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FAST TRACK

Assembly of the QM Protein Onto the 60S Ribosomal Subunit Occurs in the Cytoplasm

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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. The QM gene encodes a 24 kDa basic protein that peripherally associates with the ribosomes. Recently, the gene for this protein has also been shown in *Saccharomyces cerevisiae* to encode an essential 60S ribosomal subunit protein that is required for the joining of the 40S and 60S subunits. Since the association of QM with ribosomes can be disrupted with 1M NaCl, which has no effect on the association of core ribosomal proteins, indirect immunofluorescent cell staining was performed to colocalize the QM protein with the human large P-antigen, a core ribosomal protein of the 60S subunit, and to determine whether the assembly of the QM protein onto the 60S ribosomal subunit occurs in the nucleolus or in the cytoplasm. Our results reveal that QM co-localizes with the large P-antigen only to the cytoplasm where the rough endoplasmic reticulum is found and not to the nucleolus where ribosome assembly occurs. This finding suggests that the QM protein is most likely involved in a late step of the 60S subunit assembly and is added to the 60S ribosomal subunit in the cytoplasm and not in the nucleolus. J. Cell. Biochem. 68:281–285, 1998.

Key words: QM; large P-antigen; 60S ribosomal subunit; colocalization

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 QM cDNA encodes a 24 kDa protein that is very rich in charged and basic amino acids, most notably arginine and lysine (predicted pI of 10.5). Highly conserved homologs of QM have been isolated from more than 10 different species from the plant, animal, and fungal kingdom. The degree of conservation shared between diverse species, such as human and *Saccharomyces cerevisiae*, is almost 70% [Farmer et al., 1994]. In spite of its ubiquitous nature and high degree of conservation, the function of the QM gene product, until just recently, remained elusive. An early study by Monteclaro and Vogt [1993] reported that the chicken homolog of QM (Jif-1) was able to bind to c-Jun in vitro and repress c-Jun's ability to transactivate a promoter containing the AP-1 binding sequence. However, recent studies have indicated that this is not a relevant function in vivo and probably represents an experimental artifact [Loftus and Stanbridge, manuscript in preparation]. The yeast homolog of QM (QSR1/GRC5) has been reported by two groups. Tron et al. [1995] identified this 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 a defect in protein synthesis, growth arrest, abnormalities of the actin cytoskeleton, and accumulation of cell wall material at the septum. Recently, a series of studies have suggested a role for QM in protein synthesis. Loftus et al. [1997] demonstrated that the QM protein peripherally associates with ribosomes in the rough endoplasmic reticulum. Amino acid sequence of the rat ribosomal protein L10 is almost identical to that of the QM protein [Chan et al., 1997]. Furthermore, it has been shown in yeast that Qsr1p specifically associates with

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the 60S ribosomal subunit [Dick et al., 1997] and is required for joining of the 40S and 60S subunits [Eisinger et al., 1997]. The mammalian QM protein and the yeast homolog Qsr1p associate with the 60S ribosomal subunit only peripherally, as this association can be disrupted with 1M NaCl and 0.5 M KCl (-Mg^{2+}), respectively, while core ribosomal proteins are unaffected by this treatment [Loftus et al., 1997; Dick et al., 1997]. Ribosome assembly occurs in the nucleolus [Kruiswijk et al., 1978; Melese and Xue, 1995; Warner, 1990] and only four ribosomal proteins have been shown to be exchangeable in the cytoplasm after assembly [Zinker and Warner, 1976]. Whether the QM protein is added to the 60S subunit in the nucleolus or the cytoplasm is not known, but the N-terminus of Qsr1p has been suggested to have a putative nuclear localization signal [Eisinger et al., 1997]. In this paper, we report the use of indirect immunofluorescent cell staining to clearly colocalize the QM protein with the human large P-antigen (a core ribosomal protein of the 60S subunit) in mammalian cultured cells and to assess whether the assembly of the QM protein onto the 60S ribosomal subunit occurs in the nucleolus or in the cytoplasm.

MATERIALS AND METHODS

Indirect Immunofluorescent Cell Staining and Microscopy

Prior to the immunofluorescent staining, fibrosarcoma HT1080.6TG cells were cultured in growth medium (Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum) and maintained in a water-jacketed incubator at 37°C and 5% CO_2 for two days on acid-washed glass coverslips to allow for firm attachment of cells. Following this incubation, the HT1080.6TG cells were washed twice in PBS and fixed by a 15-min immersion in 2% paraformaldehyde, pH 7.4, prepared freshly in PBS. The fixed cells were then rinsed with PBS five times and permeabilized by a 3-min incubation in 0.1% Triton X-100/PBS at room temperature. The permeabilized cells were washed extensively again in PBS. For the intracellular localization of the QM protein or the ribosomal large P-antigen, the cells were incubated with either the rabbit C-17 α-QM primary antibody [Santa Cruz, 4 mg/ml in 1 mg/ml bovine serum albumin (BSA) in PBS] or with the human α-ribosomal large P-antigen primary antibody [ImmunoVision, 1:200 dilution in 1 mg/ml BSA in PBS] for 45 min at room temperature in a humid chamber. Cells were then washed four times in PBS, once in water, and incubated for 45 min in a dark humid chamber with either sheep α-rabbit FITC antibody conjugate (Sigma, St. Louis, MO; 1:160 dilution) or goat α-human TRITC antibody conjugate (Sigma, 1:8 dilution) respectively. For the double-labeling experiment used to visualize potential colocalization of the QM protein and the ribosomal large P-antigen, the staining was performed and completed for one protein prior to initiating the staining on the second. The coverslips were given final washes four times in PBS, once in water, mounted in antifade, and viewed using confocal microscopy.

RESULTS

Subcellular Localization of the QM Protein

Confocal fluorescence microscopy of fibrosarcoma HT1080.6TG cells, cultured on glass cover slips, fixed, permeabilized, and stained with the rabbit C-17 α-QM antibody and a sheep α-rabbit FITC conjugated secondary antibody, demonstrates that the QM protein is present in the cytoplasm, as indicated by the green fluorescence stain of FITC observed in Figure 1A. Also apparent from this figure is the complete absence of the QM protein from the nucleus. The staining of QM is not diffusely distributed throughout the cytoplasm but is seen in a punctate perinuclear staining pattern which suggests that the QM protein may be associated with the endoplasmic reticulum. This punctate staining pattern of QM observed in HT1080.6TG, like the one seen in the Wilms' tumor cell line G401.6TG.C6 [Loftus et al., 1997], is consistent with the localization pattern reported for the yeast homolog Qsr1p in *Saccharomyces cerevisiae* [Tron et al., 1995]. Recently, the QM protein was found to be peripherally associated with the ribosomes in the rough endoplasmic reticulum [Loftus et al., 1997; Dick et al., 1997] and is required for the joining of the 40S and 60S ribosomal subunits [Eisinger et al., 1997] in yeast. Core ribosomal assembly is known to occur in the nucleolus [Kruiswijk et al., 1978; Melese and Xue, 1995; Warner, 1990]. So as a peripheral ribosomal protein, is QM added onto the 60S ribosomal subunit in the cytoplasm or in the nucleolus? Despite the claim by Eisinger et al. [1997] that the N-terminal of Qsr1p has a putative nuclear localization signal, it is unlikely that the QM protein is assembled onto the 60S subunit in the nucleolus, as our immunohistochemical

study reveals the presence of the QM protein only in the cytoplasm and not in the nucleolus or the nucleus.

Subcellular Localization of the Human Ribosomal Large P-Antigen

The human ribosomal large P-antigen is a core ribosomal protein of the 60S large ribosomal subunit with which QM recently has been found to copurify [Loftus et al., 1997]. To assess the subcellular localization of the human ribosomal large P-antigen, cultured fibrosarcoma HT1080.6TG cells grown on glass cover slips were fixed, permeabilized, and stained with the human α-ribosomal large P-antigen and a goat α-human TRITC conjugated secondary antibody. Figure 1B clearly displays the Texas Red staining pattern of the human large P-antigen in the HT1080.6TG fibrosarcoma cell line. What is evident from this figure is the clear presence of the large P-antigen in the cytoplasm, as indicated by the bright red color. The cytosolic localization of the P-antigen in a punctate perinuclear staining pattern is consistent with known literature about the presence of ribosomes on the rough endoplasmic reticulum in the cytoplasm. Interestingly, the Texas Red signal representing the presence of the large P-antigen is seen also in the nucleolus (Fig. 1B), indicating that the large P-antigen is found not only in the cytoplasm where the rough endoplasmic reticulum resides but also in the nucleolus where core ribosomal assembly occurs. The staining pattern for the core ribosomal large P-antigen observed here is not surprising but rather predicted, as the nucleolus serves as the site for ribosome assembly. Nonetheless, it undoubtedly marks the similarity as well as the difference between the subcellular localization of the P-antigen versus that of the QM protein. If QM is found as a stoichiometric component of the 60S ribosomal subunit and is required for the joining of the 40S and the 60S subunits, where in the cell and at what stage of ribosome assembly is the QM protein added onto the 60S ribosomal subunit?

Colocalization of the QM Protein and the Human Ribosomal Large P-Antigen

In order to address the question of where in the cell and at what stage of ribosome assembly is the QM protein added onto the 60S ribosomal subunit, indirect immunofluorescent cell staining was again performed to colocalize the QM protein and the human large ribosomal P-antigen. As in the previous experiments, the green FITC staining represents the presence of the QM protein and the red TRITC staining indicates the presence of the large P-antigen in the cells. However, in this double-labeling experiment, both the QM protein and the ribosomal large P-antigen are simultaneously stained using their respective primary and conjugated secondary antibodies. Where these two proteins colocalize in the cell can be visualized by an orange-yellow color given off by the overlapping green and red fluorescent stains. As can be seen in Figure 1C, the QM protein colocalizes with the P-antigen only to the cytoplasm, as represented by the orange-yellow punctated perinuclear staining. However, the QM protein does not colocalize with the ribosomal large P-antigen to the nucleolus, as only the red stain representing the presence of the P-antigen and not the orange-yellow stain indicating the presence of both QM and the P-antigen is observed in the nucleolus of these cells. This finding is consistent with the QM protein being found only in the cytoplasm and not in the nucleolus (Fig. 1A). The colocalization of the QM protein and the large P-antigen to only the cytoplasm suggests that QM, a peripheral 60S ribosomal subunit protein, unlike the core 60S ribosomal large P-antigen, is not assembled onto the 60S large ribosomal subunit in the nucleolus. The data futher imply that QM is added onto the 60S ribosomal subunit only after the core ribosomal proteins have been assembled in the nucleolus and transported out into the cytoplasm.

DISCUSSION

Recently, the QM protein was found to be peripherally associated with ribosomes in the rough endoplasmic reticulum [Loftus et al., 1997]. Consistent with this finding is the report that Qsr1p, the yeast homolog of QM, specifically associates with the large ribosomal subunit as a peripheral protein and is required for joining of the 40S and 60S subunits [Dick et al., 1997; Eisinger et al., 1997]. This association of both the mammalian QM protein and the yeast Qsr1p to ribosomes can be disrupted with 1M NaCl and 0.5M KCl (-Mg^{2+}), respectively, while core ribosomal proteins are unaffected by this treatment [Loftus et al., 1997; Dick et al., 1997]. It has been known for quite some time that ribosome assembly occurs in the nucleolus

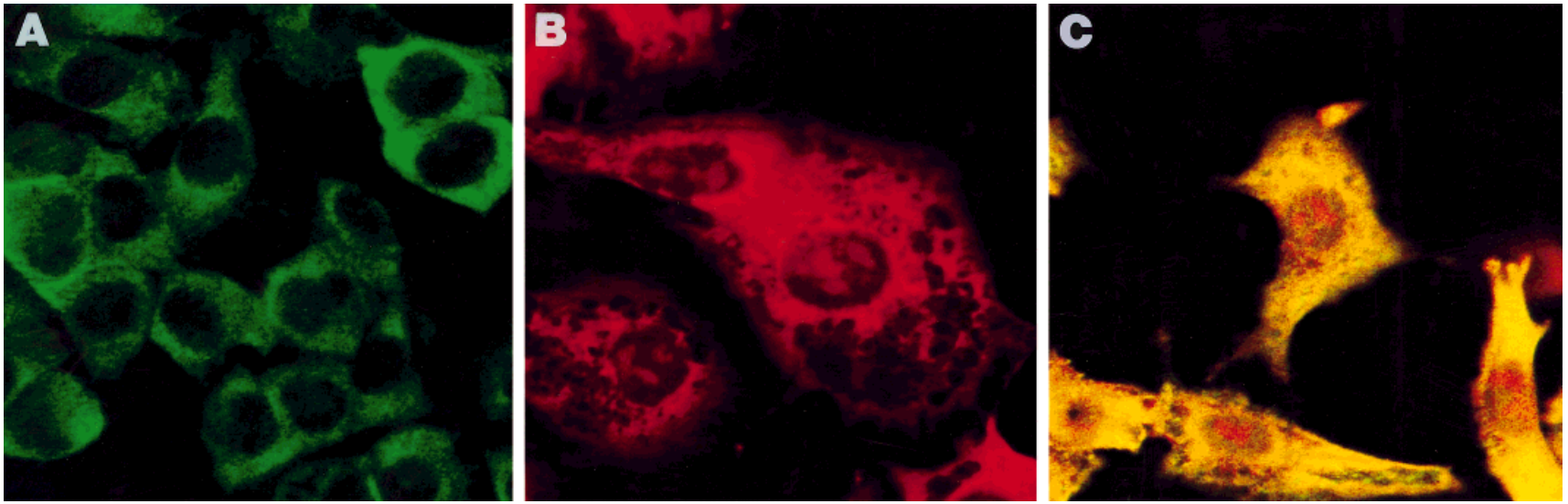

Fig. 1. Localization of the QM protein and the human ribosomal large P-antigen by indirect immunofluorescent cell staining. **A:** Cytoplasmic localization of the QM protein. Cultured fibrosarcoma HT1080.6TG cells were grown on glass cover slips, fixed in 2% paraformaldehyde, permeabilized with 0.1% Triton X-100/PBS, and stained with a rabbit C-17 α-QM primary antibody and a sheep α-rabbit FITC conjugated secondary antibody. The green fluorescence detects the presence of the QM protein in the cytoplasm. **B:** Cytoplasmic and nucleolar localization of the human ribosomal large P-antigen. Cultured fibrosarcoma HT1080.6TG cells were grown on glass cover slips, fixed in 2% paraformaldehyde, permeabilized with 0.1% Triton X-100/PBS, and stained with a human α-ribosomal large P-antigen primary antibody (1:200 dilution) and a goat α-human TRITC conjugated secondary antibody. The red staining clearly localizes the ribosomal large P-antigen to the cytoplasm, where the rough endoplasmic reticulum is found, as well as to the nucleolus where core ribosomal assembly occurs. **C:** Colocalization of the QM protein and the human ribosomal large P-antigen. Cultured fibrosarcoma HT1080.6TG cells were grown on glass cover slips, fixed in 2% paraformaldehyde, permeabilized with 0.1% Triton X-100/PBS, and stained with the rabbit C-17 α-QM and the human α-ribosomal large P-antigen primary antibodies. These cells were subsequently double-stained with sheep α-rabbit FITC and goat α-human TRITC conjugated secondary antibodies, respectively, to visualize the colocalization of the QM protein and the P-antigen. The red staining (TRITC) represents the presence of only the ribosomal large P-antigen where as the green staining (FITC) represents the presence of only the QM protein. Areas in which the green stain overlaps with the red stain to give an orange-yellow color indicate the subcellular region(s) where the two proteins are colocalized. The colocalization of the QM protein and the large P-antigen is only observed in the cytoplasm where the rough endoplasmic reticulum resides.

[Kruiswijk et al., 1978; Melese and Xue, 1995; Warner, 1990] and only four ribosomal proteins have been shown to cycle on and off the ribosome in the cytoplasm [Zinker and Warner, 1976]. Whether the QM protein is added to the 60S subunit in the nucleolus or the cytoplasm was not known. However, in this present study, using indirect immunofluorescent cell staining, we have demonstrated that QM, a peripheral 60S ribosomal protein, unlike the core 60S human ribosomal large P-antigen, is not assembled onto the large ribsomal subunit in the nucleolus. Instead, our data strongly suggest that QM is added onto the 60S ribosomal subunit only after the core ribosomal proteins have been assembled in the nucleolus and transported out into the cytoplasm (Fig. 1A–C). It seems logical and energy-efficient for the cells to keep QM in the cytoplasm and not transporting it into the nucleolus for assembly onto the 60S subunit, since QM is only a peripherally associated ribosomal protein that is required only in the cytoplasm for the ribosomal subunit joining. The prospect that QM's sole function is the joining of the small and large ribosomal subunits in eukaryotes is conceivable but unlikely. For example, the functional relationship between the nuclear-encoded inner mitochondrial membrane protein (Qcr6p) and the cytoplasmic ribosome-associated protein (Qsr1p) remains unclear. It is not known how a single chromosomal copy of QCR6 can rescue an otherwise lethal missense mutation in the cytoplasmic ribosomal protein, Qsr1p [Tron et al., 1995; Eisinger et al., 1997]. Furthermore, in the present study, not all of the QM proteins in the cytoplasm are found associated with ribosomes, as specks of the green FITC staining are still noticeably apparent in certain areas of the cytoplasm (Fig. 1C). It is conceivable that these fluorescent green specks may represent newly synthesized QM proteins that have not yet associated with ribosomes. However, we cannot completely rule out the possibility that ribosomally free QM protein may have other, as yet unknown, cellular function(s). Recently, it has been reported that the QM protein is a novel zinc-binding protein and can be phosphorylated by protein kinase C in vitro [Inada et al., 1997]. Determining whether protein kinase C does in fact phosphorylate QM in vivo may aid in the elucidation of the mechanism by which the QM protein is functionally regulated.

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REFERENCES

Chan Y-L, Diaz J-J, Denoroy L, Madjar J-J, Wool IG (1996): The primary structure of rat ribosomal protein L10: Relationship to a Jun-binding protein and to a putative Wilms' tumor suppressor. Biochem Biophy Res Commun 225:952–956.

Dick FA, Karamanou S, Trumpower BL (1997): QSR1, an essential yeast gene with a genetic relationship to a subunit of the mitochondrial cytochrome bc1 complex, codes for a 60s ribosomal subunit protein. J Biol Chem 272:13372–13379.

Dowdy SF, Lai KM, Weissman BE, Matsui Y, Hogan BL, Stanbridge EJ (1991): The isolation and characterization of a novel cDNA demonstrating an altered mRNA level in nontumorigenic Wilms' microcell hybrid cell. Nucleic Acids Res 19:5763–5769.

Eisinger DP, Dick FA, Trumpower BL (1997): Qsr1p, a 60S ribosomal subunit protein, is required for joining of 40S and 60S subunit. Mol Cell Biol 17:5136–5145.

Farmer AA, Loftus TM, Mills AA, Sato KY, Neill JD, Tron T, Yang M, Trumpower, BL, Stanbridge EJ (1994): Extreme evolutionary conservation of QM, a novel c-Jun associated transcription factor. Hum Mol Genet 3:723–728.

Inada H, Mukai J, Matsushima S, Tanaka T (1997): QM is a novel zinc-binding transcription regulatory protein: Its binding to c-Jun is regulated by zinc ions and phosphorylation by protein kinase C. Biochem Biophys Res Commun 230:331–334.

Koller HT, Klade T, Ellinger A, Breitenbach M (1996): The yeast growth control gene GRC5 is highly homologous to the mammalian putative tumor suppressor gene QM. Yeast 12:53–65.

Kruiswijk T, Planta RJ, Krop JM (1978): The course of assembly of ribosomal subunits in yeast. Biochim Biophys Acta 517:378–389.

Loftus TM, Nguyen YH, Stanbridge EJ (1997): The QM protein associates with ribosomes in the rought endoplasmic reticulum. Biochemistry 36:8224–8230.

Melese T, Xue Z (1995): The nucleolus: An organelle formed by the act of building a ribosome. Curr Opin Cell Biol 7:319–324.

Monteclaro FS, Vogt PK (1993): A Jun-binding protein related to a putative tumor suppressor. Proc Natl Acad Sci U S A 90:6726–6730.

Tron T, Yang M, Dick FA, Schmitt ME, Trumpower BL (1995): QSR1, an essential yeast gene with a genetic relationship to a subunit of the mitochondrial cytochrome bc1 complex, is homologous to a gene implicated in eukaryotic cell differentiation. J Biol Chem 270:1–10.

Warner JR (1990): The nucleolus and ribosome formation. Curr Opin Cell Biol 2:521–527.

Zinker S, Warner JR (1976): The ribosomal proteins of *Saccharomyces cerevisiae*. J Biol Chem 251:1799–1807.