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Characterization of the Sea Urchin Major Vault Protein: A Possible Role in Nucleocytoplasmic Transport

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Vaults are large ribonucleoprotein particles that have been identified in a wide range of eukaryotic organisms. Although present in thousands of copies per cell, their function remains unknown. In this report, we identify the major vault protein in sea urchins as a 107-kDa polypeptide that copurifies with microtubules and ribosomes. Although initially identified in microtubule preparations, the sea urchin major vault protein is not predominantly microtubule-associated *in vivo*. Rather, the sea urchin major vault protein is present throughout the cytoplasm in eggs and embryos and in the nucleus in adult somatic cells. Within the nucleus, the sea urchin major vault protein is concentrated in the region of the nucleolus and to punctate regions of the nuclear envelope. In addition, the vault protein localizes to short linear strings juxtaposed to the exterior of the nucleus and extending outward into the cytoplasm. Based on their copurification and intracellular distribution, vaults may be involved in the nucleocytoplasmic transport of ribosomes and/or mRNA.

INTRODUCTION

A vault is a large ribonucleoprotein (RNP) particle nearly three times the size of a ribosome and 10-fold larger than the signal recognition particle (SRP) (Kedersha and Rome, 1986; Rome *et al.*, 1991). On sucrose density gradients, vaults sediment as 150S particles and have an average mass of 13 MDa (Kedersha *et al.*, 1991). Vaults are present in thousands of copies per cell and are largely composed of an approximately 100-kDa polypeptide and a small RNA of 141 nucleotides in mammals (Kedersha and Rome, 1986; Kickhoefer *et al.*, 1993). Negative staining for electron microscopy reveals a complex morphology consisting of multiple arches reminiscent of cathedral vaults (Kedersha and Rome, 1986). The morphology and protein composition of vaults is highly conserved from slime molds to humans, indicating that their function must be essential to eukaryotes (reviewed by Rome *et al.*, 1991; Kickhoefer *et al.*, 1996; Izquierdo *et al.*, 1996b).

The function of vaults is not known. Subcellular fractionation (Kedersha and Rome, 1986; Kedersha *et al.*, 1990) and immunocytochemistry (Kedersha and Rome, 1990; Chugani *et al.*, 1993) indicate that vault RNPs are located predominantly in the cytoplasm. In rat fibroblasts, there are thousands of punctate, vault-specific loci, some of which are coincident with, and clustered at, the distal ends of actin stress fibers (Kedersha and Rome, 1990). Vault immunoreactivity appears to be excluded from the nucleus (Kedersha and Rome, 1990). However, immunogold staining for electron microscopy indicates that there is a small amount of vault reactivity at the nuclear periphery with a small number of gold clusters associated with the nuclear pores (Chugani *et al.*, 1993). The structural similarity between the octagonally symmetric vault particle (Kedersha *et al.*, 1991) and the central plug of the nuclear pore complex (NPC) (Unwin and Milligan, 1982; Akey, 1990) prompted their suggestion that the vault particle might be the central plug or nuclear pore complex transporter (Chugani *et al.*, 1993). In addition, vaults have been implicated in general cellular homeostasis (Vasu and Rome, 1995), cell motility (Kedersha *et al.*, 1990), and cytoplasmic-mediated RNA events (Kedersha and Rome, 1990).

Recently, vaults were identified in cholinergic nerve terminals in the electric organ of the electric ray, *Torpedo marmorata*. (Herrmann *et al.*, 1996). A 100-kDa polypeptide copurified with cholinergic synaptic vesicles and sequence analysis identified it as the major vault protein. Sedimentation profiles indicated the 100-kDa polypeptide was in a particle distinct from synaptic vesicles. Enrichment at the nerve terminals suggests the vault particles move to the

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presynaptic terminal via axonal transport. The role of vaults in these cells is still to be established.

An intriguing hypothesis for vault function is that they may mediate multidrug resistance in P-glycoprotein-negative tumor cells (Scheffer *et al.*, 1995). Selected multidrug-resistant cancer cells overexpress a 110-kDa polypeptide, originally named the lung-resistance-related protein (LRP) (Scheper *et al.*, 1993; Izquierdo *et al.*, 1996a). LRP overexpression has a high predictive value for a poor response to chemotherapy in acute myeloid leukemia and advanced ovarian carcinoma. A full-length cDNA for LRP was recently sequenced and identified as the human major vault protein (Scheffer *et al.*, 1995). The deduced amino acid sequence shows 87.7% identity with the rat vault sequence (Kickhoefer and Rome, 1994). Although the function of vaults is uncertain, these new observations suggest that one function of vaults may be to assist in the transport or sequestration of cytotoxic drugs.

While identifying components involved in the association of ribosomes and mRNA with sea urchin microtubules (Suprenant *et al.*, 1989; Suprenant, 1993; Hamill *et al.*, 1994), we discovered that sea urchin vaults copurified with both microtubules and ribosomes. In this study we report the identification and characterization of the sea urchin major vault protein (SU-MVP). A strength of this system is that we are able to study the SU-MVP in the embryo as well as in adult somatic cells, and we can obtain sufficient material for biochemical analyses. In contrast to previous vault studies, sea urchin vaults localize to both cytoplasmic and nuclear compartments, with strong staining in the nucleolar region. Our results indicate that vaults may be involved in the nucleocytoplasmic transport of ribosomes or mRNPs.

MATERIALS AND METHODS

Antibodies for Immunoblotting

Primary antibodies used in this study included a monoclonal anti-α-tubulin antibody (DM1A from ICN Biomedicals Inc., Costa Mesa, CA) which was used at 1:1000. A polyclonal antiserum against the sea urchin 40S ribosomal subunit was kindly provided by M. Winkler and J. Drawbridge (UT-Austin) and was used at 1:250 or 1:500 on immunoblots. We generated a rabbit polyclonal antiserum against the 107-kDa sea urchin microtubule-binding protein as previously described (Suprenant *et al.*, 1993). Blot affinity purification of an IgG-enriched fraction (50% ammonium sulfate cut) was carried out as described in Smith and Fisher (1984) and Suprenant *et al.* (1993). The anti-107-kDa antiserum was used at 1:500 to 1:1000 unless otherwise stated. Antiserum against *Dictyostelium* vaults was a kind gift from L. Rome (UCLA) and was used at 1:10,000 on immunoblots. Secondary antibodies (alkaline phosphatase-conjugated or HRP-conjugated goat anti-rabbit or -mouse IgG) were purchased from Zymed Laboratories (San Francisco, CA) and used at 1:2000.

Microtubule Protein

Microtubule protein was purifed from sea urchin eggs (*Strongylocentrotus pupuratus*, *Lytechinus pictus*) by cycles of pH- and temperature-dependent assembly and disassembly, as previously described (Suprenant and Marsh, 1987; Suprenant *et al.*, 1989).

Egg Ribosomes

Ribosomes were prepared as described in Hille and Danilchik (1986), except that phenylmethylsulfonyl fluoride (PMSF) (200 μM), rather than soybean trypsin inhibitor, was included in the homogenization buffer and sucrose gradients.

Sucrose Gradients

Sea urchin egg microtubule protein or egg ribosomes were separated on linear 15–40% (w/v) sucrose gradients. After a brief centrifugation (10 min, 39,000*g*, 4°C) to remove aggregated protein, one ml of each sample (approximately 1–2 mg) was loaded onto each 12-ml gradient. The gradients were centrifuged in a SW41Ti swinging bucket rotor (Beckman Instruments, Palo Alto, CA) at 32,500 rpm (150,000*g*) at 4°C for 2–8 hr. Fractions were collected from the bottom of the gradient and analyzed by SDS–PAGE and immunoblotting (see below).

Sea Urchin Extracts

Embryos. Eggs and sperm from the sea urchin *L. pictus* (Marinus, Inc., Long Beach, CA) were collected after injection of 0.55 *M* KCl into the body cavity. The sperm were collected "dry" and stored on ice, and eggs were collected in Instant Ocean (Aquarium Systems, Mentor, OH). Eggs were collected from more than one female to obtain a sufficient quantity of embryos at each developmental stage. The eggs were fertilized and allowed to develop in Instant Ocean at 16°C with gentle stirring. Embryos were harvested at the following time points: 0 hr (unfertilized eggs), 2 hr (2-cell), 8 hr (morula), 23 hr (swimming blastula), 27 hr (mesenchyme blastula), 31 hr (early gastrula), 34 hr (1/3 gastrula), 46 hr (prism stage), and 72 hr (pluteus stage). At each time point, an aliquot of eggs/embryos was pelleted (100*g*), resuspended in an equal volume of homogenization buffer (100 m*M* Pipes/K^+, pH 7.3, 1 m*M* $MgSO_4$, 4 m*M* EGTA, 1 m*M* GTP, 2 m*M* DTT, 0.2 m*M* PMSF, 10 μg/ml leupeptin, and 1 μg/ml pepstatin), and homogenized. The homogenate was centrifuged at 39,000*g*, 45 min at 4°C in a Beckman TLA100.3 rotor. The supernatants and resuspended pellets were analyzed by SDS–PAGE and immunoblotting.

Other cells and tissues. Several sea urchin cells and tissues were isolated and characterized by SDS–PAGE and immunoblotting as described below. Sperm heads and tails were prepared by the methods of Stephens (1970). Coelomocytes were isolated as described by Otto *et al.* (1979). Tube feet were harvested *en masse* from the sides of the aquarium.

Embryonic Nuclei Isolation

Mesenchyme blastula-stage embryos (*L. pictus*) were cultured in artificial seawater and harvested at 24 hr. Nuclei were prepared as described by Hinegardner (1962) and modified by Drawbridge *et al.* (1990). In brief, the embryos were washed three times in 1.5 *M* dextrose, homogenized in 1.5 *M* dextrose containing 2 m*M* $MgSO_4$, and centrifuged at 1000*g*. The crude nuclear fraction (1000*g* pellet) was layered over a sucrose step gradient (1.25, 1.5, 1.75, 2.0, and 2.375 *M* sucrose in water) and centrifuged in a Beckman SW41Ti rotor at 56,000*g* (45 min, 4°C). A band at the interface between 2

and 2.375 *M* sucrose was collected, diluted in an equal volume of 2 m*M* $MgSO_4$, and centrifuged at 12,000*g* (3 min, 4°C), and the pellets were resuspended by gently pipeting in a small volume of 2 m*M* $MgSO_4$. The sample was determined to contain nuclei based on phase-contrast microscopy and fluorescence microscopy with the DNA-binding dye daimidinophenolindole (DAPI).

Electrophoresis and Immunoblotting

Proteins were separated on 8% sodium dodecyl sulfate–polyacrylamide gels (Laemmli, 1970). Isoelectric focusing and 2D SDS–PAGE were carried out as described by O'Farrell (1975). Bio-Rad (Richmond, CA) ampholytes of 5/7 and 3/10 were used at a ratio of 4:1. Electrophoretic transferring of proteins to nitrocellulose was done as described in Towbin *et al.* (1979). Blots were blocked with 1% bovine serum albumin (fraction V) in PBS (0.17 *M* NaCl, 3 m*M* KCl, 10 m*M* Na_2HPO_4, 2 m*M* KH_2PO_4, pH 7.4) for at least 1 hr at room temperature. Primary and secondary antibodies were diluted in PBST (PBS containing 0.05% (v/v) Tween 20) with 100 μg/ml BSA. Blots were incubated in primary antibodies overnight at 4°C and then washed with PBST (3 × 15 min, room temperature). Secondary antibodies (HRP or AP conjugates) were diluted 1:2000 and the blots were incubated for 3 hr at room temperature. Blots that were incubated with AP-conjugated secondary antibodies were washed in PBST (3 × 15 min) and developed for color detection in alkaline phosphatase developer (Blake *et al.*, 1984). Blots that were incubated with HRP-conjugated antibodies were washed in TBS (10 m*M* Tris–HCL, pH 7.4, 0.15 *M* NaCl) and developed 5–15 min in freshly prepared developer (20 m*M* TBS, 4 ml 3 mg/ml 4-chloro-1-napthol in methanol and 20 μl of 30% hydrogen peroxide) (Hawkes *et al.*, 1982).

Immunofluorescence Microscopy

Immunofluorescence microscopy on sea urchin embryos and coelomocytes was performed as described in Suprenant *et al.* (1993).

Negatively Stained Samples for Electron Microscopy

Samples were fixed with 2% (v/v) glutaraldehyde and negatively stained with 2% aqueous uranyl acetate on carbon-coated Formvar grids. The grids were observed on a JOEL 1200 EX II microscope at an accelerating voltage of 80 keV.

RESULTS

Identification of a 107-kDa Microtubule-Binding Protein

To identify proteins that may be involved in the targeting of polyribosomes to microtubules (Hamill *et al.*, 1994), the protein profiles of microtubules and ribosomes were compared by SDS–PAGE (Fig. 1). Egg microtubules, purified by three cycles of pH- and temperature-dependent assembly, are composed of α- and β-tubulin, the 77-kDa EMAP (Suprenant *et al.*, 1993), a few low M_r polypeptides, and two polypeptides with M_rs of 100 and 107 kDa. We have shown previously that the 100- and 107-kDa polypeptides copurify with egg microtubules with a constant stoichiometry through multiple polymerization cycles (see Fig. 5, Suprenant and Marsh, 1987). The identities of these microtubule-binding proteins were not known at that time. In this report, we concentrate on the identity and characterization of the 107-kDa polypeptide.

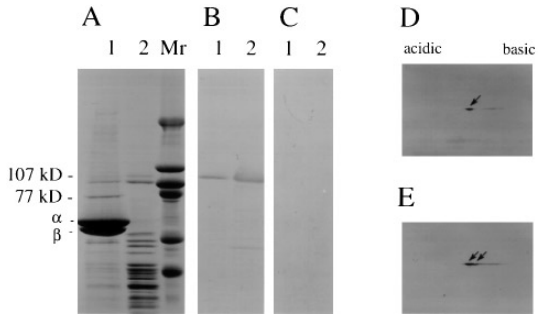

FIG. 1. Comparison of a 107-kDa microtubule- and ribosome-binding protein by 1D SDS–PAGE (A–C) and 2D SDS–PAGE (D, E). Microtubule proteins (lane 1) and ribosomes (lane 2) were purified from sea urchin eggs and separated by SDS–PAGE. The gels were stained with Coomassie blue (A) or transferred to nitrocellulose and immunostained (B, C). B was probed with an antiserum raised against the 107-kDa sea urchin microtubule-binding protein. This antiserum recognizes a single polypeptide of 107 kDa in both microtubule and ribosome preparations. C was probed with the preimmune serum. The molecular mass standards (M_r) in A are as follows: 205, 116, 97.4, 66, 45, and 29 kDa. In addition, the positions of the 77-kDa echinoderm microtubule-associated protein (EMAP) and α- and β-tubulin are shown. In D and E, the 107-kDa microtubule- and ribosome-binding proteins were compared by two-dimensional SDS–PAGE and immunoblotting with the anti-107-kDa antiserum. The 107-kDa polypeptide has a p*I* of 5.8 in both purified microtubules (D) and purified ribosomes (E). In ribosome preparations, there is a less abundant, slightly more basic isoform.

Ribosomes purified from egg cytosol are composed of many proteins ranging in M_r from 20 to 60 kDa (Fig. 1). In addition, sea urchin ribosomes are associated with two polypeptides of 100 and 107 kDa. An antiserum was generated against the 107-kDa microtubule-binding polypeptide to determine whether the 107-kDa ribosome and microtubule proteins are antigenically related. Figures 1A–1C illustrate that the anti-107-kDa antiserum reacts strongly with a 107-kDa polypeptide in both ribosome and microtubule preparations. The antiserum does not recognize the 100-kDa polypeptide in either microtubule or ribosome preparations.

To further address the relatedness of these 107-kDa proteins, ribosomes and microtubule proteins were analyzed by isoelectric focusing followed by SDS–PAGE. In both

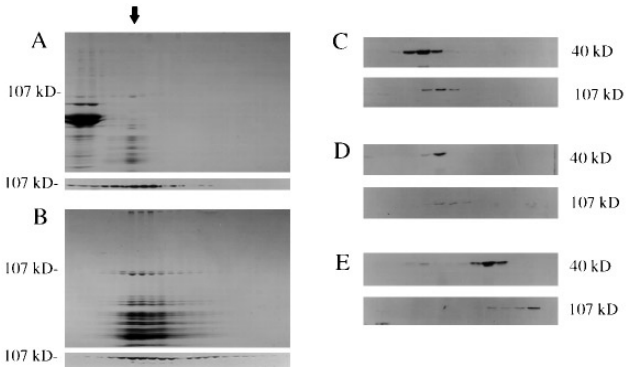

FIG. 2. The 107-kDa microtubule- and ribosome-binding protein is part of a large sedimentable particle. Purified microtubule proteins (A) or purified ribosomes (B) were fractionated on 15–40% sucrose gradients for 2 hr. Individual fractions were analyzed by SDS–PAGE and immunoblotting with the anti-107-kDa antiserum. In A and B, the corresponding immunoblots are shown directly below the Coomassie-stained gels. Because these gradients were run in the cold, the microtubules were depolymerized and the soluble microtubule proteins are present at the top of the gradient (A, left). Monoribosomes present in these microtubule preparations migrate to a position approximately one-third from the top of the sucrose gradients (arrow). In both microtubule (A) and ribosome (B) preparations, the 107-kDa polypeptide appears to cosediment with the ribosomes. When identical gradients are centrifuged for longer than 2 hr, the 107-kDa polypeptide can be separated from the ribosome peak. In C–E, microtubule protein was sedimented on ice-cold 15–40% sucrose gradients for 3, 4, and 8 hr, respectively. Corresponding immunoblots with the anti-107-kDa and an anti-40-kDa ribosomal antiserum are shown. By 8 hr, the 107-kDa peak is separated from the 40-kDa ribosomal protein, indicating that the two polypeptides are part of two separate structures.

preparations, the 107-kDa polypeptide migrates with a p*I* of 5.8 (Figs. 1D and 1E), although there appears to be a second, slightly more basic spot (p*I* of 5.9) in the ribosome preparation as well (Fig. 1E). Thus, the immune serum appears to recognize the same polypeptide in both microtubule and ribosome preparations.

To examine whether the 107-kDa antigen is a soluble polypeptide, depolymerized microtubule protein and purified ribosomes were subjected to sucrose-gradient centrifugation. These gradients were run in the cold in order to depolymerize the microtubules into soluble components, such as tubulin, which remain at the top of the gradient. This was done because polymerized microtubules would have migrated throughout a velocity gradient due to their heterogeneous lengths and mass. With microtubules distributed throughout the gradient, it would be difficult to separate a microtubule interaction from a ribosome interaction. Figure 2A illustrates that very little of the 107-kDa polypeptide appears to remain with the soluble microtubule components such as tubulin and EMAP. Rather, the 107-kDa polypeptide is associated with a particulate structure that appears to migrate approximately one-third of the way into the gradient. The peak of 107-kDa immunoreactivity appears to cosediment with ribosomes in both microtubule and ribosome preparations (Figs. 2A and 2B). These results indicate that the 107-kDa polypeptide is part of a particulate structure or is associated with a particulate structure, such as the ribosome. To distinguish between the two possibilities, identical sucrose gradients were centrifuged for successively longer periods of time. Over an 8-hr time course the 107-kDa polypeptide sediments further into the sucrose density gradient and no longer comigrates with the 80S monoribosome peak (Fig. 2C). The migration of the 107-kDa polypeptide is consistent with it being part of a 150S structure.

The 107-kDa Microtubule-Binding Protein Is the Sea Urchin Major Vault Protein (SU-MVP)

The identity of the 107-kD polypeptide was presaged by the presence of intact vault particles in negatively stained microtubule preparations (Fig. 3). The multiply arched structure of the barrel-shaped sea urchin vault appears indistinguishable from the previously described rat vaults (Kedersha and Rome, 1986). The vaults appear to lie close to the microtubule wall; however, no physical connection is observed between these two organelles.

The similarity in molecular mass between the major rat vault polypeptide (Kedersha and Rome, 1986) and the sea urchin 107-kDa microtubule-binding polypeptide indicated that the 107-kDa polypeptide could be the sea urchin major vault protein. To examine this possibility, the 107-kDa antiserum was affinity-purified and immunoblotted to sea urchin egg extracts, sea urchin microtubule proteins, and purified rat vaults (kindly provided by Leonard Rome, UCLA) (Figs. 4A and 4B). The affinity-purified anti-107-kDa antibodies recognize a single band of M_r 107 kDa in unfertilized egg extracts and purified microtubule protein, as well as the major rat vault protein. Conversely, an antiserum against *Dictyostelium* vaults recognizes the 107-kDa microtubule-binding protein (Fig. 4C).

The Sea Urchin MVP Is Expressed in Embryonic and Somatic Tissues

To determine the function of sea urchin vaults, it is important to know where and when the major vault protein is expressed. Initially, sea urchin vaults were detected in egg extracts and in purified microtubules and ribosomes prepared from egg extracts (see Figs. 1–4). It is likely that these vaults were assembled from proteins coded by maternal transcripts during oogenesis. However, vaults are not unique to embryos and are abundant in adult tissues such as coelomocytes, a macrophage-like cell of the invertebrate immune system, as well as tube feet, a locomotory tissue (Fig. 5). In both tissues, the anti-107-kDa antiserum recognizes the 107-kDa SU-MVP. We were unable to detect the SU-MVP in sperm, neither in heads nor in tails.

Vaults Are Not Microtubule-Associated in Situ

The presence of the 107-kDa polypeptide in preparations of microtubules assembled *in vitro* from sea urchin, sug-

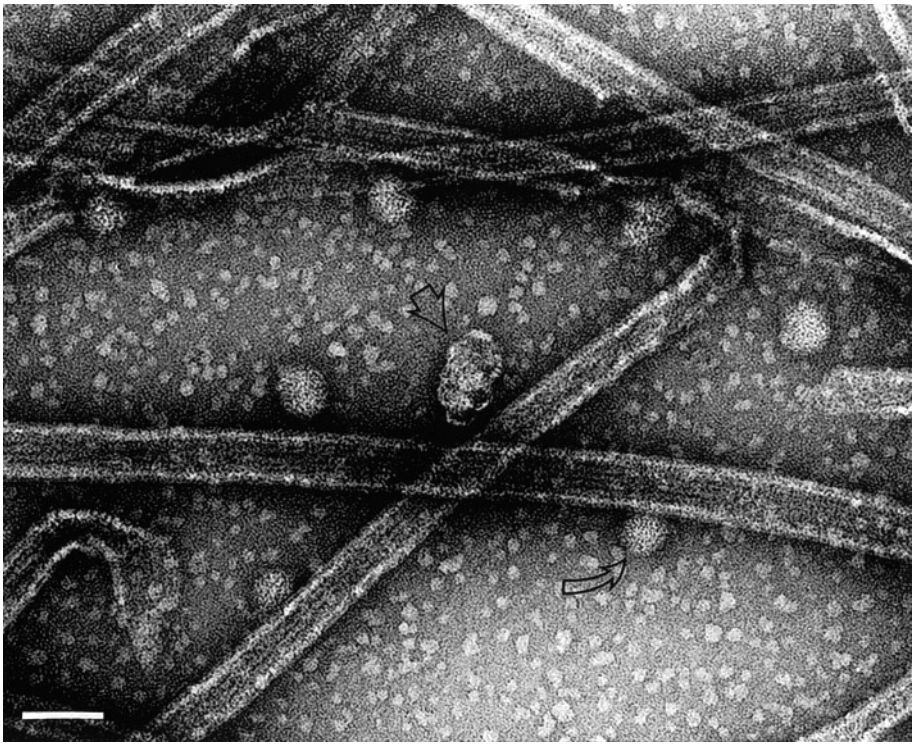

FIG. 3. Electron micrograph of vaults and ribosomes in sea urchin egg microtubule preparations. Individual protofilaments can be seen running longitudinally through these negatively stained microtubules. Numerous ribosomes are associated with the walls of the microtubule (curved arrow). Straight arrow points to a distinctive structure called a vault. Bar, 50 nm.

gests that the vaults might be microtubule-associated in sea urchin embryos. To examine this possibility, first-cleavage mitotic embryos were immunostained with the anti-107-kDa antiserum. In contrast to the anti-tubulin antibody that strongly stains the mitotic apparatus (Fig. 6a), the anti-107-kDa localization pattern is diffuse and throughout the entire cytoplasm. Anti-107-kDa staining is slightly stronger at the centrosomal region of the mitotic apparatus (Fig. 6b). The diffuse cytoplasmic staining with the anti-107-kDa antiserum also is observed in the unfertilized egg, as well as in 2- and 4-cell embryos (data not shown). The staining is lost from the centrosomal region as well as the entire cytoplasm when the mitotic embryos are extracted with a non-ionic detergent, Nonidet P-40, under microtubule-stabilizing conditions. These data indicate that the vault particles are not tightly associated with the microtubule cytoskeleton or the centrosome.

Vault Localization to the Nucleus in Somatic Cells

Cellular immunity is maintained in adult sea urchins by a collection of body-cavity cells called coelomocytes, the invertebrate equivalent of a macrophage. The petaloid coelomocyte is a highly flattened cell with a prominent nucleus (Fig. 7A). In contrast to the early embryonic stages, the SU-MVP is prominent in the nucleus of petaloid coelomocytes. Immunofluorescence localization reveals punctate staining of the nuclear periphery as well as intense staining of the nucleolus (Fig. 7B, inset). In addition, some vault staining appears to extend into the ruffled cytoplasm from the nucleus in linear strings. As in eggs and embryos, the SU-MVP is not localized to the cytoskeleton of petaloid coelomocytes.

The immunofluorescence staining patterns observed in eggs and early embryos gives the impression that the SU-MVP is found only in the cytoplasm of embryonic cells and not in the nucleus. However, the high level of vault staining in the cytoplasm of embryonic cells obscured any potential vault immunoreactivity within the nucleus. To examine whether the SU-MVP is associated with the embryonic nucleus, nuclei were purified from mesenchyme-blastula-stage embryos. This embryonic stage was chosen because nuclei can be purified in large numbers from the approximately 500 nucleated cells. The purity of the nuclei preparation was confirmed by fluorescence microscopy with the DNA-binding dye DAPI (see Materials and Methods). In addition, the nuclei were shown to be free of contaminating cytoplasm by immunoblotting with a monoclonal antibody against a known cytosolic protein, α-tubulin (Fig. 8). At this developmental stage, the 107-kDa SU-MVP is detectable in purifed nuclei as well as cytosolic fractions, although only about 2% of the SU-MVP by mass is associated with the

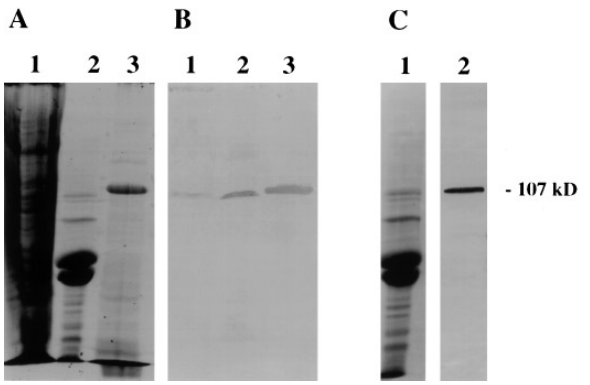

FIG. 4. The major vault proteins from rat and *Dictyostelium* are antigenically related to the 107-kD sea urchin microtubule-binding protein. Sea urchin egg extract (lane 1), sea urchin microtubule proteins (lane 2), and purified rat vaults (lane 3) were separated by SDS–PAGE and either stained with Coomassie blue (A) or immunoblotted with affinity-purified antibodies against the sea urchin 107-kDa microtubule-binding protein and HRP-conjugated secondary antibodies (B). In C, sea urchin microtubule protein (lane 1) was immunoblotted with an antiserum against the *Dictyostelium* major vault protein and visualized with an AP-conjugated secondary antibody (lane 2). The anti-107-kDa antiserum binds to rat vaults and the anti-vault antiserum binds strongly and specifically to the sea urchin 107-kDa polypeptide. The intensity of the antibody staining in B and C cannot be compared directly due to the different methods used to develop the blots.

nuclei fraction. These results demonstrate that a small fraction of the 107 kDa SU-MVP is localized to the nucleus in mesenchyme-blastula-stage embryos.

To examine whether the SU-MVP distribution changes during development, we investigated whether the sedimentation of the SU-MVP changes from fertilization to the formation of the pluteus-stage larvae. Initially, total embryo homogenates were analyzed for the presence of the 107-kDa SU-MVP by quantitative immunoblotting. Figure 9A indicates that the total level of the SU-MVP remains relatively constant during early development. After determining that the SU-MVP is an abundant protein throughout sea urchin development, we quantitated the relative amounts of the SU-MVP in soluble extracts and insoluble, sedimentable fractions. Surprisingly, the amount of soluble SU-MVP drops dramatically at the onset of gastrulation, in the experiment shown in Fig. 9B. Concomitant with the loss of soluble protein, the levels of the sedimentable 107-kDa SU-MVP increase in the 39,000*g* pellet (containing nuclei, cytoskeletal components, and unbroken cells) (Fig. 9C). While the loss of the soluble MVP fraction is always observed during embryogenesis, it is not always coincident with the onset of gastrulation and can occur as early as the onset of blastulation (Fig. 10). The variability between batches of embryos may reflect differences in individual sea urchins or a slight asynchrony of the cultures. The asynchrony may be due to the fact that eggs from at least two females were pooled for a single embryonic time course. The shift from a soluble to a predominately insoluble form may reflect a change in the intracellular distribution of the SU-MVP during embryogenesis, perhaps from the cytosol to the nucleus.

DISCUSSION

A 107-kDa Polypeptide Is the Sea Urchin Major Vault Protein

In this report we identify the SU-MVP as a 107-kDa polypeptide with a p*I* of 5.8. A monospecific antiserum against this 107-kDa polypeptide recognizes both the MVPα and MVPβ vault proteins in *Dictyostelium* (data not shown), as well as the 104-kDa MVP in rat liver. In sea urchins, the 107-kDa MVP is abundant in embryonic and adult tissues and is part of a bona fide vault particle. Structures with the characteristic vault morphology are seen in sea urchin eggs, and the the 107-kDa immunoreactivity is coincident with a 150S particle in sucrose density gradients. Vaults are phylogenetically conserved and broadly distributed among a wide variety of cell and tissue types, indicating that vaults must play an essential role for eukaryotic cells (for reviews see Rome *et al.*, 1991; Kickhoefer *et al.*, 1996; Izquierdo *et al.*, 1996b).

The SU-MVP copurified with microtubules through mul-

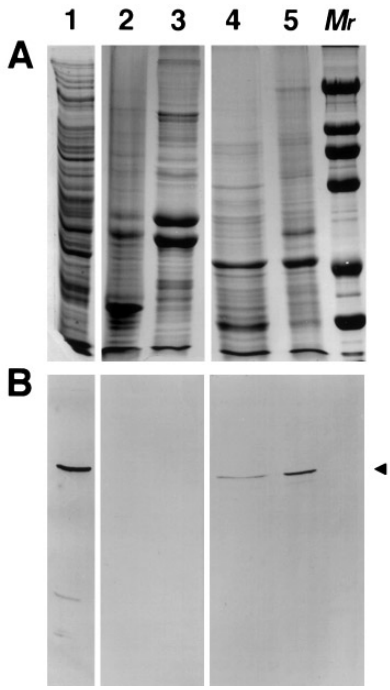

FIG. 5. The 107-kDa SU-MVP is present in several sea urchin cell types and tissues. Homogenates of sea urchin eggs (lane 1), sperm heads (lane 2), sperm tails (lane 3), tube feet (lane 4), and coelomocytes (lane 5) were separated by SDS–PAGE (A) and immunoblotted with affinity-purified antibodies to the 107-kDa microtubule-binding protein (B). M_r, 205, 116, 97.4, 66, 45, and 29 kDa.

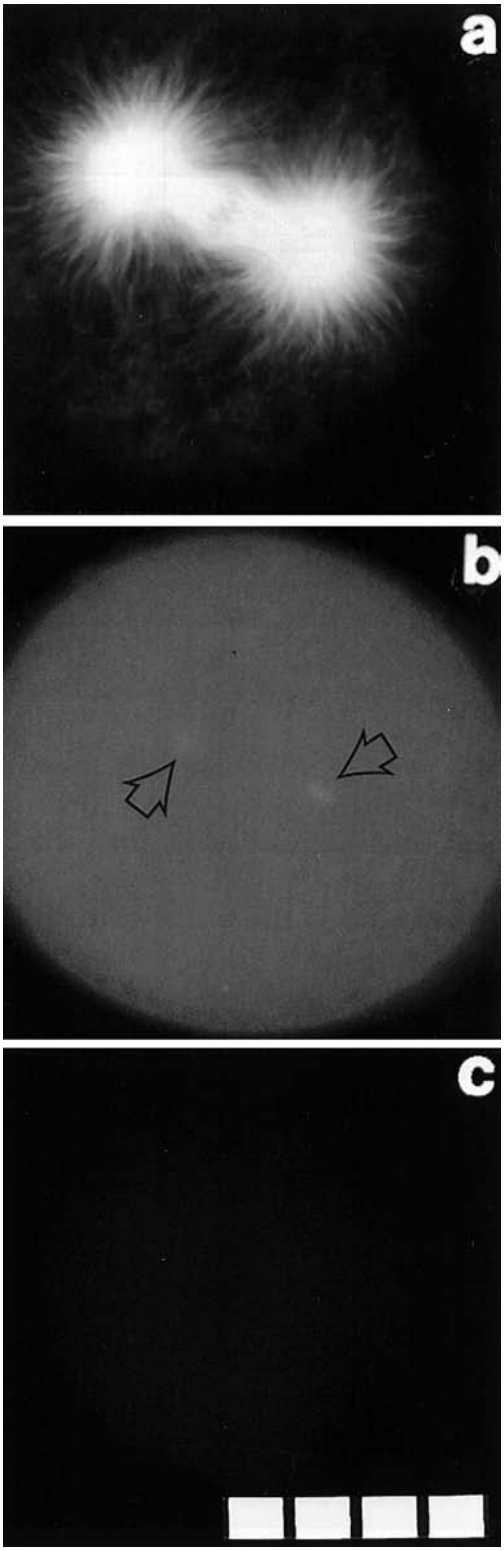

FIG. 6. Immunofluorescence localization of the 107-kDa SU-MVP in mitotic embryos. Embryos at the first mitotic metaphase were methanol-fixed and immunostained with: (a) anti-tubulin antibodies, (b) anti-107-kDa antiserum, or (c) preimmune control serum. Arrows point to the spindle poles of the first mitotic spindle. Magnification bar is in 10-μm divisions.

tiple cycles of assembly and disassembly from postmeiotic sea urchin eggs, as well as from starfish oocytes that had not completed their meiotic reduction divisions (unpublished observations). Therefore, the copurification of vaults with microtubules does not appear to be restricted to a single organism or stage of the embryonic cell cycle. We were unable to detect the SU-MVP in microtubules purified from squid optic lobe and bovine brain (unpublished observations). These results are consistent with a relatively lower abundance of vaults in neuronal tissue (Kedersha *et al.*, 1990), although vaults are enriched in rat brain microglia (Chugani *et al.*, 1991) and synaptic nerve termini of electric rays (Hermann *et al.*, 1996).

Vaults copurify with microtubules with a constant stoichiometry through three cycles of assembly and disassembly *in vitro*, and yet vaults do not appear to be microtubule-associated *in vivo*. The SU-MVP is not enriched along microtubules of the first cleavage mitotic apparatus, but rather is diffusely localized throughout the cytoplasm. Moreover, the SU-MVP staining is lost upon detergent extraction, indicating that the SU-MVP is not tightly associated with the cytoskeleton. While colocalization of vaults with microtubules *in situ* would have been a strong indication of their interaction, a lack of colocalization does not rule out a transient or weak interaction of vaults with microtubules. In addition, it is not known what fraction of the vaults would be associated with microtubules at any given time. A small amount of microtubule staining would be obscured by the cytoplasmic vault pool.

Interaction between Vaults and Ribosomes

If vaults are not microtubule-associated *in situ*, how do vaults copurify with microtubules *in vitro*? Vaults are probably too small (150S) to have sedimented on their own during the microtubule purification cycles, so it is likely that they copurify with microtubules through an interaction with a microtubule-associated component. The most obvious components are the numerous ribosomes attached to the walls of the microtubules by a thin stalk (Suprenant *et al.*, 1989; Hamill *et al.*, 1994). Consistent with this observation is the copurification of vaults with ribosomes that were isolated in the absence of microtubules (see Figs. 1 and 2). In addition, we have partially purified vaults from sea urchin eggs, using the methods described for the purification of rat vaults (Kedersha *et al.*, 1991), and have found that ribosomes are a major component of these vault preparations (Hamill, 1997).

If vaults and ribosomes interact *in situ*, one would predict that they should function in the same compartment and be present at comparable levels. Both of these conditions have been met. The mature sea urchin egg contains approximately 4×10^8 ribosomes that are synthesized and accumulated during oogenesis (Griffith *et al.*, 1981). Similarly, we have demonstrated that the SU-MVP is an abundant protein in the unfertilized egg cytoplasm. From immunoblots we estimate the concentration of SU-MVP to be on the order of 10 μM and the number of molecules of SU-MVP to be

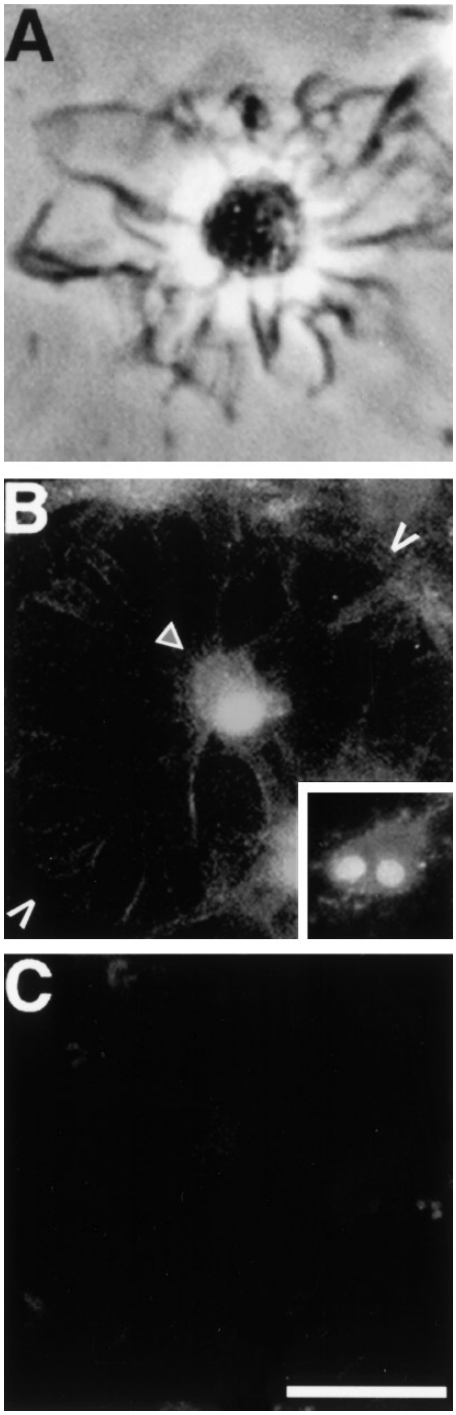

FIG. 7. Immunofluorescence localization of the SU-MVP in adult coelomocytes. (A) Phase-contrast micrograph of a petaloid coelomocyte. (B) Coelomocyte stained with antiserum against the 107-kDa SU-MVP. The open arrowheads indicate the border of the cell. The small triangle points to the nucleus that is brightly stained with the 107-kDa antiserum. Punctate staining of the nuclear envelope region and the intense staining of the nucleolus are observed. The inset shows a nucleus with two brightly stained nucleoli. (C) Coelomocyte stained with preimmune control serum. Magnification bar, 10 μm.

on the order of 10^9/egg. With a minimum of 96 copies of the MVP per vault particle (Kedersha *et al.*, 1991), there should be enough of the SU-MVP to assemble 10^7 vaults. If the interaction of vaults with ribosomes is stoichiometric, each vault particle could associate with 10 ribosomes individually in succession or simultaneously in a large complex.

The copurification of vaults and ribosomes using various isolation conditions indicates that vaults and ribosomes may interact. However, the data shown in Fig. 2 indicate that the interaction is not maintained in these sucrose-density gradients. These data by themselves might indicate that the vault–ribosome association is artifactual or a very weak affinity interaction. However, several lines of evidence support the idea that vaults and ribosomes are associated. First, we show by immunofluorescence microscopy that the major vault protein is localized to the nucleolar region, the site of ribosome synthesis and assembly. Second, the major vault protein is present in nuclei purified from mesenchyme-blastula-stage embryos. Third, the solubility of the SU-MVP changes during development, shifting to a predominantly insoluble fraction in embryo homogenates after the mesenchyme blastula stage. Interestingly, nucleoli are first observed around this stage in sea urchin embryos, which also corresponds with the onset of embryonic rRNA synthesis (Karasaki, 1967). Therefore, the shift to the insoluble fraction observed in embryonic development may result from the association of the SU-MVP with the newly formed nucleoli.

Our interpretation of the gradient results is that vaults

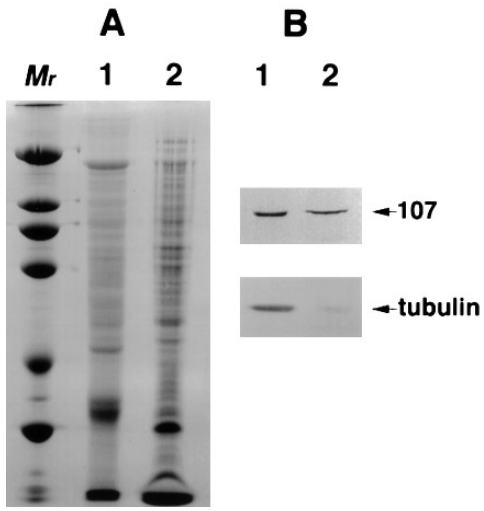

FIG. 8. Immunoblot analysis of the subcellular distribution of the 107-kDa SU-MVP. Mesenchyme-blastula-stage sea urchin embryos were fractionated on a sucrose step gradient. The cytosolic fraction (1) and nuclei (2) were separated by SDS–PAGE (A) and immunoblotted (B). Blots were probed with affinity-purified antibodies against the 107-kDa sea urchin major vault protein or with an anti-tubulin antibody. Unlike tubulin, the SU-MVP is present in purified nuclei. Molecular mass standards (M_r), 205, 116, 97.4, 66, 45, and 29 kDa.

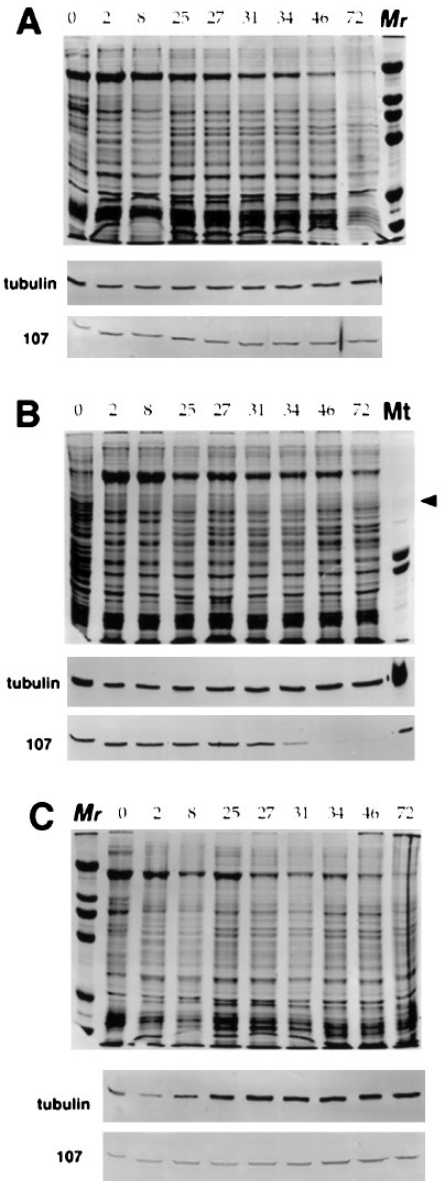

FIG. 9. Immunoblot analysis of the SU-MVP over a developmental time course. Homogenates from eggs and embryos were centrifuged, and the supernatant (soluble extract) and pellet were analyzed by SDS–PAGE. The total homogenates are shown in A, the supernatants in B, and the pellets in C, all loaded at equal protein concentrations. The gels were either stained with Coomassie blue or transferred to nitrocellulose. Blots were probed with affinity-purified antibodies against the 107-kDa sea urchin major vault protein or with anti-tubulin antibody. The blot strips are shown below each of the stained gels. The embryonic stages used in this study: unfertilized egg (0 hr), 2-cell (2 hr), morula (8 hr), swimming blastula (25 hr), mesenchyme blastula (27 hr), early gastrula (31 hr), one-third gastrula (34 hr), prism (46 hr), and pluteus (72 hr). Mt is sea urchin microtubule protein; arrowhead points to 107-kDa vault protein. M_r are standards: 205, 116, 97.4, 66, 45, and 29 kDa.

and ribosomes may interact through a labile intermediate such as mRNA. No extra precautions were taken to preserve mRNA in these microtubule or ribosome preparations. If the vault–ribosome interaction is mediated by mRNA or proteins associated with mRNA, the loss of mRNA could destroy the ribosome and vault interaction. Furthermore, Drawbridge (1989) demonstrated that density gradient centrifugation stripped poly(A)-binding proteins from a poly(A^+)-RNA–poly(A)-binding protein complex in sea urchins. Proteins important for the maintenance of a vault–ribosome interaction may have been stripped from this complex during gradient centrifugation.

While these data point to a role for vaults in some aspect of ribosome function, it should be pointed out that it remains an open question as to how all the individual vault components are assembled and whether this takes place in the nucleus or the cytoplasm. One alternative explanation for the nucleolar localization of the SU-MVP is that, like ribosomes, vaults may be assembled in the nucleolus.

Vault Function

There is still much speculation about the function of vaults. As an apparently ubiquitous and highly conserved organelle (Rome *et al.*, 1991; Kickhoefer *et al.*, 1993), vaults must have an important function. In terms of the high copy number of the major vault protein, vaults are cytoskeletal-like in nature, which suggests that vaults may assemble and disassemble during their lifetime (Kedersha *et al.*, 1990). Further, variations exist in the vault structure, indicating that vaults may unfold into two symmetrical vault flowers, each with eight petals (Kedersha *et al.*, 1991). As reported in previous studies (Kedersha and Rome, 1986; Kedersha *et al.*, 1990), the majority of vaults appear to be in the cytoplasm, although a small amount of vault immunoreactivity (<5%) is associated with the nuclear envelope in tissue sections and with nuclear pore complexes (NPCs) (Chugani *et al.*, 1993). The symmetry and geometry of the vault particle led to the suggestion that vaults may be the central plug/transporter of the NPC (Kedersha *et al.*, 1991). Alternatively, with their eightfold symmetry, vaults may interact with the central plug/transporter and, as such, may play a role in nucleocytoplasmic transport (Chugani *et al.*, 1993).

Vaults appear to be most abundant in motile cells such as macrophages and *Dictyostelium* amoebae (Kedersha *et al.*, 1990). In *Dictyostelium*, disruption of two vault genes encoding MVPα and MVPβ reveals a growth defect suggesting that vaults play a central role in cellular homeostasis in these cells (Vasu *et al.*, 1993; Vasu and Rome, 1995). Other ideas regarding vault function include their involvement in translational control, mRNA localization, or cytoplasmic RNA-mediated events (Kedersha and Rome, 1990). A possible interaction between vaults and the cytoskeleton as also been suggested. A subpopulation of vaults appears to be associated with the distal ends of actin stress fibers (Kedersha and Rome, 1990). In addition, vault proteins are highly enriched in the actin-rich cholinergic nerve terminals of electric rays (Herrman *et al.*, 1996).

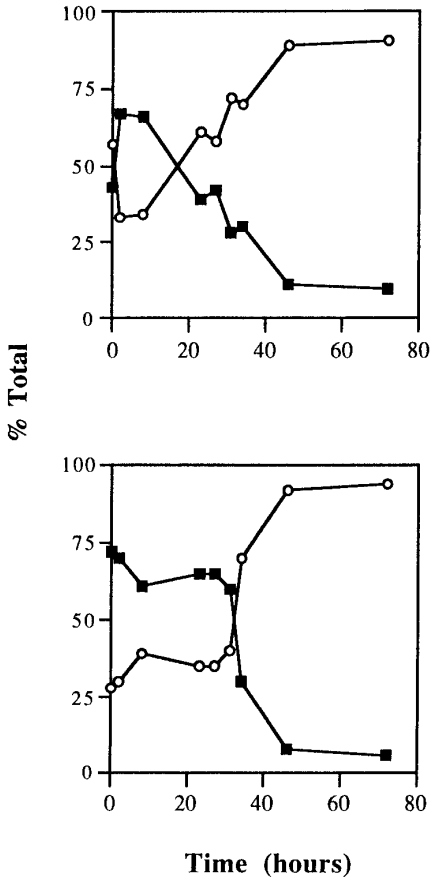

FIG. 10. The SU-MVP becomes less soluble over a developmental time course. The percent of the SU-MVP in the soluble (supernatant) and sedimentable (pellet) fractions was quantitated by densitometry of immunoblots. Soluble MVP (closed squares) and particulate MVP (open circles) fractions are shown at each developmental time point from two separate experiments. In the top panel, a dramatic drop in the soluble vault fraction occurs during blastulation, while in the bottom panel, the decrease does not occur until gastrulation.

One of the more intriguing observations is the overexpression of the human major vault protein in multidrug-resistant cells *in vitro* (Scheper *et al.*, 1993; Scheffer *et al.*, 1995). The human vault protein is overexpressed in a variety of drug-resistant tumor cells that are negative for the P-glycoprotein or multidrug-resistance-associated protein, two members of the ABC family of transporters usually associated with the aquisition of drug resistance. Moreover, the human major vault protein is overexpressed in drug-resistant human cancer cell lines that were not subjected to laboratory drug selection (Izquierdo *et al.*, 1996a). Human vaults may play a role in drug resistance by regulating the vesicular and nucleocytoplasmic transport of drugs (Izquierdo *et al.*, 1996b). For example, overexpression of the human vault protein is associated with the redistribution of daunorubicin away from the nucleus to unidentified punctate perinuclear and cytoplasmic structures (Schuurhuis *et al.*, 1991). The implication is that vaults are a component of these punctate structures.

From the above discussion it is clear that vaults are expressed in many eukaryotic cells and must perform an important and universal function. It is attractive to postulate that the vaults in different organisms participate in related cellular functions, perhaps nucleocytoplasmic transport. Alternatively, different vaults may participate in overlapping cellular functions such as cell growth, cell motility, and even intracellular transport.

In this report, we demonstrate vault immunostaining at the nuclear surface and strong reactivity in the nucleolus, the site of ribosomal subunit assembly (reviewed by Melese and Xue, 1995; Shaw and Jordan, 1995). One possibility is that vaults are involved in the assembly and/or nucleocytoplasmic transport of ribosomal subunits or mRNPs. There is very little information available regarding the export of ribosomal subunits and mRNPs from the nucleus, although the process is saturable and likely to be facilitated. Recent work has focused on the role of nonribosomal nucleolar proteins that appear to shuttle between the nucleolus and the cytoplasm, as it is likely that they may function in ribosomal subunit assembly and nucleocytoplasmic transport (Xue and Melese, 1994).

The nucleolus also plays an important role in mRNA transport in yeast (Tani *et al.*, 1995). During severe heat shock in *S. pombe*, mRNA transport is blocked and poly(A^+) RNA accumulates in the condensed nucleolar regions of the cell. In addition, there are several RNA transport mutants in *S. cerevisiae* that accumulate poly(A^+) RNA in the nucleus coincident with nucleolar fragmentation (Kadowaki *et al.*, 1994; Schneiter *et al.*, 1995). In mammalian cells transcripts for c-myc, N-myc, and Myo D localize to the nucleoli (Bond and Wold, 1993). In addition, UV irradiation of HeLa and BSC-1 nuclei blocks the export of nonribosomal RNAs from the nucleus to the cytoplasm (Sidebottom and Harris, 1969; Deak, 1973). These results indicate that the nucleolar region of the nucleus may be involved in RNA transport in addition to being the site of ribosomal RNA synthesis and ribosomal subunit assembly.

Freeze-etch electron microscopy illustrates that vaults can unfold into two halves (Kedersha *et al.*, 1991), and if the vaults were to open and close *in vivo*, as has been suggested, the interior would probably be large enough to accommodate either the 40S or 60S ribosomal subunit. Vaults are of the right size and symmetry to dock with the nuclear pore complex (Rome *et al.*, 1991) and perhaps even move between the nuclear envelope and cytoplasmic compartments while shuttling ribosomal subunits or mRNPs. In the cytoplasm, vaults might transiently or weakly associate with microtubules or a microtubule-associated component.

The mRNP or ribosomal transport hypothesis is consistent with the recent observation that the overexpression of human major vault protein in acute myeloid leukemia and advanced ovarian carcinoma is associated with a poor response to chemotherapy (Scheper *et al.*, 1993). Scheffer and Scheper suggest that vaults may be able to transport and

sequester the chemotherapeutic agents. However, malignant cells also undergo a dramatic increase in protein synthesis and ribosome biogenesis and as a result have an increased number of nucleoli (Schwarzacher and Wachtler, 1993). Therefore, another possibility is that the overexpression of the major vault protein in highly proliferating cancer cells may reflect a greater need for ribosomal synthesis and transport during a rapid cell cycle. In this regard, overexpression of the LRP may be proliferation-associated and not necessarily sine qua non for drug resistance. The fact that transfection with the LRP gene itself appears to be insufficient for a drug-resistance phenotype supports this suggestion (Scheffer *et al.*, 1995).

In summary, the cellular function(s) of vaults still remains to be established. We present in this report new information about vaults that may lead to a better understanding of these ubiquitous organelles. In addition to identifying and characterizing vaults in another organism, we present new evidence for nucleolar localization of the major vault protein and also demonstrate a likely interaction between vaults and ribosomes. We suggest that vaults may play a role in ribosome assembly and/or transport of ribosomes or mRNPs from the nucleus.

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REFERENCES

Akey, C. W. (1990). Visualization of transport-related configurations of the nuclear pore transporter. *Biophys. J.* **58,** 341–355.

Blake, M. S., Johnston, K. H., Russell-Jones, G. J., and Gotschlich, E. C. (1984). A rapid, sensitive method for detection of alkaline phosphatase-conjugated anti-antibody on Western blots. *Anal. Biochem.* **136,** 175–179.

Bond, V. C., and Wold, B. (1993). Nucleolar localization of myc transcripts. *Mol. Cell Biol.* **13,** 3221–3230.

Chugani, D. C., Rome, L. H., and Kedersha, N. L. (1993). Evidence that vault ribonucleoprotein particles localize to the nuclear pore complex. *J. Cell Sci.* **106,** 23–29.

Deak, I. I. (1973). Further experiments on the role of the nucleolus in the transfer of RNA from nucleus to cytoplasm. *J. Cell Sci.* **13,** 395–401.

Drawbridge, J. (1989). ''Characterization of the Poly(A)-Binding Proteins from Eggs and Embryos of the Sea Urchin, *Strongylocentrotus purpuratus*.'' Ph. D. dissertation, The University of Texas at Austin.

Drawbridge, J., Grainger, J. L., and Winkler, M. M. (1990). Identification and characterization of the poly(A)-binding proteins from the sea urchin: A quantitative analysis. *Mol. Cell. Biol.* **10,** 3994–4006.

Griffith, J. K., Griffith, B. B., and Humphreys, T. D. (1981). Regulation of ribosomal RNA synthesis in sea urchin embryos and ooctyes. *Dev. Biol.* **87,** 220–228.

Hamill, D. R. (1997). ''Association of Proteins, RNAs, and RNPs with Sea Urchin Microtubules.'' UMI Dissertation Information Service, Ann Arbor, Michigan.

Hamill, D., Davis, J., Drawbridge, J., and Suprenant, K. A. (1994). Polyribosome targeting to microtubules: Enrichment of specific mRNAs in a reconstituted microtubule preparation from sea urchin embryos. *J. Cell Biol.* **127,** 973–984.

Hawkes, R., Niday, E., and Gordon, J. (1982). A dot-immunobinding assay for monoclonal and other antibodies. *Anal. Biochem.* **119,** 142–147.

Herrmann, C., Volknandt, W., Wittich, B., Kellner, R., and Zimmermann, H. (1996). The major vault protein (MVP100) is contained in cholinergic nerve terminals of electric ray electric organ. *J. Biol. Chem.* **271,** 13908–13915.

Hille, M. B., and Danilchik, M. V. (1986). The protein synthetic machinery: ribosomes and cell-free systems. *Methods Cell Biol.* **27,** 175–188.

Hinegardner, R. T. (1962). The isolation of nuclei from eggs and embryos of the sea urchin. *J. Cell Biol.* **15,** 503–508.

Izquierdo, M. A., Scheffer, G. L., Flens, M. J., Schoemaker, R. H., Rome, L. H., and Scheper, R. J. (1996a). Relationship of LRP-human major vault protein to in vitro and clinical resistance to anticancer drugs. *Cytotechnology* **19,** 191–197.

Izquierdo, M. A., Shoemaker, R. H., Flens, M. J., Scheffer, G. L., Wu, L., Prather, T. R., and Scheper, R. J. (1996b). Overlapping phenotypes of multidrug resistance among panels of human cancer-cell lines. *Int. J. Cancer* **65,** 230–237.

Kadowaki, T., Hitomi, M., Chen, S., and Tartakoff, A. M. (1994). Nuclear mRNA accumulation causes nucleolar fragmentation in yeast mtr2 mutant. *Mol. Biol. Cell* **5,** 1253–1263.

Karasaki, S. (1968). The ultrastructure and RNA metabolism of nucleoli in early sea urchin embryos. *Exp. Cell Res.* **52,** 13–26.

Kedersha, N. L. (1995). An intranuclear isoform of the major vault protein and its possible involvement in RNA transport. *Mol. Biol. Cell* **6**(Suppl.), 196a.

Kedersha, N. L., and Rome, L. H. (1986). Isolation and characterization of a novel ribonucleoprotein particle: Large structures contain a single species of small RNA. *J. Cell Biol.* **103,** 699–709.

Kedersha, N. L., and Rome, L. H. (1990). Vaults: Large cytoplasmic RNP's that associate with cytoskeletal elements. *Mol. Biol. Rep.* **14,** 121–122.

Kedersha, N. L., Heuser, J. E., Chugani, D. C., and Rome, L. H. (1991). Vaults. lll. Vault ribonucleoprotein particles open into flower-like structures with octagonal symmetry. *J. Cell Biol.* **112,** 225–235.

Kedersha, N. L., Miquel, M.-C., Bittner, D., and Rome, L. H. (1990). Vaults. ll. Ribonucleoprotein structures are highly conserved among higher and lower eukaryotes. *J. Cell Biol.* **110,** 895–901.

Kickhoefer, V. A., and Rome, L. H. (1994). The sequence of a cDNA encoding the major vault protein from *Rattus norvegicus*. *Gene* **151,** 257–260.

Kickhoefer, V. A., Searless, R. P., Kedersha, N. L., Garber, M. E., Johnson, D. L., and Rome, L. H. (1993). Vault ribonucleoprotein particles from rat and bullfrog contain a related small RNA that is transcribed by RNA polymerase lll. *J. Biol. Chem.* **268,** 7868–7873.

Kickhoefer, V. A., Vasa, S. K., and Rome, L. H. (1996). Vaults are the answer, what is the question? *Trends Cell Biol.* **6,** 174–178.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227,** 680–685.

Melese, T., and Xue, Z. (1995). The nucleolus: an organelle formed

by the act of building a ribosome. *Curr. Op. Cell Biol.* **7,** 319–324.

O'Farrell, P. H. (1975). High resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem.* **250,** 4007–4021.

Otto, J. J., Kane, R. E., and Bryan, J. (1979). Formation of filopodia in coelomocytes: localization of fascin, a 58,000 dalton actin cross-linking protein. *Cell* **17,** 285–293.

Rome, L., Kedersha, N., and Chugani, D. (1991). Unlocking vaults: Organelles in search of a function. *Trends Cell Biol.* **1,** 47–50.

Scheffer, G. L., Wijngaard, P. L. J., Flens, M. J., Izquierdo, M. L., Slovak, M. L., Pinedo, H. M., Meijer, C. J. L. M., Clevers, H. C., and Scheper, R. J. (1995). The drug resistance-related protein LRP is the human major vault protein. *Nature Med.* **1,** 578–582.

Scheper, R. J., Broxterman, H. J., Scheffer, G. L., Kaaijk, P., Dalton, W. S., van Heijningen, T., H. M., van Kalken, C. D., Slovak, M. L., de Vries, E. B. E., van der Valk, P., Meijer, C. J. L. M., and Pinedo, H. (1993). Overexpression of a *Mr* 110,000 vesicular protein in non-P-glycoprotein-mediated multidrug resistance. *Cancer Res.* **53,** 1475–1479.

Schneiter, R., Kadowaki, and Tartakoff, A. M. (1995). mRNA transport in yeast: Time to reinvestigate the functions of the nucleolus. *Mol. Biol. Cell* **6,** 357–370.

Schuurhuis, G. L., Broxterman, H. J., de Lange, J. H. M., Pinedo, H. M., van Heijningen, T. H. M., Kuiper, C. M., Scheffer, G. L., Scheper, R. J., van Kalken, C. K., Baak, J. P. A., and Lankelman, J. (1991). Early multidrug resistance, defined by changes in intracellular doxorubicin distribution, independent of P-glycoprotein. *Br. J. Cancer* **64,** 857–861.

Schwarzacher, H. G., and Wachtler, F. (1993). The nucleolus. *Anat. Embryol.* **188,** 515–536.

Shaw, R. J., and Jordan, E. G. (1995). The nucleolus. *Annu. Rev. Cell Dev. Biol.* **11,** 93–121.

Sidebottom, E., and Harris, H. (1969). The role of thenucleolus in the transfer of RNA from nucleus to cytoplasm. *J. Cell Sci.* **5,** 351–364.

Stephens, R. E. (1970). Thermal fractionation of outer fiber doublet microtubules into A- and B-subfiber components: A- and B- tubulin. *J. Mol. Biol.* **47,** 353–363.

Suprenant, K. A. (1993). Microtubules, ribosomes, and RNA: Evidence for cytoplasmic localization and translational regulation. *Cell Motil. Cytoskeleton* **25,** 1–9.

Suprenant, K. A., and Marsh, J. C. (1987). Temperature and pH govern the self-assembly of microtubules from unfertilized sea-urchin egg extracts. *J. Cell Sci.* **87,** 71–84.

Suprenant, K. A., Dean, K., McKee, J., and Hake, S. (1993). EMAP, an echinoderm microtubule-associated protein found in microtubule-ribosome complexes. *J. Cell Sci.* **104,** 445–456.

Suprenant, K. A., Tempero, L. B., and Hammer, L. E. (1989). Association of ribosomes with in vitro assembled microtubules. *Cell Motil. Cytoskeleton* **14,** 401–415.

Tani, T., Dearby, R. J., Hiraoka, Y., and Spector, D. L. (1995). Nucleolar accumulation of poly(A)+RNA in heat-shocked yeast cells: Implication of nucleolar involvement in mRNA transport. *Mol. Biol. Cell* **6,** 1515–1534.

Tiwari, S. C., and Suprenant, K. A. (1993). A pH- and temperature-dependent cycling method that doubles the yield of microtubule protein. *Anal. Biochem.* **215,** 96–103.

Towbin, H., Staehelin, T., and Gordon, J. (1979). Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. *Proc. Nat. Acad. Sci. USA* **76,** 4350–4354.

Unwin, P. N. T., and Miligan, R. A. (1982). A large particle associated with the perimeter of the nuclear pore complex. *J. Cell Biol.* **93,** 63–75.

Vasu, S. K., and Rome, L. R. (1995). *Dictyostelium* vaults: Disruption of the major proteins reveals growth and morphological defects and uncovers a new associated protein. *J. Biol. Chem.* **270,** 16588–16594.

Vasu, S. K., Kedersha, N. L., and Rome, L. H. (1993). cDNA cloning and disruption of the major vault protein a gene (mvpA) in *Dictyostelium discoideum*. *J. Biol. Chem.* **268,** 15356–14360.

Xue, Z., and Melese, T. (1994). Nucleolar proteins that bind NLSs: A role in nuclear import or ribosome biogenesis? *Trends Cell Biol.* **14,** 414–417.

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