptide.

Peptide Mapping of mAb 414-Binding Proteins. It is clear that mAbs 414 and 457 recognize epitopes common to at least three different proteins. Because mAb 414 still reacts after removal of the sugar residues with glycosidase (unpublished result), the shared epitope probably reflects related protein sequence in this case. To examine the relationship between these polypeptides, we performed peptide mapping of material immunoprecipitated by mAb 414. An immunoprecipitate from [^{3}H]galactose-labeled rat liver nuclei was subjected to $NaDodSO_4$/PAGE, after which the lane was excised and laid horizontally over a second $NaDodSO_4$/polyacrylamide gel. The gel slice was overlaid with *Staphylococcus aureus* V8 protease before reelectrophoresis essentially as described (5). The results of this digestion are shown in Fig. 6. The three polypeptides gave very distinct peptide maps and, in fact, shared no peptides containing labeled sugars. In addition, p62 was extremely resistant to proteolysis with V8 protease, yielding a single, large cleavage product.

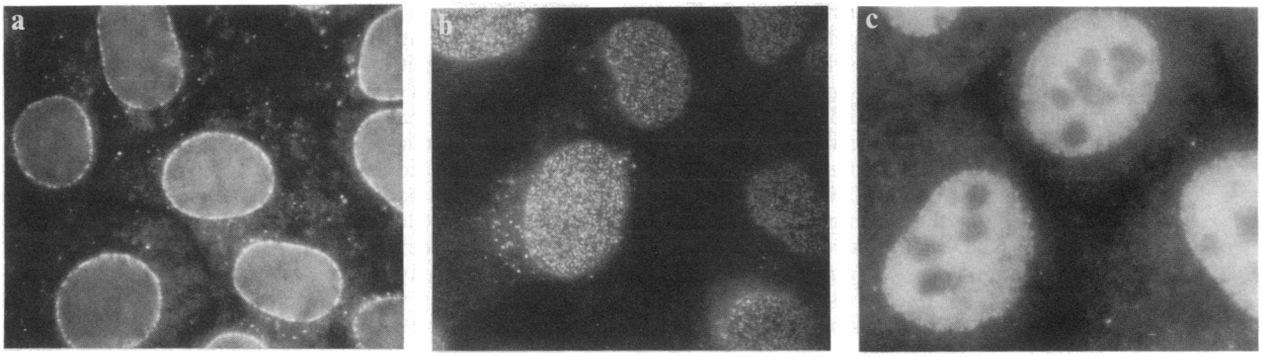

FIG. 4. Immunofluorescence labeling of tissue culture cells. BRL cells grown on coverslips were fixed with 2% formaldehyde and made permeable with methanol as described (1). The cells were incubated with either mAb 414 (*a* and *b*) or mAb 457 (*c*) and then with a fluorescein isothiocyanate-conjugated goat anti-mouse IgG (1). Samples were viewed under a Zeiss photomicroscope III. Kodak Tri X-pan film was used at ASA 800.

DISCUSSION

The results presented here provide additional evidence for our previous proposal (1) that the nuclear pore complex protein, p62, belongs to a family of glycoproteins shown by Holt and Hart (2) to contain monosaccharidic O-linked GlcNAc residues. We show here that the molecular mass of p62 is reduced by about 3 kDa as the result of incubation with GlcNAcase, which cleaves terminal GlcNAc residues. The reduction in mass of p62 is unlikely to be due to protease contamination in the glycosidase preparation because this shift was completely inhibited when incubation with glycosidase was carried out in the presence of GlcNAc. Further evidence for the existence of terminal GlcNAc residues on p62 comes from our finding that p62 can be galactosylated *in vitro* from UDP-Gal in the presence of galactosyltransferase. Holt and Hart (2) and Schindler *et al.* (13) have carried out definitive chemical analysis of the sugar residues present on the galactosylatable protein(s) migrating at 62–63 kDa (which must include p62). They have shown that the sugar consists of monosaccharidic O-linked GlcNAc residues.

An important result of our studies concerns the time and the topology of sugar addition. We found that most of the sugar residues are added to p62 within 5 min of its synthesis, either during translation or shortly thereafter. Because there is a further, albeit smaller, increase in mass after p62 becomes part of the pore complex (1) (which can be abolished by incubation with GlcNAcase), we assume that a small number of GlcNAc residues are added much later.

Most importantly, we found that newly synthesized p62, even when analyzed within 5 min after synthesis, is a soluble cytosolic protein. Most likely, it is synthesized by free (not

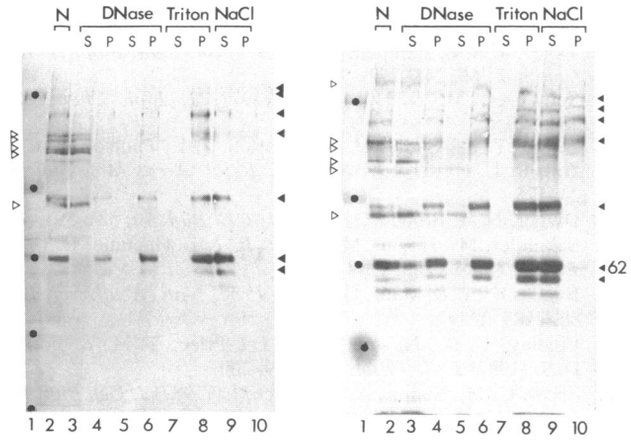

FIG. 5. Protein blots of subnuclear fractions probed with mAb 457 or WGA. Isolated rat liver nuclei were fractionated as described (1, 12). The samples were centrifuged each time for 10 min at 20,000 × g_{avg} to yield supernatant (lanes S) and pellet (lanes P) fractions. Nuclei (N) (lanes 2) were digested twice with DNase I (lanes 3–6). The pellet from the second digest was extracted with 10% sucrose/10 mM triethanolamine hydrochloride, pH 7.4/5 mM $MgCl_2$/2% Triton X-100 (lanes 7 and 8). The resultant pellet was extracted with 10% sucrose/10 mM triethanolamine hydrochloride, pH 7.4/5 mM $MgCl_2$/0.5 M NaCl (lanes 9 and 10). All buffers contained 1 mM dithiothreitol and 0.3 mM $PhMeSO_2F$. The equivalents (1 equivalent corresponds to that amount of material derived from 3 × 10^6 nuclei) loaded onto the lanes were 1 (lanes 2–5), 2 (lanes 5 and 6), and 4 (lanes 7–10). The samples were electrophoresed on a $NaDodSO_4$/7% polyacrylamide gel, which was then electroblotted to nitrocellulose. Blots were probed with either mAb 457 (*Left*) or biotinylated WGA (*Right*) as described (1). Lanes 1 contain prestained molecular mass markers (●): myosin, 200 kDa; phosphorylase B, 97.4 kDa; bovine serum albumin, 68 kDa; ovalbumin, 43 kDa; and α-chymotrypsinogen, 25.7 kDa. ▷, Proteins extracted by nuclease digestion; ◀, proteins fractionating with the pore complex–lamina.

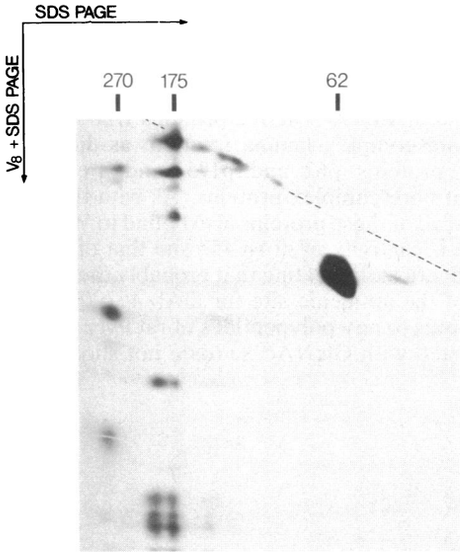

FIG. 6. Protease mapping of proteins immunoprecipitated by mAb 414. Isolated rat liver nuclei were galactosylated *in vitro* and immunoprecipitated with mAb 414. The immunoprecipitate was subjected to $NaDodSO_4$ (SDS)/PAGE, after which the lane was excised and laid horizontally over a second $NaDodSO_4$/polyacrylamide gel. Sample buffer containing *Staphylococcus aureus* V8 protease was layered over the gel slice, and the sample was reelectrophoresed. The polypeptides generating the fragments are indicated at the top by their molecular mass (in kDa) in the first dimension. The dotted line represents the diagonal upon which undigested material migrates in the second dimension.

membrane bound) ribosomes and subsequently released into the cytosol. Therefore, addition of GlcNAc residues is probably carried out by a cytosolic glycosyltransferase. This is in contrast to proteins irreversibly segregated into the lumen of the endoplasmic reticulum and Golgi compartments, which are glycosylated by membrane-bound transferases. Thus, the addition of GlcNAc residues to p62 is topologically distinct from the glycosylation pathways involving endoplasmic reticulum and Golgi membranes.

mAb 414 precipitated p62 as well as two other proteins, p175 and p270, out of a sample derived from [^{3}H]galactosylated rat liver nuclei. p175 had also been observed previously to react with mAb 414 on protein blots and by immunoprecipitation from [^{35}S]methionine-labeled tissue culture cells (1). Since mAb 414 decorated only the nuclear pore complex in immunoelectron microscopy, we assume that both p62 and p175 are localized to this structure. Our failure to detect p270 repeatedly on protein blots is most likely due to its high molecular weight; however, it also could be that we only detected it in [^{3}H]galactosylated samples because galactosylation of p270 increased its affinity for mAb 414. Thus, since the possibility exists that mAb 414 may not react with p270 *in situ*, we cannot draw any conclusions as to the location of this antigen. From one-dimensional peptide mapping of p62, p175, and p270 (Fig. 6) and the other GlcNAc-containing proteins of the nucleus (data not shown), it appears that each polypeptide represents a unique protein. Nevertheless, the crossreactivity of several proteins with mAb 414 indicates that at least these polypeptides share common epitopes. These epitopes must be at least partly composed of protein because mAb 414 reacts with p62 even after digestion with glycosidase (data not shown).

mAb 457 precipitated at least six galactosylated proteins (Fig. 3), among them being p62, p175, and p270. Immunofluorescence with mAb 457 showed diffuse granular staining of the nucleus, with exclusion of the nucleolar region. Thus, mAb 457 reacts with polypeptides located in the nuclear pore complexes and others in the nuclear interior. This conclusion was supported by data on nuclear subfractionation (Fig. 5). DNase treatment of rat liver nuclei quantitatively extracted at least four major mAb 457-reactive polypeptides, all of which also bound to WGA. The remainder of the mAb 457-reactive proteins fractionated with the crude pore complex–lamina fraction, as did the mAb 414-reactive proteins p62 and p175, and are thus likely to represent pore complex proteins. As with the internal polypeptides, all of these proteins also bound to WGA. In fact, the pattern of reactivity of mAb 457 and that of WGA are more or less identical, indicating that probably the GlcNAc moiety is part of the antigenic site for mAb 457. This antibody no longer binds to any polypeptides of rat liver nuclei after they are digested with GlcNAcase (data not shown).

The fundamental significance of this modification remains unknown. One intriguing possibility is that GlcNAc residues provide a recognition site along the nucleocytoplasmic pathway for transport of proteins and/or RNA. It should be noted here that WGA has been shown to inhibit both RNA export (14) and protein import (15) *in vitro*.

At the conclusion of this work, it was reported that a set of monoclonal antibodies that reacted with various members of the "O-linked GlcNAc" containing proteins of the nuclear envelope had been produced (16, 17). All of these antibodies bound specifically to the pore complex when immunoelectron microscopy was performed on isolated rat liver nuclei. However, as in the case of mAbs 414 and 457, none of the mAbs appear to be entirely monospecific. Thus, the definitive localization of each of the proteins to the pore complex has to await the production of monospecific antibodies.

We thank Nilabh Chaudhary for many helpful suggestions regarding glycosylation. This work was supported by National Institutes of Health Grant GM27155. L.I.D. was supported in part by National Science Foundation Training Grant GM07982.

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