The QM Protein Associates with Ribosomes in the Rough Endoplasmic Reticulum†

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ABSTRACT: QM is a human cDNA originally isolated as a transcript elevated in a nontumorigenic Wilms' tumor microcell hybrid, relative to the tumorigenic parental cell line. Homologs of this gene have been identified from a large number of diverse eukaryotic species which demonstrate a high degree of conservation. The functional importance implied by this strong conservation is supported by the observation that the disruption of the yeast homolog is lethal. In spite of its apparent importance, the function of the encoded protein remains elusive. Indirect immunofluorescent cell staining of cultured human, G401 cells with an antibody to the QM protein shows a punctate staining pattern in the cytoplasm with much of the signal in a perinuclear pattern. Subcellular fractionation demonstrated an association of QM protein with the rough endoplasmic reticulum. It was possible to disrupt this association by washing microsomal membranes with 1M NaCl, suggesting a peripheral association. Proteolytic latency studies showed the protein to be exposed on the cytoplasmic face of the membrane. *In situ* cross-linking followed by diagonal SDS gel analysis indicates that QM exists as a member of a large protein complex. In agreement with this, QM was found to copurify with the ribosome complex. Incubation with 1 M NaCl was found to disrupt this association while having no effect on the association of core ribosomal proteins.

QM is a 753 base pair human cDNA originally isolated as a transcript that was expressed at elevated levels in a nontumorigenic microcell hybrid, relative to the tumorigenic parental cell line (Dowdy *et al*., 1991). The cDNA encodes a 24 kDa protein that is very rich in basic amino acids and shows no significant homology to any other known human protein (Dowdy *et al*., 1991). Southern blot analysis and genomic cloning indicate that QM is a member of a multigene family; however, many of these loci appear to result from retrotransposition events (Stanbridge *et al.*, 1994). To date, no other QM family members have been found to be expressed.

Although no functional QM homologs have been identified in humans, highly conserved homologs have been isolated from more than 10 different species from the plant, animal, and fungal kingdoms. This conservation approaches 70% identity in as diverse species as human and the yeast, *Saccharomyces cerevisiae* (Farmer *et al.*, 1994). In addition to being expressed in all eukaryotic species examined to date, the QM transcript has been found to be expressed in all tissues and developmental stages that have been evaluated. This expression is not wholly constitutive as QM expression shows a high degree of variability between different tissues and elevated expression in those tissues undergoing rapid proliferation (Dowdy *et al*., 1991; Kaneko *et al.*, 1992). This is consistent with studies in mouse 3T3-L1 adipocytes (Eisinger *et al.*, 1993), tobacco (Marty *et al.*, 1993), and NIH-3T3 cells (Loftus and Stanbridge, unpublished observations) which all show an association of QM (or homolog) expression with proliferation.

In spite of its ubiquitous nature and high degree of conservation, the function of the QM gene product remains elusive. Monteclaro and Vogt reported that the chicken homolog of QM (JIF-1) could bind to the proto-oncoprotein c-Jun *in vitro* and inhibit its function as a DNA-binding transcription factor (Monteclaro and Vogt, 1993). However, our studies indicate that this is not a relevant function *in vivo* (Loftus and Stanbridge, manuscript in preparation). A yeast homolog of QM has been reported by two groups (*QSR1*/*GRC5*). Tron *et al.* (1995) identified the yeast form as a point mutant which gave rise to a synthetic lethal in combination with a disruption of subunit 6 of the mitochondrial bc_1 complex. Koller *et al.* (1996) reported a temperature-sensitive mutation which results in growth arrest, abnormalities of the actin cytoskeleton, and accumulation of cell wall material at the septum.

Considering its high degree of sequence conservation, the function of QM is likely to be important. This is supported by the observation that disruption of the QM homolog (QSR1/GCR5) in yeast is lethal (Tron *et al*., 1995). In order to gain a better understanding of the role QM plays in the cell, we have undertaken a characterization of the subcellular localization, topology, and physical characteristics of the protein. We have found it to be peripherally associated with the cytoplasmic face of the endoplasmic reticulum and to copurify with the ribosomal fraction.

EXPERIMENTAL PROCEDURES

Materials. All tissue culture reagents were obtained from Gibco/BRL. Acrylamide−bisacrylamide was from Bio-Rad. Unless otherwise indicated, all remaining reagents were obtained from Sigma Chemical Co.

Mammalian Cell Lines and Culture Conditions. The mammalian cell lines employed in these studies were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 8% fetal bovine serum (FBS) (G401.6TG.C6

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and HT1080.6TG) or with 5% FBS and 10% donor horse serum (DHS) (PC-12). Cultures were maintained in a water-jacketed incubator at 37 °C and 5% CO_2. PC-12 cells were harvested by gently spraying the cells off of the culture surface with a stream of fresh medium from a 10 mL pipet. The remaining cell lines were harvested by first washing with phosphate-buffered saline−EDTA (PBS−EDTA) and then treating with 0.05% trypsin in PBS−EDTA. All cell lines were routinely tested for mycoplasma by culture and DNA staining methods (Stanbridge, 1981) and found to be negative.

Indirect Immunofluorescent Cell Staining. Prior to cell staining, G401.6TG.C6 cells were grown for 2 days on acid-washed glass coverslips. The cells were washed in PBS, fixed by a 15 min incubation in 2% paraformaldehyde in PBS, and permeabilized by incubation with 0.1% Triton X-100/PBS for 3 min. The fixed cells were washed extensively in PBS and incubated for 1 h with the C-17 anti-QM primary antibody [Santa Cruz, 2 μg/mL in 0.5 mg/mL bovine serum albumin (BSA) in PBS] or a no antibody control. The cells were then washed with PBS and incubated for 1 h in the dark with a 1:70 dilution of goat anti-rabbit Texas Red antibody conjugate (Vector). The coverslips were washed again and mounted in antifade and viewed using a Zeiss Axiophot fluorescent microscope.

Protein Quantitation, Gel Electrophoresis, and Immunoblot Analysis. Protein concentration was determined using the Bio-Rad DC protein assay system, a modification of the colorimetric protein assay of Lowry *et al.* (1951), with BSA as a standard. For electrophoresis, protein samples were combined with an equal volume of 2× SDS sample buffer [20 mM Tris, pH 6.8, 20% glycerol, 2% SDS, 0.1% bromphenol blue, and 2% β-mercaptoethanol (BME)] and boiled for 5 min. Samples were electrophoresed on 12% polyacrylamide−SDS gels using the discontinuous buffer system described by Laemmli (1970). For immunoblot analysis, proteins were transferred to an Immobilon polyvinylidine difluoride membrane (Millipore) using a Bio-Rad semidry blotting apparatus with 25 mM Tris, pH 9.5, and 20% methanol as the transfer buffer. Blots were blocked with 5% BSA and probed with the C-17 anti-QM primary antibody (0.1 μg/mL) and a goat anti-rabbit IgG−horseradish peroxidase conjugate (Santa Cruz) secondary antibody or with human antiribosomal P-antigen antibody (ImmunoVision, 1:100 dilution) and a rabbit anti-human IgG−horseradish peroxidase conjugate (Jackson ImmunoResearch Laboratories). Blots were washed as described by Burnette (1981) and visualized using the ECL chemiluminescent system (Amersham). Equivalence of protein loading was assessed by staining of proteins on the blot with 0.2% Coomassie blue G-250 in 50% methanol and 10% acetic acid and destaining in 50% methanol and 10% acetic acid prior to blocking with BSA.

Subcellular Fractionation. Broad fractionation by differential centrifugation was performed with homogenates from HT1080.6TG cells as described by Graham (1992). Cell pellets were suspended in 5 mL of homogenization buffer (0.25 M sucrose, 10 mM HEPES, pH 7.5) and homogenized by 20 strokes in a Dounce homogenizer with a tight-fitting pestle. The crude homogenate was subjected to several rounds of centrifugation at progressively increasing centrifugal force. At each step, the pellet represented the fraction of note while the supernatant was carried onto the next centrifugation step. The supernatant following the P5 fraction was taken to represent the soluble fraction of the cell. The centrifugation steps were as follows: P1, 1000*g* for 10 min; P2, 3000*g* for 10 min; P3, 10000*g* for 10 min; P4, 20000*g* for 10 min; and P5, 150000*g* for 50 min.

Density gradient separation of the P5 fraction was performed using the livers from female athymic nu/nu mice by the method of Ahlers (1992). Briefly, the 40000*g* supernatant of a mouse liver homogenate was centrifuged for 50 min at 150000*g* to create a crude microsomal fraction. The microsomal fraction was applied to a discontinuous density gradient comprised of 0.6 M sucrose/15 mM CsCl and 1.3 M sucrose/15 mM CsCl and centrifuged for 90 min at 102000*g*. The resulting pellet containing high-density microsomes was collected, while the isolated band was further purified by application to a second density gradient containing 0.25 M sucrose/15 mM CsCl and 0.9 M sucrose/15 mM CsCl, pH 7.5, and centrifugation for 14 h at 56000*g*. The resulting purified low-density microsome pellet was made up primarily of smooth endoplasmic reticulum while the isolated band contained mainly Golgi and plasma membrane vesicles.

Enzyme Assays. NADPH-dependent cytochrome *c* reductase activity was assayed in 1 mL of a reaction buffer containing 0.1 mM NADPH, 0.1 mM KCN, 0.1 mM cytochrome *c* (purified from horse heart, Sigma Chemical Co.), and 50 mM KH_2PO_4, pH 7.5, in a polystyrene disposable cuvette. To initiate the reaction, 40 μg of membrane protein was added and activity monitored as the increase in absorbance at 550 nm in an LKB Ultrospec spectrophotometer.

Glucose-6-phosphatase activity was assayed by the method of Aaronson and Touster (1974). To perform the assay, 25 μL of the protein to be assayed was combined with 225 μL of assay buffer (22 mM glucose 6-phosphate, 20 mM histidine, pH 6.5, and 1 mM EDTA) and incubated for 20 min at 37 °C. The reaction was terminated by the addition of 1 mL of 10% TCA, and samples were placed on ice for 5 min before a 5 min centrifugation at 13000*g* and 4 °C.

The supernatant was then assayed for free phosphate by adding 1−1.5 mL of a solution of 0.4% ammonium molybdenate in 0.833 N H_2SO_4. After 10 min, the absorbance of the sample at 340 nm was determined relative to a blank of 1 mL of water plus 1.5 mL of ammonium molybdenate/H_2SO_4. As controls, the assay buffer alone and the protein extract alone were assayed for free phosphate. Phosphate quantitation was standardized to a curve of known solutions of sodium phosphate.

Salt Treatment of Membranes. Density gradient purified rough endoplasmic reticulum membranes were suspended in a buffer containing 0.25 M sucrose and 10 mM HEPES−NaOH, pH 7.6 (minus salt), or in a similar buffer containing 1 M NaCl (plus salt). The membranes were then pelleted by centrifugation at 150000*g* for 30 min. The supernatants were then obtained and analyzed by immunoblot analysis.

Trypsin Treatment of Membrane Preparations. Proteolytic latency studies were performed independently on crude homogenate and partially purified microsomes prepared from mouse liver as described for subcellular fractionation. To test for the latency of proteins to protease digestion (Mandon *et al.*, 1992), three samples were set up for each membrane fraction. These contained (1) membranes with buffer only, (2) membranes with 1 mg/mL trypsin (bovine pancreas), or

(3) membranes with 1 mg/mL trypsin and 0.1% Triton X-100. Samples were incubated for 20 min at 37 °C followed by the addition of soybean trypsin inhibitor to 2 mg/mL and incubation for 10 min on ice. The concentration of Triton X-100 was then adjusted to 0.1% in all samples, and these were subjected to immunoblot analysis and glucose-6-phosphatase assays.

Myristylation Analysis. A 60% confluent T75 flask of PC-12 cells was labeled with 0.1 mCi/mL [9,10(N)-^{3}H]myristic acid (1.0 mCi/mL in ethanol, 51 Ci/mmol, Amersham) in 10 mL of DMEM supplemented with 5% dialyzed FBS according to the method of McIlhiney (1992). The cells were permitted to label for 6 h before harvesting and lysing in Tris-buffered saline−Triton X-100 (TBS−Triton 20 mM Tris, pH 7.4, 150 mM NaCl, 1% Triton X-100) supplemented with 1 μg/mL pepstatin and 2 μg/mL aprotinin. The lysate was clarified by centrifugation for 20 min at 100000*g* and the supernatant was precleared by incubation with 50 μL of washed protein A−agarose for 30 min at 4 °C, followed by centrifugation. The precleared lysate was then split into two microcentrifuge tubes and incubated with 1 μg of either the C-17 anti-QM antibody or the c-Src N-16 antibody (Santa Cruz) along with 20 μL of protein A−Sepharose. The samples were centrifuged to obtain the protein A−Sepharose pellets with antibody bound. The pellets were washed with TBS−Triton, resuspended in 40 μL of SDS sample buffer without BME, and boiled for 5 min, and 20 μL was loaded in duplicate on a 12% polyacrylamide gel for autoradiography or immunoblot analysis.

In the case of immunoblot analysis, the unreduced antibody, migrating at 160 kDa, was cut off of the blot to avoid cross-reaction with the secondary antibody. For autoradiography of labeled proteins, gels were impregnated with EN^{3}HANCE autoradiography enhancer (New England Nuclear) as recommended by the manufacturer and exposed to Kodak XAR-5 film at −80 °C for 1 month.

Diagonal SDS Gel Analysis. Prior to electrophoretic analysis, 5×10^5 HT1080.6TG cells were cross-linked with the thiol-cleavable homobifunctional cross-linking agent, dithiobis(succinimidyl propionate) (DSP, also known as Lomant's reagent, Pierce). DSP was added to a final concentration of 1 mM in 1 mL of 1% dimethyl sulfoxide in PBS, and the cells were incubated for 1 h at 0 °C. The reaction was terminated by the addition of 100 μL of 1 M Tris, pH 7.4. The cells were collected by centrifugation and lysed by boiling in SDS sample buffer lacking BME.

The sample was separated on a nonreducing 10% polyacrylamide gel. Following electrophoresis, the lane containing the cross-linked proteins was excised and placed into 20 mL of 125 mM Tris-HCl, pH 6.8, 0.1% SDS, and 100 mM BME and incubated for 20 min at 37 °C. The lane was then placed horizontally on a 12% polyacrylamide separating gel, electrophoresed, and subjected to immunoblot analysis. The protein diagonal was visible on the Coomassie-stained blot and was used to orient the spots visualized by immunoblot analysis.

Preparation of Ribosomal Proteins. Ribosomal proteins were prepared using a modification of the method of Madjar (1994). Briefly, 1×10^7 cells were washed in iced PBS, trypsinized, and collected by centrifugation at 500*g*. The cells were then lysed in 0.7% Nonidet P-40 in buffer A (0.25 M sucrose, 0.5 M KCl, 5 mM $MgCl_2$, 50 mM Tris-HCl, pH 7.4) and centrifuged at 750*g* and 12500*g* to pellet the nuclei and mitochondria, respectively. The resulting supernatant was centrifuged through a cushion of 1 M sucrose in buffer A for 2 h at 245000*g* in a TLA-100.3 ultracentrifuge rotor. For elution of ribosome-associated proteins, the prepared ribosomes were resuspended in buffer A containing 1 M NaCl and recentrifuged through a 1 M sucrose cushion in the same buffer.

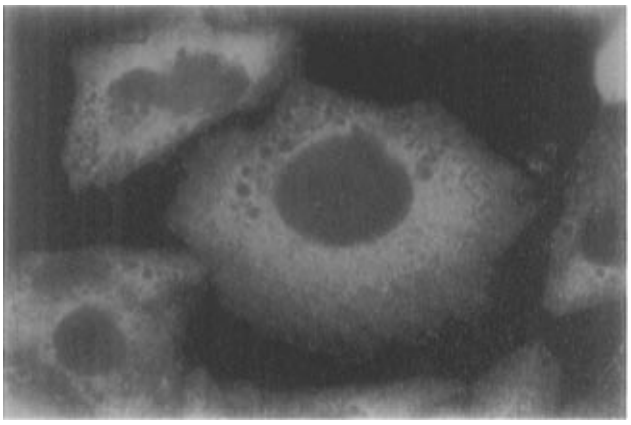

FIGURE 1: Indirect immunofluorescent cell staining of QM. G401.6TG.C6 cells grown on glass coverslips were stained with a 1:500 dilution of the C-17 QM antibody and a Texas Red conjugated anti-rabbit secondary antibody.

RESULTS

A primary goal in the physical characterization of QM was to determine its subcellular localization. This was initially performed using indirect immunofluorescent cell staining. To accomplish this, G401.6TG.C6 cells were grown on glass coverslips, fixed, and permeabilized. These were the cells from which the original QM cDNA was cloned (Dowdy *et al*., 1991). The cells were then stained with the C-17 QM antibody and an anti-rabbit Texas Red antibody conjugate. The stained cells were observed by fluorescence microscopy. A representative view is shown in Figure 1. It is obvious from the staining pattern that the protein is in the cytoplasm. Furthermore, by the pattern of staining it appears that the QM protein is associated with some cellular structure rather than being diffusely distributed throughout the cytoplasm. There is no apparent association with the plasma membrane. The perinuclear staining pattern suggests that the protein may be associated with the endoplasmic reticulum.

In order to determine the nature of the structures with which QM associates, the protein was analyzed by subcellular fractionation. To determine the rough cellular fraction with which QM associates, fibrosarcoma HT1080.6TG cell homogenates were fractionated by differential centrifugation. This relies on the differential sedimentation rates of the various organelles. Preliminary studies had indicated abundant QM protein in these cells. Western blot analysis of the differential centrifugation samples is shown in Figure 2A. The most obvious result is the total lack of QM signal in the soluble fraction. This supports the observation from the immunofluorescence studies that QM is not a diffuse soluble protein. By far, the strongest signal was observed in the P5 fraction. This is the crude microsomal fraction, containing vesicles of endoplasmic reticulum, Golgi, and plasma membrane. With the exception of endoplasmic reticulum, larger, partially intact forms of these structures

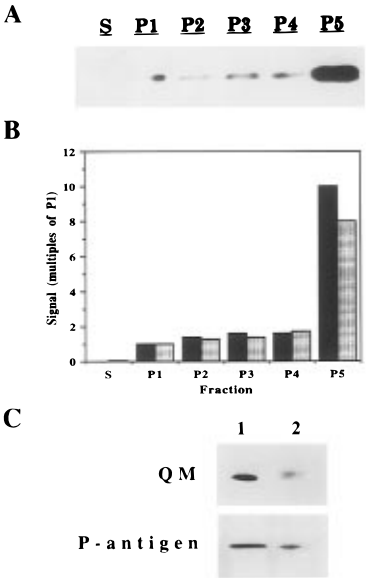

FIGURE 2: Subcellular fractionation of QM protein. (A) Differential centrifugation fractions were analyzed by western blot using the C-17 QM antibody: P1, 1000*g*; P2, 3000*g*; P3, 10000*g*; P4, 20000*g*; P5, 150000*g*; S, 150000*g* supernatant. (B) Densitometric analysis of western signal is compared with activity of NADPH-dependent cytochrome *c* reductase in the various fractions. The solid bars represent QM concentrations, and the shaded bars represent NADPH−cytochrome *c* reductase activity. (C) High-density (1) and low-density (2) ER microsomes were isolated by density gradient centrifugation and subjected to western blot analysis using antibodies to the QM protein or to ribosomal P-antigen.

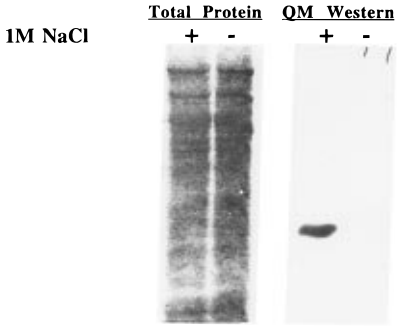

FIGURE 3: Displacement of QM from microsomal membranes by salt. Purified endoplasmic reticulum microsomes were subjected to two rounds of freeze thaw, followed by treatment with homogenization buffer or high salt buffer (homogenization buffer plus 1 M NaCl). The membrane fractions were then centrifuged for 30 min at 150000*g*. The supernatants were subjected to 12% polyacrylamide−SDS gel electrophoresis (left) and western blot analysis using the C-17 anti-QM antibody (right).

Table 1: Glucose-6-phosphatase Activity

incubation conditions	glucose-6-phosphatase activity (nmol mg^{-1} min^{-1})[a]	
	crude membranes	microsomes
buffer	156	920
buffer + trypsin	169	892
buffer + trypsin and Triton X-100	26	548

[a] Values represent the mean of three determinations performed as described in Experimental Procedures.

also sediment in the earlier fractions. There is some QM signal in the earlier fractions; however, only the P5 fraction shows enrichment for the signal. In these preparations, the microsomal fraction often contaminates the earlier fractions as vesicles are entrapped by the bulk of the sedimenting material. In Figure 2B, densitometric analysis of QM distribution is compared with the distribution of NADPH-dependent cytochrome *c* reductase, an enzyme associated with the endoplasmic reticulum. The QM signal directly correlates with the activity of the marker enzyme, suggesting that QM resides within the microsomal fraction.

To further separate the microsomal fraction, a mouse liver homogenate was subjected to differential centrifugation to generate a crude microsomal preparation. These microsomal membranes were then applied to sucrose/CsCl density gradients. The rough endoplasmic reticulum, being more dense due to its associated ribosomes, sediments ahead of the remaining vesicles. The band containing these remaining membranes could then be separated on a lower density gradient to yield a purified low-density microsome fraction. Western blot analysis of these fractions for QM and ribosomal P-antigen is shown in Figure 2C. As expected, the ribosomal protein is enriched in the dense microsomes. The QM signal shows a similar distribution, consistent with a localization in the rough endoplasmic reticulum. Preparations of Golgi and plasma membrane vesicles showed no QM signal (data not shown).

Having established that QM associated with a membranous structure, it became important to determine the nature of that association. QM could be a transmembrane protein; however, this seems unlikely as the protein has no structural elements which would serve as a transmembrane domain. Alternatively, QM may associate peripherally with the membrane, on either the cytoplasmic or luminal face. To distinguish between these possiblities, an ER membrane preparation was subjected to salt treatment. If QM is an integral membrane protein, it should require detergent solubilization to remove it from the membrane. If, however, it is peripherally associated with the membrane, it might be removed by disrupting electrostatic interactions with salt. The ability of 1 M NaCl treatment to dissociate QM from the ER vesicles was defined as a shift from the pellet to the supernatant of a 150000*g* centrifugation. Western blot analysis of this separation is shown in Figure 3. The QM protein was clearly displaced by the salt treatment. This indicates that QM is a peripheral membrane protein.

As a peripheral membrane protein, QM could be associating with either the cytoplasmic or the luminal face of the membrane. Proteolytic latency studies were performed to determine which was the case. Proteins which are exposed on the cytoplasmic face of the membrane are accessible to proteases while those on the luminal faces are protected by the membrane of the vesicle. These protected proteins are said to be latent to proteolytic digestion and are degraded only after disruption of the membrane by detergents. The latency of QM to trypsin digestion was determined with a crude liver homogenate and with a liver microsomal fraction.

As an indication of the integrity of the membrane preparations, the various digestion reactions were assayed for glucose-6-phosphatase activity. This is a gluconeogenic enzyme, the active site of which is found within the lumen of the endoplasmic reticulum. As a result, it is latent to protease digestion in intact membrane preparations. The results of the glucose-6-phosphatase assays are shown in Table 1. In both liver membrane preparations the activity is degraded in the presence of detergent but not in its absence, indicating that the preparations are intact. Western blot

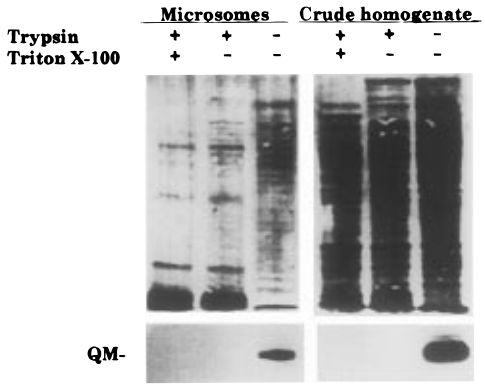

FIGURE 4: Proteolytic latency of QM protein. Crude liver homogenate or the microsomal fraction was treated with or without the protease trypsin (1 mg/mL) in the presence or absence of Triton X-100 (0.1%). Samples were boiled in SDS sample buffer and subjected to western blot analysis using the C-17 anti-QM antibody.

analysis of the liver membrane preparation is shown in Figure 4. In both cases, the QM protein is degraded in both the presence and absence of detergent, indicating a cytoplasmic localization.

Thus, QM had been found to be a peripheral membrane protein; yet it remained to be seen how this association was mediated. Myristylation occurs commonly among proteins peripherally associated with the cytoplasmic face of membranes, and several ER-associated proteins are myristylated. The fatty acylation occurs via a stable amide linkage to a glycine residue found at the penultimate position in the nascent polypeptide, and QM has just such a glycine residue. Furthermore, these proteins are often held onto the membranes by electrostatic interactions, as the lipid alone is insufficient to effect the interaction, and can be displaced by disruption of these interactions.

To determine whether QM was myristylated, PC-12 cells were labeled with [^{3}H]myristic acid. QM was then isolated from labeled protein extracts by immunoprecipitation and analyzed for incorporation of label by fluorography. A second immunoprecipitation was performed and visualized by western blot analysis to ensure that the protein had been successfully precipitated. The results of this are shown in Figure 5A. As a control for the success of the labeling, a known myristylated protein, c-Src, was immunoprecipitated from the same extracts and analyzed in parallel. Western blot analysis using the anti-Src antibody detected two specific bands of approximately 60 kDa in the PC-12 cell line. The fluorography is shown in Figure 5B. While the c-Src bands are clearly evident, the QM does not appear to have become labeled, indicating that QM is not myristylated.

An alternate possibility is that QM associates with the membranes as part of a protein complex. To investigate this possibility, the QM protein was examined by diagonal SDS gel analysis. This technique uses cleavable cross-linking agents to resolve proteins as covalently cross-linked complexes in the first dimension and as their monomeric constituents in the second. The proteins which are not cross-linked have the same migration in both the first and second dimensions, and together these form a diagonal band across the gel. The proteins which are cross-linked into a larger complex are displaced from the diagonal. The extent of this displacement along the first dimension is indicative of the size of the cross-linked complex.

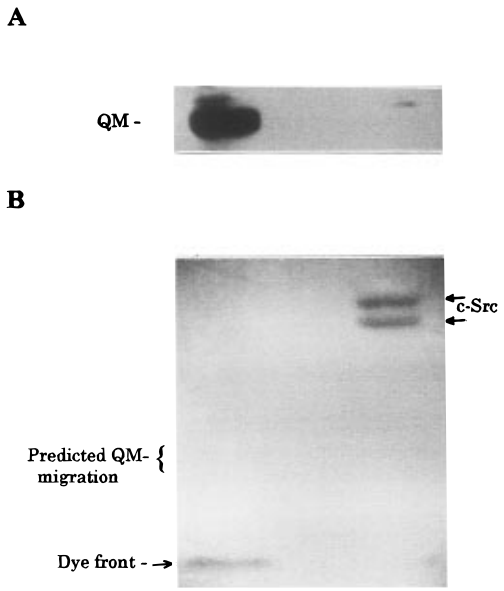

FIGURE 5: Evaluation of QM for myristylation. PC-12 cells were labeled by incubation with [^{3}H]myristate for 6 h. The cells were then lysed with detergent, and the precleared lysates were immunoprecipitated with the C-17 anti-QM antibody or the N-16 anti-c-Src antibody. Immunoprecipitates were analyzed in parallel by western blot analysis using the C-17 anti-QM antibody (A) and by 12% polyacrylamide−SDS gel electrophoresis and fluorography (B).

The cross-linking of the QM protein was achieved using the homobifunctional cross-linking agent dithiobis(succinimidyl propionate) (DSP). This is an NHS imido ester that is reactive with primary amines. It also contains a disulfide linkage which renders it sensitive to cleavage by reducing agents. In addition, the hydrophobic nature of this agent allows it to cross cell membranes. This agent was used to cross-link whole HT1080.6TG cells. As a result, it would be expected to penetrate the cell and link protein complexes in their natural setting.

Following cross-linking, the cells were lysed in a nonreducing buffer and electrophoresed on a standard Laemmli SDS gel under nonreducing conditions. This lane was then excised, reduced with BME, and electrophoresed. The resulting two-dimensional gel was analyzed by western blot. The results of this analysis are shown in Figure 6.

The largest source of QM signal is found on the diagonal. This represents QM monomers that were not involved in any intermolecular linkages. The large amount of un-cross-linked QM may be the result of the whole cell cross-linking. Under these conditions, the reagent must penetrate a considerable distance into the cell, past many other potentially reacting molecules, and this may result in incomplete cross-linking. Alternatively, the un-cross-linked QM may represent monomers which were not involved in a complex at the time of the cross-linking.

The other notable QM signal is displaced considerably from the diagonal. The migration of the displaced QM signal, relative to molecular weight standards for the first dimension, indicates a molecular mass of greater than 250 kDa for the cross-linked complex. This suggests that QM is part of a large protein complex.

One large protein complex within the rough endoplasmic reticulum is the ribosome. To evaluate the association of QM with ribosomes, ribosomes and associated proteins were purified by differential centrifugation followed by sedimenta-

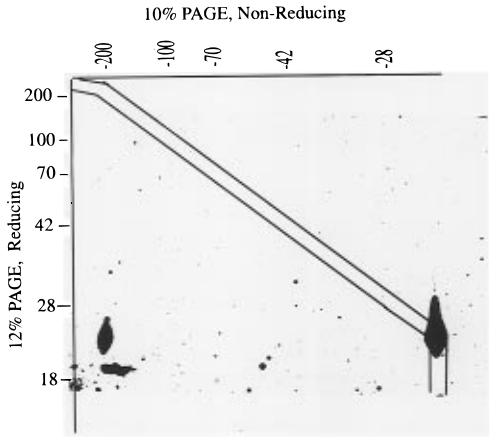

FIGURE 6: Diagonal SDS gel analysis of the QM protein. HT1080.6TG cells were cross-linked *in situ* with 1 mM DSP. Lysates from cross-linked cells were separated by SDS−polyacrylamide gel electrophoresis, under nonreducing conditions in the first dimension and under reducing conditions (BME) in the second dimension. The gel was then subjected to western blot analysis using the C-17 anti-QM antibody. The diagonal representing proteins that ran as monomers under both reducing and nonreducing conditions is indicated by the double line.

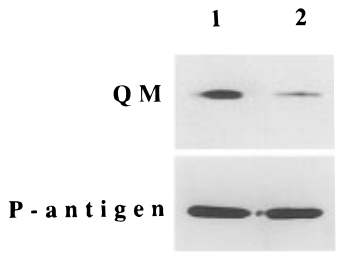

FIGURE 7: Western blot analysis of isolated ribosomes. Partially purified ribosomes (1) or ribosomes treated with 1 M NaCl (2) were subjected to western blot analysis using antibody to QM and to ribosomal P-antigen.

tion through a sucrose cushion. Western blot analysis of the prepared ribosomal fraction is shown in Figure 7. QM protein copurifies with the ribosomal P-antigen, suggesting that QM is a ribosome-associated protein. However, incubation of the ribosome preparation with 1 M NaCl, followed by a second sedimentation, displaces the majority of the QM from the ribosome fraction. Under these conditions, the core ribosomal P-antigen protein is unaffected. This suggests that QM is peripherally associated with the ribosome, rather than being a component of the ribosomal core.

DISCUSSION

QM is a highly conserved protein (Farmer *et al.*, 1994) which has been shown to be essential in yeast (Tron *et al.*, 1995). In spite of its apparent importance, there has been little evidence to indicate the function of the QM protein. In this report, we have analyzed the subcellular localization, membrane topology, and membrane association of the QM protein in order to better understand its situation within the cellular milieu.

Immunofluorescent cell staining and subcellular fractionation results are both consistent with an association between QM and the rough endoplasmic reticulum. However, this association is readily disrupted by 1 M NaCl. This indicates that QM is peripherally associated with the membrane rather than being an integral membrane protein. The sensitivity of the QM protein to protease digestion further suggests that QM is on the cytoplasmic face of the membrane.

Analysis of the nature of the interaction with the membrane has shown that while QM is not myristylated, it does exist as a member of a large protein complex. This is consistent with the observation that QM copurifies with ribosomes, a notable large protein complex associated with the rough endoplasmic reticulum.

Although the QM protein remains consistently associated with the membrane under the fractionation conditions employed, it remains unclear whether QM has a direct association with the membrane or an indirect association mediated by the ribosomal complex. It is interesting to note that QM was never observed in the soluble fraction as would be expected if the protein was interacting with free ribosomes. The association between QM and the ribosome complex is disrupted by salt concentrations which have no effect on core ribosomal proteins. Taken together, these data suggest that QM is not a member of the basic translational machinery but may be involved with membrane-associated translational processes such as cotranslational processing and membrane translocation. The association of QM with the endoplasmic reticulum is probably due to the preponderance of ribosomes found there. It is not known whether QM associates with other organelles under conditions in which ribosomes associate with them. It will be important to elucidate the mechanisms regulating the association between QM and ribosomes and the role this association may play in translation.

The *S. cerevisiae* homolog of QM, *QSR1*, was identified as a point mutation which gave rise to a synthetic lethal phenotype in combination with a disruption of subunit 6 of the mitochondrial cytochrome bc_1 complex (*QCR6*). The functional relationship between this nuclear-encoded mitochondrial protein and a cytoplasmic ribosome-associated protein, such as QM, is unclear. One possibility is a role for QM in the cotranslational import of *QCR6*. Ribosomes have been reported to associate with the outer mitochondrial membrane (Ades & Butow, 1980). Additionally, Cascarano *et al.* (1995) have reported association between ribosomes and the rough endoplasmic reticulum in hepatocytes. Recent studies on the QSR1 protein have also been indicative of ribosomal association (B. Trumpower, personal communication).

The only putative function that has been suggested for QM is an inhibition of c-Jun-activated transcription. While Monteclaro and Vogt (1993) demonstrated an *in vitro* interaction between the chicken homolog of QM, JIF1, and c-Jun, results in our laboratory have shown that QM has no effect on c-Jun-activated transcription *in vivo* (Loftus and Stanbridge, manuscript in preparation). The studies presented here also argue against a role for QM as a direct regulator of nuclear transcription but instead suggest a role in the process of translation. QM expression has been shown to correlate positively with proliferation with very high expression in growing cells. This is inconsistent with an inhibitor of c-Jun, which would be predicted to inhibit proliferation (Angel & Karin, 1991), but fits well with the expression of proteins known to be involved in translation (Woolford, 1991).

Evidence from *S. cerevisiae* suggests that the absence of QM-like activity is incompatible with cell growth. This is consistent with evidence from mouse embryonic stem cells

(A. Mills, personal communication). Furthermore, the introduction of additional copies of QM under the control of highly active promoters fails to yield increased QM expression in cultured mammalian cells. Taken together, these results suggest that future molecular analysis of QM function may require the use of an organism more amenable to genetic manipulation than mammalian cells.

QM was originally identified as a candidate tumor suppressor gene (Dowdy *et al*., 1991). The true role of QM in the regulation of tumorigenicity is unclear; however, if QM is a tumor suppressor, this suggests that components of the translation machinery may play a role in the suppression of the tumorigenic phenotype.

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