An N-terminal Truncated Form of Orp150 Is a Cytoplasmic Ligand for the Anti-proliferative Mushroom Agaricus bisporus Lectin and Is Required for Nuclear Localization Sequence-dependent Nuclear Protein Import*

Received for publication, April 12, 2002
Published, JBC Papers in Press, April 17, 2002, DOI 10.1074/jbc.M203550200

Lu-Gang Yu‡, Nigel Andrews‡, Mike Weldon¶, Oleg V. Gerasimenko§, Barry J. Campbell‡, Ravinder Singh‡, Ian Grierson‡, Ole H. Petersen§, and Jonathan M. Rhodes§||

From Departments of Medicine and Physiology, The Henry Wellcome Laboratory of Molecular and Cellular Gastroenterology, University of Liverpool, Liverpool L69 3GA and Department of Biochemistry, University of Cambridge, 80 Tennis Court Rd., Cambridge CB2 1GA, United Kingdom

Nuclear localization sequence-dependent nuclear protein import is essential for maintaining cell function and can be selectively blocked in epithelial cells by mushroom (Agaricus bisporus) lectin. Here we report that a major intracellular ligand for this lectin is an N-terminally truncated form of oxygen-regulated protein 150 (Orp150), which lacks the endoplasmic reticulum translocation signal peptide of full-length Orp150. This cytoplasmic form of Orp150 expresses the lectin carbohydrate ligand (sialyl-2,3-galactosyl-1,3-N-acetylgalactosamine-α) and is shown to be essential for nuclear localization sequence-dependent nuclear protein import.

Nuclear compartmentation is one of the key features that differentiate eukaryotic cells from prokaryotic cells. Genetic information in the nucleus is separated from its cytoplasmic protein regulators by the nuclear envelope. Molecular communication across the nuclear envelope occurs through nuclear pore complexes and is essential for maintaining cell function and growth.

In previous studies, we demonstrate that a lectin (ABL) from the common edible mushroom Agaricus bisporus, which recognizes the Thomsen-Friedenreich antigen (Galβ1,3GalNAccα) and its sialylated form (sialyl-L,3-galactosyl-β1,3-N-acetylgalactosamine-α) (1–3), produces reversible inhibition of proliferation in a range of epithelial cells (4). The lectin has to be internalized to produce this effect and selectively blocks the classical NLS1-dependent nuclear protein import (5). This implied that this lectin could be a very useful tool in further elucidation of this system.

The classical NLS-dependent nuclear protein import is to date the best characterized nucleocytoplasmic transport pathway that mediates nuclear import of large proteins (>40–60 kDa) (6–8). The classical NLS, which contains short stretches of basic amino acids, is recognized by the “adapter” protein importin α. Importin α (karyopherin α) binds to importin β (karyopherin β) (9), and the cargo-importin complex then translocates into the nucleus through nuclear pore complexes. The cargo-importin complex is then dissociated by RanGTP (10), thus releasing the cargo from its carrier. Importin α then forms a trimeric complex with an importin β-related protein CAS (cellular apoptosis susceptibility gene protein) (11) and RanGTP, and this together with the importin β–RanGTP complex returns to the cytoplasm.

In the cytoplasm, conversion of RanGTP to RanGDP by GT-Pase-activating protein (RanGAP1) and Ran-binding protein (RanBP1) results in dissociation of the Ran-importin complexes and recycling of the carrier importin molecules (12–14). RanGDP then transports back to the nucleus after forming a complex with nuclear transport factor 2 (NTF2) (15–17) and is converted back to RanGTP inside the nucleus (14, 18).

Thus, transport of cargo proteins requires three types of soluble factors, transport receptor molecule (importin β), adapter molecule (importin α), and Ran and its binding proteins. Identification of these soluble transport factors has largely been achieved using digitonin-permeabilized cells in which these factors have been shown to be essential for nuclear protein import.

We have demonstrated in this study that one of the intracellular ABL binding ligands is a truncated, hence cytoplasmically localized form of oxygen-regulated protein 150 (Orp150) that is also shown to express the ABL ligand, sialyl-L,3-galactosyl-β1,3-N-acetylgalactosamine-α. Introduction of an antibody against Grp170 (the hamster homologue of Orp150) or depletion of Orp150 from the transport system by prior immunosorption inhibited nuclear accumulation of NLS-bovine serum albumin (BSA)-fluorescein isothiocyanate (FITC) in digitonin-permeabilized cells. This suggests that the N-terminal truncated form of Orp150 is essential for NLS-dependent nuclear protein import.

EXPERIMENTAL PROCEDURES

Materials—Peroxidase-ABL, agaro-ABL, FITC-ABL, and peroxidase-FNA were from EY Laboratories Inc. (San Mateo, CA). Sialidase (EC 3.2.1.18) from Arthrobacter ureafaciens, sialidase from Newcastle Disease virus (EC 3.2.1.18), and O-glycanase (endo-α-N-acetylgalactosaminidase) (EC 3.2.1.97) from Streptococcus pneumoniae were obtained from Oxford GlycoSciences (Abingdon, UK). Fluorescent mounting medium was purchased from Vector Laboratories Inc. (Peterborough, UK). Anti-Orp150/Grp170 polyclonal antibody gener-
centrifugations of the supernatant at 40,000g. Binding to ABL-agarose was released by elution with 5 mM galactose-

Confocal Microscopy—To assess the localization of Orp150 and ABL by ConfoCal Microscopy—To assess the localization of Orp150 and ABL, subconfluent HT29 cells were cultured on glass coverslips in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum for 2 days. The medium was changed to serum-free medium (Dulbecco’s modified Eagle’s medium containing 0.25% mg/ml BSA), and FITC-ABL (10 μg/ml) was added. After 6 h at 37°C, the cells were washed once with culture medium, twice with PBS, and fixed with ethanol for 10 min at −20°C. The cells were then treated with 0.5% BSA, 0.5% Triton-X-100, PBS, and 1% BSA, PBS each for 30 min at room temperature. Anti-Grp170 antibody (1500) in 1% BSA, PBS was then applied for 1 h. After 4 washes with PBS, Texas Red conjugated anti-rabbit antibody (1.50) in 1% BSA, PBS was applied for 1 h. After extensive washes, the cells were mounted, and the subcellular distributions of FITC and Texas Red were monitored simultaneously by confocal microscopy (Noran Instruments Inc., Middleton, WI) with 60x Nikon objective (1.4 NA) with excitation at 488 nm and emission 500–560 nm for FITC and above 610 nm for Texas Red. Image acquisition in slow mode (800 ns) was used with a slit of 25 μm, and images were processed using Two-D Analysis software (Noran Instruments Inc.).

Conjugation of NLS Peptide to BSA-FITC—A peptide containing the SV40 large T antigen wild type NLS CCGGGPKKKRKVED was synthesized as previously described (4). The NLS peptide was then conjugated to BSA-FITC (Sigma) as previously described (5) with modification. Briefly, 10 mg/ml BSA-FITC was activated by incubation with a 100-fold molar excess of sulfo-SMCC (sulfosuccinimidyl 4-[N-maleimidyl-ethyl]cysteine-1-carboxylate) (Pierce and Warriner, Chester, UK) for 90 min at room temperature in 100 mM HEPES-NaOH (pH 7.3). Excess cross-linker was removed by gel filtration on a PD10 column containing 9.1 ml of Sephadex-G25 (Amersham Biosciences). The activated BSA solution was reacted with a 30-fold molar excess of NLS peptide in 10 mM HEPES-NaOH (pH 7.5) for 2 h at 37°C. Uncoupled peptide was removed by gel filtration on a PD10 column equilibrated in 150 mM NaCl. The molar ratio of coupling was 15–25 peptides/BSA molecule as estimated from the electrophoretic mobility.

Nuclear Import of NLS-BSA-FITC in Digtinogin-permeabilized HT29 Cells—Preparation of HT29 cell cytosol extracts for nuclear protein import was performed as described previously (5). Cell permeabilization and in vitro nuclear protein import was carried out by a modification of the method previously described (5). HT29 cells were cultured on glass coverslips inserted into 24-well cell culture plates for 3 days in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum. The cells were rinsed twice with PBS and twice with cold transport buffer (20 mM HEPES-NaOH (pH 7.3), 110 mM potassium acetate, 5 mM sodium acetate, 2 mM magnesium acetate, 1 mM EGTA, 2 mM dithiothreitol, 1 μg/ml aprotinin, and 1 μg/ml leupeptin) and then permeabilized by incubation with 40 μg ml −1 digitonin (Sigma) in transport buffer for 6 min at 4°C. After 5 washes with ice-cold transport buffer, transport mixture (final volume, 200 μl) was applied (20% cytosol fraction, 1 mM ATP, 5 mM creatine phosphate, 20 units/ml creatine phosphokinase, 2 mM BSA-NLS-fluorescein in transport buffer), and a protein transport reaction was allowed to proceed for 45 min in a 30°C incubator. Protein transport was stopped by the addition of 1-ml of ice-cold transport buffer. After extensive washes with ice-cold transport buffer, the slides were fixed with ice-cold ethanol and mounted with Vectashield mount-
ing medium H-1000 (Vector). Intracellular fluorescein localization was detected by fluorescence microscopy (Polyvar, Reichert-Jung, Austria), and images were recorded using a Kodak DC120 digital camera (Eastman Kodak Co.). Distribution of fluorescence in the nucleus and cytoplasm was analyzed as described previously (5). Each experiment was repeated at least three times with at least six randomly selected cells analyzed on each occasion.

For inhibition experiments, the cytosol extract was first incubated with 100 µg/ml wheat germ agglutinin or anti-Grp170 antibody (1:100) for 20 min at room temperature before mixing with the other transport components and introduced to the digitonin-permeabilized cells. Immunoprecipitation and Immunoabsorption—100 µl of cell cytosol extract (120 µg protein) obtained as described above was “precleaned” by mixing with 10 µl of protein A-agarose beads (Sigma) for 10 min. The beads were discarded after centrifugation at 16,000 × g for 3 min. The precleaned cytosol extract was incubated with anti-Grp170 antibody (1:50) for 1 h before introduction of 10 µl of protein A-agarose beads for another hour. After centrifugation at 16,000 × g for 5 min, the supernatant was kept as Orp150-depleted cytosol extract to be used in nuclear transport. The immunoprecipitate was washed thoroughly with PBS and kept as Orp150 immunoprecipitate.

### RESULTS

**Affinity Purification of ABL Intracellular Binding Ligands**—Using ABL-agarose affinity purification, eight cytosolic proteins within the molecular mass range 46–300 kDa were obtained from HT29 cytosolic protein extracts, of which the four lower molecular weight proteins were stained strongly and the four higher molecular weight proteins were stained weakly by Coomassie Blue (Fig. 1A). A lectin blot of the eluate showed that only 4 of the 8 eluted proteins with molecular weights of 90, 120, 160, and >250 kDa were directly recognized by ABL (Fig. 1B). The four proteins directly recognized by ABL were those that stained weakly with Coomassie Blue (Fig. 1B).

To determine whether any of these proteins was a known cytoplasmic transport factor, the apparent molecular sizes of the ABL-binding proteins were compared with those of the known transport factors. It was clear that only the 90-kDa protein appeared to be close in size to one of the known trans-
port factors, importin $\beta$. However, immunoblotting using anti-importin $\beta$ monoclonal antibody (Transduction Laboratories, Lexington, KY) indicated that this 90-kDa intracellular protein obtained from ABL affinity purification was not importin $\beta$ (data not shown).

**Glycosylation Characterization of ABL Intracellular Binding Ligands—**Our previous studies have shown that the ABL-mediated effect on nuclear protein import is prevented by the addition of glycoproteins that express the ABL carbohydrate ligand, galactosyl-$\beta$1,3-N-acetylgalactosamine-$\alpha$, the Thomsen-Friedenreich (TF) blood group antigen (4). This suggests that the intracellular ABL-ligand interaction is mediated through lectin-carbohydrate interactions. Therefore, to further identify the functional intracellular ligand of ABL, we investigated the glycosylation of the intracellular ABL-binding proteins obtained from ABL affinity purification.

It was found that none of the proteins eluted from ABL-agarose were recognized by anti-TF-monomoclonal antibody (Fig. 2A, first lane) or by the TF binding PNA lectin (Figs. 2B and 2C, first lane), suggesting the absence of unsubstituted TF antigen on these intracellular proteins. To determine whether any of the intracellular ABL-binding proteins might express sialyl-TF antigen, the alternative carbohydrate binding ligand of ABL (1–3), the eluate was treated with $A$. ureafaciens sialidase to cleave sialic acid $\alpha$2–3-linked to galactose or 2–6 to N-acetylgalactosamine. It was found that removal of sialic acid residues resulted in strong binding of both anti-TF antibody (Fig. 2A, second lane) and PNA (Fig. 2B, second lane) to the 160-kDa protein in addition to weak binding to the 90- and >250-kDa proteins. Further treatment of the sialidase-treated eluate with O-glycanase (endo-$\alpha$-N-acetylgalactosaminidase) to allow specific removal of terminal Gal$\beta$1–3GalNAc resulted in an 84% reduction in binding of anti-TF antibody (Fig. 2A, third lane, and Fig. 2D) and a 62% reduction in binding of PNA (Fig. 2B, third lane and Fig. 2E) to the sialidase-treated 160-kDa protein. Weak binding to the 90- and >250-kDa proteins by anti-TF antibody and PNA was also largely inhibited by the sialidase treatment (Fig. 2). These results suggest that the 90-, 160-, and >250-kDa intracellular proteins (but not the 120-kDa protein) are sialyl-TF antigen-expressing glycoproteins.

Although $A$. ureafaciens sialidase removes sialic acid whether $\alpha$2–3- or $\alpha$2–6-linked to galactose or N-acetylgalactosamine, it is known that ABL does not recognize galactosyl-$\beta$1,3-(sialyl$\alpha$2–6)-N-acetylgalactosamine-$\alpha$ (1–3). Moreover, PNA would be expected to bind galactosyl-$\beta$1,3-(sialyl$\alpha$2–6)-N-acetylgalactosamine-$\alpha$ (2). Therefore these results suggest that the sialic acid on these intracellular ABL-binding proteins is $\alpha$2–3-linked to galactose. This is confirmed by the following studies using sialidase from Newcastle Disease virus that specifically cleaves non-reducing terminal $\alpha$2–3-linked sialic acid residues. As shown in Fig. 2, treatment of the eluate with sialidase from Newcastle Disease virus resulted in strong binding of PNA to the 160- and >250-kDa proteins in addition to weak binding to the 90-kDa protein (Fig. 2C, second lane). Again, further treatment of the sialidase-treated eluate with O-glycanase to allow specific removal of unsubstituted terminal Gal$\beta$1–3GalNAc resulted in a 73% reduction in binding of PNA to the 160-kDa protein (Fig. 2C, third lane, and 2F) as well as significantly decreased binding to the 90- and >250-kDa proteins. These results are in keeping with the conclusion that these proteins express sialyl-2,3-galactosyl-$\beta$1,3-N-acetylgalactosamine-$\alpha$ (sialyl-TF).

**Identification and Intracellular Localization of Orp150—**Because the 160-kDa ABL-binding protein is the major intracellular sialyl-TF-expressing glycoprotein (Fig. 2), this protein was selected for N-terminal sequencing. The sequence obtained, LAVMSVDLG, matched exactly with the 33–42 amino acid sequence of oxygen-regulated protein 150 (Orp150), a member of the stress-related protein family that has more than 90% amino acid sequence identity with its hamster homologue glucose-regulated protein 170 (Grp170) (21). The amino acids 33–426 of Orp150/Grp170 protein exhibit 32 and 30% identity, respectively, with amino acids 1–380 of human and bovine Hsp70 (21, 22), a heat-stress related protein directly involved in NLS-dependent nuclear protein import (23–27). Immunoblot of the ABL affinity-purified proteins using a polyclonal antibody against Grp170 (Orp150) further supported Orp150 as the 160-kDa ABL-binding protein (Fig. 3A). Immunoblotting with anti-Grp170 (Orp150) antibody confirms that treatment with sialidase and O-glycanase induced electrophoretic shifts, in keeping with expression of sialyl-TF by Orp150 (Fig. 3B).

Orp150 is known as an ER-associated protein that is thought to function as a molecular chaperone. Because the N-terminal 32 amino acids represent the signal peptide for ER translocation (21, 28), the discovery that ABL-purified Orp150 lacks this signal peptide suggests that this may represent a free cytoplasmic form of Orp150 as predicted by Kanaeda et al. (28). Dual labeling of the cells with FITC-ABL and anti-Grp170 antibody/Texas Red-conjugated second antibody assessed by confocal microscopy revealed co-localization of ABL and Orp150 in the cytoplasm, particularly in the perinuclear region (Fig. 4A). The existence of cytoplasmic Orp150 is further demonstrated by digitonin treatment of the cells under conditions that selectively release cytoplasmic but not Golgi or ER resident proteins (43). Orp150, like Hsp70, is released from the cells by digitonin treatment, whereas the typical ER-resident proteins, Erp72 and protein disulfide isomerase, are not released by this treatment (Fig. 4B).

**Effect of Anti-Grp170 Antibody on Nuclear Localization of NLS-BSA-FITC in Digitonin-permeabilized Cells—**Given the cytoplasmic localization of the N-terminally truncated Orp150, its expression, like sialyl-TF, its co-localization with ABL, its sequence similarity with nuclear transport factor Hsp70, and the known inhibition of NLS-dependent nuclear import by ABLs, we speculated that Orp150 might be involved in NLS-dependent nuclear protein import. We therefore investigated the role of Orp150 in nuclear protein import of a NLS-peptide complex (NLS-BSA-FITC) in vitro in digitonin-permeabilized cells.
NLS-BSA-FITC accumulates in the nucleus in this transport system (Fig. 5A). Nuclear import of this NLS complex was reduced by 53% \((n/H_11005\)3) by the presence of wheat germ agglutinin (40 \(H_9262\)gm l\(H_11002\)1) (Fig. 5, A and C), a lectin that has been shown previously to block nuclear protein import by binding to N-acetylglucosamine on p62 nucleoporin (29). The transport process was also inhibited by 52% \((n/H_11005\)3) in the absence of ATP (Fig. 5, A and C). Introduction of an anti-Grp170 antibody to the transport system largely blocked the nuclear accumulation of NLS-BSA-FITC (Fig. 5A and C). Introduction of irrelevant antibodies such as anti-Erk1/2 antibody (Promega UK Ltd., Southampton, UK) (1:50) (not shown) or normal rabbit serum (1:50) to the system did not show any effect on the nuclear accumulation of NLS-BSA-FITC.

**Fig. 4.** Intracellular localization of Orp150 assessed by confocal microscopy and digitonin treatment. A, intracellular co-localization of ABL with Orp150 by confocal microscopy. HT29 cells were incubated with FITC-ABL for 6 h before being fixed and probed with anti-Orp150 antibody followed by Texas Red-conjugated secondary antibody. Shown is co-localization (yellow) of ABL (green) and Orp150 (red) in the perinuclear region (typical example from one of three experiments). Bar, 10 \(\mu\)m. B, detection of cytoplasmic proteins released by digitonin treatment. Lysed whole cells (4 \(\times\) 10⁴), digitonin-permeabilized cells (4 \(\times\) 10⁴), or digitonin-released fractions from 4 \(\times\) 10⁴ cells were separated by electrophoresis followed by immunoblotting using antibodies against Orp150, ERp72, protein disulfide isomerase (PDI), and Hsp70. Orp150 and Hsp70 are released by digitonin permeabilization, whereas the ER luminal proteins ERp72 and protein disulfide isomerase are not released.

**Fig. 5.** Effect of the addition of antibody against Orp150 on nuclear accumulation of NLS-BSA-FITC in digitonin-permeabilized HT29 cells. A, panel 1 shows NLS-BSA-FITC accumulated in the nucleus in the presence of cytosol extracts and ATP regeneration system. NLS-BSA-FITC nuclear accumulation was inhibited in the presence of wheat germ agglutinin (WGA) (2) and in the absence of ATP (3). Introduction of anti-Grp170 antibody (4) or depletion of Orp150 from the cytosolic extracts by immunoabsorption (5) both inhibited NLS-BSA-FITC nuclear accumulation. The addition of irrelevant antibody or normal rabbit serum (6) did not affect NLS-BSA-FITC nuclear accumulation. Each microphotograph shows representative cells from one of three (2 and 3) and five (1, 4–6) experiments. Bar, 10 \(\mu\)m. B, detection of cytoplasmic proteins released by digitonin treatment. Lysed whole cells (4 \(\times\) 10⁴), digitonin-permeabilized cells (4 \(\times\) 10⁴), or digitonin-released fractions from 4 \(\times\) 10⁴ cells were separated by electrophoresis followed by immunoblotting using antibodies against Orp150, ERp72, protein disulfide isomerase (PDI), and Hsp70. Orp150 and Hsp70 are released by digitonin permeabilization, whereas the ER luminal proteins ERp72 and protein disulfide isomerase are not released.
NLS-BSA-FITC (Fig. 5A). Similar results were observed also in human gastric cancer cell line AGS cells (not shown).

To exclude the possibility that the inhibition of nuclear import by the addition of anti-Grp170 antibody to the digitonin-permeabilized cells might have been due to binding to ER-associated Orp150, NLS-BSA-FITC nuclear import was also investigated in the presence of free cytosolic Orp150-depleted cytosol extracts obtained by Orp150 immunoabsorption. As shown in Fig. 5B, more than 95% Orp150 was removed from the cytosol extracts by this immunoabsorption process, whereas ER-associated Orp150, which remained within the permeabilized cells, would not be affected by this process. In the presence of these Orp150-free cytosol extracts, nuclear accumulation of NLS-BSA-FITC was reduced by 41% \((\text{n} / \text{H}11005)3\) (Fig. 5A and C). To check that the inhibition of NLS-BSA-FITC nuclear accumulation observed using Orp150-depleted cytosol extract compared with that which resulted from direct introduction of an anti-Grp170 antibody to the system may be due to the presence of residual Orp150 remaining within the cells after permeabilization.

Immunoprecipitation of Orp150 to Identify Its Possible Association with Other Known Cytoplasmic Nuclear Transport Factors—Having demonstrated the involvement of Orp150 in NLS-dependent nuclear import process, the possible association of Orp150 with the other known cytoplasmic nuclear transport factors was investigated by immunoprecipitation (Fig. 6). No importin \(\alpha\) (Fig. 6B), importin \(\beta\) (Fig. 6C), or Hsp70 (Fig. 6D) was detected in the Orp150 immunoprecipitate. However, a small amount of Ran (Fig. 6E) was found to be co-immunoprecipitated with Orp150. This suggests that Orp150 may form a functional complex with Ran inside cells. This is supported by the observation that ABL affinity purification of the cell cytosol extracts, which pulls out Orp150, also pulls out a small amount of Ran (Fig. 6F), as identified by immunoblotting. To estimate the relative amount of Ran in the Orp150 immunoprecipitate, optical density of the relevant bands was quantified by densitometry. The Ran band in the Orp150 immunoprecipitate (from 100 \(\mu g\) of cytosolic extract proteins) was 10 times less dense than the Ran band in the control cytosol (10 \(\mu g\) of protein).
DISCUSSION

The present studies show that Orp150, a highly diverged Hsp70-like protein, has an N-terminal truncated form that is present in cytoplasm, expresses sialyl-TF, and acts as a major ligand for ABL. Introduction of anti-Orp150 antibody or depletion of Orp150 from the transport system largely inhibited nuclear accumulation of NLS-BSA-FITC in digitonin-permeabilized cells. This suggests that the truncated Orp150 functions as a cytosolic transport factor that is essential for the NLS-dependent nuclear transport process.

The Orp150 protein family was first identified in Chinese hamster ovary cells and termed glucose-regulated protein Grp170 (19). It has subsequently been identified in rat (30) and human (21). Alignment of the deduced amino acid sequences of human Orp150, rat Orp150, and hamster Grp170 indicates greater than 91% identity (21), suggesting that they are functionally homologous proteins. The N-terminal 33–426 amino acids of Orp150/Grp170 are 32% identical to the 1–380 amino acids of both inducible human Hsp70 and constitutive bovine Hsp70 (21, 22). Its N-terminal 400 residues also contain an ATPase domain similar to both Hsp70 and Hsp110 (22). The full-length Orp150 is an ER-associated glycoprotein (19, 31) with a C-terminal ER retention sequence of NDEF and an N-terminal ER-translocation sequence (21, 22). Induction of Orp150 expression has been demonstrated in human (32, 33) and rat cells (30) by hypoxia and hypoglycemia but not by heat. The physiological function of Orp150 is still not well understood. It has been suggested that Orp150/Grp170 is involved in protein folding and translocation into the endoplasmic reticulum (31, 34–37) and release of proteins from the ER (38). Recently it has been reported that Orp150 plays a cytoprotective role in hypoxia-induced apoptosis in human embryonic kidney cells (33) and hypoxia/ischemia-induced neuronal death in human brain (39).

It is intriguing to find that a protein thought to be ER-associated could be actively involved in nuclear translocation. The explanation of this apparent paradox seems to be that cells also contain a cytosolic form of Orp150. Recently Holaska et al. (43) discovered that calreticulin, an ER-associated protein involved in protein folding (40, 41) and protection of nonglycosylated proteins from thermal denaturation in the lumen of the ER (42), also has a cytosplastic form that acts as a receptor, mediating NES-related nuclear export by forming an assembly with NES-protein and RanGTP. Although the presence of cytosolic Orp150/Grp170 has not been reported before, Kaneda et al. (28) recently cloned and sequenced the entire human Orp150 gene and discovered three distinct Orp150 mRNAs produced by alternative promoters. Analysis of transcription initiation sites and transcriptional regulatory sequences revealed that two of the Orp150 mRNAs are started from exon 1 (1A and 1B), and the transcript beginning from exon 1B is preferentially induced by hypoxia. The third mRNA, which starts from alternative exon 2, was predicted to produce a cytosolic protein lacking the N-terminal 32 amino acid ER translocation signal peptide. In the present study, N-terminal protein sequencing of ABL affinity-purified Orp150 has revealed just such a truncated form of Orp150.

One property expected for a transport factor is its association, at least at some stage during the transport process, with the other known transport factors such as importins or the Ran protein family. The discovery that Orp150 forms a complex inside cells with the small GTPase Ran implies that Orp150 may be involved in the nuclear transport process by interacting with Ran and perhaps has a role in the cycling of Ran-GTP and Ran-GDP between the cytoplasm and nucleus that plays a key role in controlling the directionality of nucleocytoplasmic transport (10, 53).

During the last decade, evidence has accumulated to suggest that many cytosolic and nuclear proteins are modified by glycosylation (44–46). The best characterized intracellular glycosylation so far is O-linked N-acetylgalactosamine, which has been found on many cytosplastic and nuclear proteins such as transcription factors, nucleoporins, cytoskeletal proteins, onco- gene products, and chromatin-associated proteins (44, 47). It has been suggested that intracellular O-GlcNAc glycosylation, like phosphorylation, may play an important role in many key cellular processes (44, 45, 47). Other sugars, such as galactose, glucose, mannose, N-acetylgalactosamine, and sialic acid, have been occasionally reported on intracellular structures, usually demonstrated by lectin binding (48). However, the cytoplasmic or nuclear glycoconjugates that express these sugars have not been identified, and in particular, no intracellular glycoproteins bearing galactose-terminated or TF-expressing oligosaccharides have been identified. The functional role of such O-linked oligosaccharides is not known, and concealment of O-linked GlcNAc on nucleoporin p62 by further extension with galactose has been shown not to affect protein import (49). The functional role of sialyl-TF expression by Orp150 glycoprotein in its role as a nuclear transport protein is currently unclear. Duverger et al. (50–52) suggest that sugars such as glucose, fucose-, mannose, or N-acetyllactosamines could serve as nuclear localization signals and showed that glycoconjugates of BSA can move rapidly into the nucleus. They have shown that, like the classical NLS-mediated nuclear import, sugar-mediated nuclear import also requires energy and is blocked by wheat germ lectin (51, 52). Unlike the classical NLS pathway, however, this sugar-mediated nuclear import process has been reported not to require cytosolic transport factors (51).

Orp150 thus seems to have at least two functions. The full-length protein, which is targeted to the ER, serves as a stress protein involved in cellular response to metabolic stress, whereas the truncated protein, which is released into the cytoplasm, has a crucial role in NLS-dependent nuclear protein import.

Acknowledgment—We are very grateful to Dr. John Subjeck (Roswell Park Cancer Institute, Buffalo, New York) for the anti-Grp170 antibody.

REFERENCES

1. Presant, C. A., and Kornfeld, S. (1972) J. Biol. Chem. 247, 6837–6845
2. Chen, Y., Jain, R. K., Chandrasekaran, E. V., and Matta, K. L. (1995) Glycoconj. J. 12, 55–62
3. Sueyoshi, S., Tsuji, T., and Osawa, T. (1988) Carbohydr. Res. 178, 213–224
4. Yu, L., Fernig, D. G., Smith, J. A., Milton, J. D., and Rhodes, J. M. (1993) Cancer Res. 53, 4627–4632
5. Yu, L. G., Fernig, D. G., White, M. R. H., Evans, R. C., Appleton, P. A., Gossling, E., Davies, H., Gerasimenko, O. V., Petersen, O. H., Milton, J. D., and Rhodes, J. M. (1999) J. Biol. Chem. 274, 4890–4899
6. Mattaj, I. W., and Englmeier, L. (1998) Annu. Rev. Biochem. 67, 265–306
7. Adam, S. A. (1999) Curr. Opin. Cell Biol. 11, 402–406
8. Nakielny, S., and Dreyfuss, G. (1999) Cell 99, 677–690
9. Gorlich, D., Henklein, P., Laskey, R. A., and Hartmann, E. (1996) EMBO J. 15, 1810–1817
10. Gorlich, D., Pante, N., Kutay, U., Aebi, U., and Bischoff, F. (1996) EMBO J. 15, 5584–5594
11. Kutay, U., Bischoff, F. R., Kostka, S., Kraft, R., and Gorlich, D. (1997) Cell 90, 1061–1071
12. Coutavas, E., Ben, M., Oppenheim, J. D., D’Eustachio, P., and Rush, M. G. (1993) Nature 366, 585–587
13. Bischoff, F. R., Krebber, H., Smirnova, E., Dong, W., and Ponstingl, H. (1995) EMBO J. 14, 705–715
14. Takai, Y., Sasaki, T., and Matozaki, T. (2001) Physiol. Res. 81, 153–208
15. Ribbeck, K., Lipowsky, G., Kent, H. M., Stewart, M., and Gorlich, D. (1998) EMBO J. 17, 6587–6598
16. Steggerda, S. M., Black, E., and Peschel, B. (2000) Mol. Biol. Cell 11, 703–719
17. Quinby, B. B., Lamitina, T., L’Hernault, S. W., and Corbett, A. H. (2000) J. Biol. Chem. 275, 28575–28582
18. Dasso, M. (2001) Cell 104, 321–324
19. Lin, H. Y., Masso-Welsh, P., Di, Y. P., Cui, J. W., Shen, J. W., and Subjeck, J. R. (1993) Mol. Biol. Cell 4, 1189–1199
Truncated Orp150 Protein and Nuclear Protein Import

20. Smith, P. K., Krohn, R. I., Hermanson, G. T., Mallia, A. K., Gartner, P. H., Provenzano, M. D., Fujimoto, E. K., Goeke, N. M., Olson, B. J., and Klenk, D. C. (1985) Anal. Biochem. 150, 76–85
21. Ikeda, J., Kaneda, S., Kuwabara, K., Ogawa, S., Kobayashi, T., Matsumoto, M., Yura, T., and Yanagi, H. (1997) Biochem. Biophys. Res. Commun. 230, 94–99
22. Chen, X., Easton, D., Oh, H. J., Lee-Yoon, D. S., Liu, X. and Subjeck, J. (1996) FEBS Lett. 380, 68–72
23. Shi, Y., and Thomas, J. O. (1992) Mol. Cell. Biol. 12, 2186–2192
24. Imamoto, N., Matsuoka, Y., Kurihara, T., Keho, K., Miya, M., Sakiyama, F., Okada, Y., Tsunasawa, S., and Yeneda, Y. (1992) J. Cell Biol. 119, 1047–1061
25. Yang, J., and DeFranco, D. B. (1994) Mol. Cell. Biol. 14, 5088–5098
26. Shulga, N., Roberts, P., Gu, Z., Spitz, L., Tabb, M. M., Nomura, M., and Roberts, P., Gu, Z., Spitz, L., Tabb, M. M., Nomura, M., and Saphire, A. C., Guan, T., Schirmer, E. C., Nemerow, G. R., and Gerace, L. (1994) J. Biol. Chem. 269, 37, 439–438
27. Saito, Y., Ihara, Y., Leach, M. R., Cohen-Doyle, M. F., and Williams, D. B. (1998) EMBO J. 17, 7218–7229
28. Dierks, T., Volkmer, J., Schlenstedt, G., Jung, C., Sandholzer, U., Zachmann, M., and Tohyama, M. (1999) J. Biol. Chem. 274, 6397–6404
29. Okada, Y., Tsunasawa, S., and Yoneda, Y. (1992) J. Biol. Chem. 267, 6535–6540
30. Takasu, S., Okabe, M., Yanagi, H., Stern, D. M., Ogawa, S., and Tohyama, M. (2001) J. Biol. Chem. 276, 3057–3063
31. Krause, K. H., and Michalak, M. (1997) Cell 88, 439–443
32. Saito, Y., Ihara, Y., Leach, M. R., Cohen-Doyle, M. F., and Williams, D. B. (1999) EMBO J. 18, 6718–6729
33. Izaurralde, E., Kutay, U., von Kobbe, C., Mattaj, I. W., and Gorlich, D. (1997) Science 275, 189–197
34. Hart, G. W. (1997) Annu. Rev. Biochem. 66, 315–335
35. Kuznetsov, G., Chen, L. B., and Nigam, S. K. (1997) J. Biol. Chem. 272, 3057–3063
36. Vassilakos, A., Michalak, M., Lehrman, M. A., and Williams, D. B. (1998) Biochemistry 37, 3480–3490
37. Saito, Y., Ihara, Y., Leach, M. R., Cohen-Doyle, M. F., and Williams, D. B. (1999) EMBO J. 18, 6718–6729
38. Hart, G. W. (1997) Annu. Rev. Biochem. 66, 315–335
39. Kuznetsov, G., Chen, L. B., and Nigam, S. K. (1997) J. Biol. Chem. 272, 3057–3063
40. Krause, K. H., and Michalak, M. (1997) Cell 88, 439–443
41. Holaska, J. M., Black, B. E., Love, D. C., Hanover, J. A., Leszcz, J., and Paschal, B. M. (2001) J. Cell Biol. 152, 127–140
42. Vassilakos, A., Michalak, M., Lehrman, M. A., and Williams, D. B. (1998) Biochemistry 37, 3480–3490
43. Saito, Y., Ihara, Y., Leach, M. R., Cohen-Doyle, M. F., and Williams, D. B. (1999) EMBO J. 18, 6718–6729
44. Holaska, J. M., Black, B. E., Love, D. C., Hanover, J. A., Leszcz, J., and Paschal, B. M. (2001) J. Cell Biol. 152, 127–140
45. Hart, G. W. (1997) Annu. Rev. Biochem. 66, 315–335
46. Vassilakos, A., Michalak, M., Lehrman, M. A., and Williams, D. B. (1998) Biochemistry 37, 3480–3490
47. Saito, Y., Ihara, Y., Leach, M. R., Cohen-Doyle, M. F., and Williams, D. B. (1999) EMBO J. 18, 6718–6729
48. Holaska, J. M., Black, B. E., Love, D. C., Hanover, J. A., Leszcz, J., and Paschal, B. M. (2001) J. Cell Biol. 152, 127–140
49. Hart, G. W. (1997) Annu. Rev. Biochem. 66, 315–335
50. Vassilakos, A., Michalak, M., Lehrman, M. A., and Williams, D. B. (1998) Biochemistry 37, 3480–3490
51. Saito, Y., Ihara, Y., Leach, M. R., Cohen-Doyle, M. F., and Williams, D. B. (1999) EMBO J. 18, 6718–6729
52. Holaska, J. M., Black, B. E., Love, D. C., Hanover, J. A., Leszcz, J., and Paschal, B. M. (2001) J. Cell Biol. 152, 127–140
53. Hart, G. W. (1997) Annu. Rev. Biochem. 66, 315–335
An N-terminal Truncated Form of Orp150 Is a Cytoplasmic Ligand for the Anti-proliferative Mushroom *Agaricus bisporus* Lectin and Is Required for Nuclear Localization Sequence-dependent Nuclear Protein Import

Lu-Gang Yu, Nigel Andrews, Mike Weldon, Oleg V. Gerasimenko, Barry J. Campbell, Ravinder Singh, Ian Grierson, Ole H. Petersen and Jonathan M. Rhodes

*J. Biol. Chem.* 2002, 277:24538-24545.

doi: 10.1074/jbc.M203550200 originally published online April 17, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M203550200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 52 references, 24 of which can be accessed free at http://www.jbc.org/content/277/27/24538.full.html#ref-list-1