Biochemistry

 Volume 41, Number 49 December 10, 2002

Current Topics

Vault Ribonucleoprotein Particles: Sarcophagi, Gondolas, or Safety Deposit Boxes?†

Kathy A. Suprenant*

Department of Molecular Biosciences, University of Kansas, Lawrence, Kansas 66045

Received August 27, 2002; Revised Manuscript Received September 25, 2002

In 1896, the great architect Louis Henri Sullivan asserted, "Form ever follows function". Although his dictum has become somewhat cliché, the maxim is applicable, even at the molecular level, where form and function are intricately intertwined. For example, the barrel-like nuclear pore complex, embedded in the nuclear envelope, provides a portal for the selective transport of macromolecules into and out of the nucleus (*1*). In bacteria and higher eukaryotes, proteins are actively folded within the cavity of another barrel-like structure known as the GroEL and GroES chaperonin complex (*2*). Moreover, the proteolysis of ubiquitin-linked proteins occurs within the hollow interior of a bicapped hollow barrel known as the 26S proteosome complex (*3*). The commonality of these barrel-shaped containers is that they sequester macromolecules for diverse functions.

In 1986, Nancy Kedersha and Leonard Rome purified, from rat liver homogenates, a beautiful and enigmatic barrel-shaped organelle called the vault ribonucleoprotein particle. Vaults were so named because, in negatively stained samples for electron microcopy, the organelle resembled the vaulted ceilings of medieval cathedrals (*4*−*7*). Their unique structure is conserved among higher and lower eukaryotes (*8*−*10*), and at 12.9 ± 1 MDa, vaults are the largest ribonucleoprotein particles known (*11*).

Vaults are abundant, with over 10^4 particles per differentiated cell (*12*) and over 10^7 vaults per embryonic cell (*9*). In addition, selected tumor cells appear to have many more vaults than their normal counterparts. For example, glioblastomas and anaplastic astrocytomas express high levels of the major vault protein (MVP)[1] when compared to normal human astrocytes (*13*). Furthermore, certain cancerous cell lines that have become resistant to a wide variety of structurally unrelated xenobiotics are rich in vaults (*12*, *14*−*18*). Whether vault particles are necessary or sufficient for multidrug resistance is currently under consideration (*15*−*17*, *19*, *20*).

Vaults also appear to be involved in the complex pathways of growth and proliferation. Disruption of vault formation in the slime mold *Dictyostelium discoidium* reveals a growth defect (*21*, *22*). When placed under nutritional stress in a minimal medium, *mvp*$^-$ strains grow to less than one-third the density of wild-type cells. Initially, wild-type and mutant (*mvp*$^-$) cells double at the same rate; however, when the cell density reaches 10^6 cells/mL, the *mvp*$^-$ cell cultures reach a stationary growth phase. There is no difference in the ability of wild-type strains and deletion strains to undergo aggregation, form migrating slugs, or generate fruiting bodies. Under nutritional stress *mvp*$^-$ *Dictyostelium* appear to have a lower threshold for exiting the cell cycle.

† Supported by NSF Grant MCB-9982377 and NIH COBRE Grant 1P20RR15563 with matching support from the State of Kansas and the University of Kansas.

* To whom correspondence should be addressed. Tel: 785-864-4580. Fax: 785-864-5321. E-mail: ksupre@ku.edu.

[1] Abbreviations: cryo-EM, cryoelectron microscopy; hVR, human vault RNA; LPS, lipopolysaccharide; LRP, lung resistance-related protein; MVP, major vault protein; MDR, multidrug resistant; NACHT, neuronal apoptosis inhibitor protein (*NA*IP), MHC class II transcription activator (*C*IITA), Podosporina anserina incompatibility locus protein (*HE*T-E), and the telomerase-associated protein (*T*EP1/TP1) domain; NAD^+, nicotinamide adenine dinucleotide; NPC, nuclear pore complex; RNP, ribonucleoprotein particle; TEP1, telomerase-associated protein; WD, tryptophan aspartate; VIT, vault inter-α trypsin domain; VPARP, vault-associated poly(ADP-ribosyl)ating polymerase; VWF, von Willebrand factor.

10.1021/bi026747e CCC: $22.00
Published on Web 10/23/2002

A recent study suggests that vaults are critical for dendritic cell differentiation and maturation (*23*). Immature dendritic cells are rich in peripheral tissues where they capture antigens for presentation to naive T-cells in the lymphoid tissues. Vault levels are dramatically increased when immature dendritic cells are cultured in the presence of lipopolysaccharide (LPS), a cell wall component of Gram-negative bacteria that can induce septic shock (*23*). Moreover, endocytosis of anti-MVP antibodies in the presence of LPS leads to a reduction in the viability of these monocyte-derived dendritic cells. The internalization of the anti-MVP antibodies also reduces the expression of distinctive cell surface markers, CD1a, CD86, CD83, and CD54, that appear during dendritic cell maturation (*23*). Therefore, vaults may be involved in the cellular stress response to endotoxins.

The abundance and conservation of vault structure among evolutionarily divergent eukaryotes indicate that vaults may have an important general cellular function in fighting infections, resisting chemotherapy, and surviving nutritional stress. Although the cellular function of vaults is unknown, it is likely that the barrel-like form of the vault has evolved from the way it performs its function.

VAULT STRUCTURE

Vaults were first observed by negative staining for electron microscopy as small ovoid contaminants in rat liver coated vesicle preparations **(***4*, *5***)**. Their subsequent purification revealed that these particles have a unique barrel-shaped structure unlike coated vesicles **(***6*, *7*, *24***)**. Individual vaults are uniform in size, shape, and overall morphology. In addition, vaults have the same distinctive appearance whether they are isolated from rat, rabbit, chicken, frog, electric ray, slime mold, sea urchin, mouse, or man (*4*, *9*, *10*, *24*, *25*).

Recently, the vault structure was determined by cryoelectron microscopy to approximately 32 Å resolution in the presence of the small vault-associated RNA and to 22 Å resolution in RNase-treated preparations (*26*, *27*). A three-dimensional image reconstruction reveals that vaults are smooth walled and hollow (Figure 1). Vaults are barrel-shaped with a bulging middle section demarcated by a central constriction. Each end of the central barrel terminates in a small protruding cap. The maximum overall dimensions of the vault RNP are 420 Å × 750 Å with each cap having a diameter of 240 Å. The smooth walls of the vault surround a central cavity with a volume of approximately 5×10^7 Å^3, sufficient in size to surround at least two intact ribosome complexes (*28*).

The cryo-EM image reconstructions reveal asymmetric densities within the vaults (*27*). As many vaults appear to be empty, the irregular material imaged within a vault is not part of the vault structure per se but part of the vault cargo. For material to enter an intact vault there must be holes of sufficient size to permit entry, or vaults must have a mechanism to open and close. The walls of the intact vault appear to be solid; however, there are eight small holes (20 Å in diameter) arranged around the circumference where the caps meet the midsection of the vault in the higher resolution cryo-EM structure of the RNase-treated vaults (Figure 1D). Material could enter the vault through these small apertures. In addition, vaults could partially unfold to allow larger complexes to enter. The biomechanics of vault opening and closing can be inferred from freeze−etch images (*11*). When absorbed to polylysine-coated mica prior to freezing, vaults open up into two halves along their central constriction. Each half-vault unfolds into a "flower-petal" shape with eight rectangular petals attached to a central disk or ring structure (*11*). Vaults in various states of opening also are observed by cryo-EM (*27*). These observations indicate that vaults may be able to open and close in vivo.

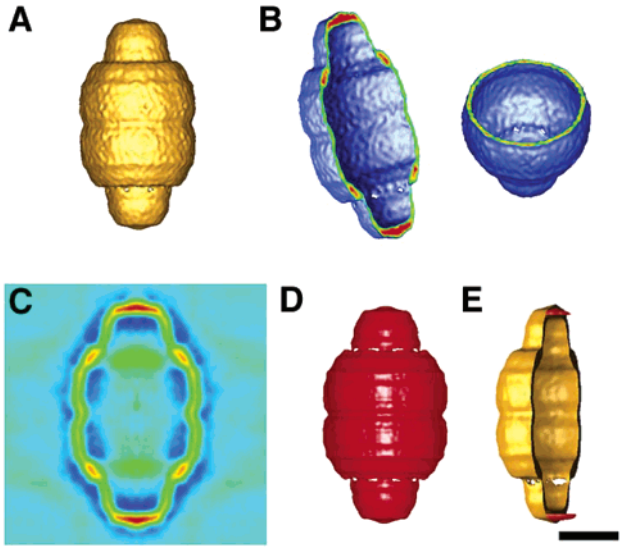

FIGURE 1: Three-dimensional cryo-EM reconstructions of the intact and RNase-treated rat vault. (A) An outer surface view of the intact rat vault reconstruction. (B) Cropped views of the intact rat vault reconstruction revealing the large internal cavity. The crop planes are displayed with the strongest density in red and the weakest density in green. (C) A central density slice from the intact rat vault reconstruction showing weak internal density (green) where the caps meet the barrel of the particle. (D) An outer surface view of the RNase-treated rat vault reconstruction. (E) Difference mapping between the intact and RNase-treated rat vault reconstructions revealing the location of the VAULT RNA (red) within the ends of the vault caps. The scale bar represents 250 Å. (A), (B), and (C) are reprinted from ref *27* with permission from Elsevier Science. (D) and (E) are reprinted from ref *26* with permission from Cambridge University Press.

Freeze−etch images of the open vault revealed two flower-petal half-vaults that appeared to be related by 2-fold symmetry, indicating dihedral 8-fold symmetry (D8 or 822) for the closed vault with an 8-fold axis along the long axis and a perpendicular 2-fold axis (*11*). In the cryo-EM study of the intact vault, cyclic 8-fold symmetry (C8), with only an 8-fold symmetry axis along the long axis of the vault, appeared to be the predominant symmetry for the particle. However, the authors indicate that the irregular material trapped inside the vault might contribute to the apparent asymmetry between the two halves of the vault (*27*). The later cryo-EM study of the RNase-treated vault indicated that the vault protein components in the absence of the vault RNA form a structure with strong D8 symmetry (*26*). This implies that the two halves of the vault are structurally identical with at least the major protein, MVP, packaged in a symmetrical manner.

VAULT COMPOSITION

By mass and by number concentration, proteins are the most abundant components of the vault particle; vault RNA components comprise less than 5% of the vault particle by

Table 1: Human Vault Components[a]

	size	accession no.	mapping	location in vault	proposed function
protein					
TEP1	2627 aa	NM_007110	14q11.2	cap complex	catalytic
VPARP	1724 aa	AF158255	13q11	cap complex	catalytic
MVP	893 aa	X79882	16p13.1-p11.2	barrel and cap	structural
RNA					
hVR1	98 nt	AF045143	5q33.1	cap complex	not structural
hVR2	88 nt	AF045144	5q33.1	cap complex	not structural
hVR3	88 nt	AF045145	5q33.1	cap complex	not structural

[a] GenBank accession numbers for nucleotide sequences and current chromosomal mapping data are shown for *Homo sapiens* vault components.

mass. For a summary of vault components, consult Table 1. Surprisingly, the complexity with respect to protein composition is rather low. Only three vault proteins have been identified. Mammalian vaults are composed of the 100 kDa major vault protein (MVP), a 193 kDa vault poly(ADP-ribosyl)ating polymerase (VPARP), and the 240 kDa telomerase-associated protein (TEP1) (*4*, *12*, *14*, *29*, *30*).

Major Vault Protein (MVP). Approximately 75% of the mass of the vault particle can be accounted for by MVP alone (*4*, *27*). The human MVP gene has 15 exons and is located on chromosome 16 (*14*, *31*). The promoter region is TATA-less and contains an inverted CCAAT box with putative SP1 and p53 binding sites (*32*). There is evidence for two mRNA isoforms that differ in 5′ leader sequences but do not differ in the final MVP coding sequence (*33*).

The conservation of vault morphology among a wide variety of organisms predicts that the MVP coding sequence should be conserved, and this is indeed what is observed. Full-length MVP orthologues have been identified in the trypanosome *Leishmania* (GenBank accession numbers for protein sequence, CAC14329), the bivalve mollusc *Mytilus* (AAD48063), the channel catfish (AAG00866), the electric ray (Q90405), and the slime mold *Dictyostelium* (P34118, P54659) as well as mammals including human, rat, and mouse (CAA56256, AAC52161, AAG43520). The primary sequence of the major vault protein is highly conserved. For example, mammalian and slime mold MVPs are approximately 60% identical over their entire coding sequence. It is puzzling that obvious MVP orthologues are notably missing from the yeast (*Saccharomyces*), the fruit fly (*Drosophila melanogaster*), and the nematode (*Caenorhabditis elegans*) databases.

Similar to the cytoskeletal proteins, tubulin and actin, MVP alone contains sufficient information to self-assemble into a polymer similar to the native structure. A stoichiometric model predicts that there are 96 copies of the MVP per vault (*11*), and the cryo-EM data corroborate this calculation (*27*). Remarkably, when the MVP alone is expressed as a histidine fusion protein in baculovirus-infected Sf9 cells, vault-like particles self-assemble in the cytosol of these insect cells, and intact vaults can be purified by sucrose density gradient centrifugation (*34*). The exogenously expressed vault particles are very similar in their overall structure to purified rat vaults and exhibit both the central barrel structure and the two caps. Although the caps are somewhat flattened when compared to endogenous rat vaults, the presence of caps indicates that some of the cap mass must be associated with the MVP.

In rats, the major vault protein is 898 amino acids in length with a theoretical molecular mass of 99531 and a calculated p*I* of 5.53 (*35*). The amino-terminal half of the mammalian MVP is comprised of seven vault repeats each approximately 50 amino acids in length (Figure 2). In addition, there are three pairs of internal repeats that overlap the vault repeats (repeat 1, aa 113−231 and aa 166−286; repeat 2, aa 58−225 and aa 281−439; repeat 3, aa 41−178 and aa 157−286). The function of each of these repeats is unknown. Within the carboxyl-terminal half of the MVP is a 150 amino acid region predicted to form a parallel two-stranded coiled coil similar to that found in myosins, tropomyosins, and keratins (intermediate filaments, types I and II). Indeed, in a yeast two-hybrid analysis, MVP molecules interact with each other through this coiled-coil domain (*36*). From the sequence analysis, it is inferred that the MVP performs a structural role and contributes to the physical properties of the vault. The vault particle is unusually stable and resists solubilization or denaturation in 2 M urea (*4*).

Purified MVP is a calcium-binding protein, and it is likely that this occurs at a single EF-hand domain located at the beginning of the third vault repeat (*36*). Usually proteins need to contain an even number of EF-hands to be calcium-binding proteins unto themselves. However, since MVP is capable of forming an oligomer with itself, a complex of two neighboring EF-hands may form the calcium-binding domain. For example, members of the penta-EF-hand protein family, which includes calpain domain VI, are capable of forming calcium-binding homodimers (*37*, *38*).

The tumor suppressor PTEN binds to MVP in a Ca^{2+}-dependent manner, and the EF-hand domain within MVP is both necessary and sufficient for this interaction (*39*). PTEN plays a pivitol role in signal transduction through dephosphorylation of the 3′-phosphoinosides (*40*). The physiological function of the PTEN and MVP interaction is not known at this time.

The primary sequence of the MVP also reveals numerous potential phosphorylation sites, so it is not surprising that MVP is a substrate for endogenous casein kinase II in CHO and PC12 cells (*41*) as well as protein kinase C in *Torpedo* electric organs (*10*) and tyrosine protein kinase in the electric ray *Discopyge* (*42*). The physiological response to these phosphorylation events is not known.

Vault-Associated Poly(ADP-ribosyl)ating Polymerase (VPARP). A yeast two-hybrid screen with a nearly full-length MVP sequence was used to identify the vault-associated PARP, and biochemical analyses confirmed that VPARP is an integral component of highly purified vaults (*43*). VPARP is a member of a larger PARP protein family that uses nicotinamide adenine dinucleotide (NAD^+) as a substrate to catalyze the addition of multiple ADP ribose moieties to a variety of protein targets (*44*). The founding member of the

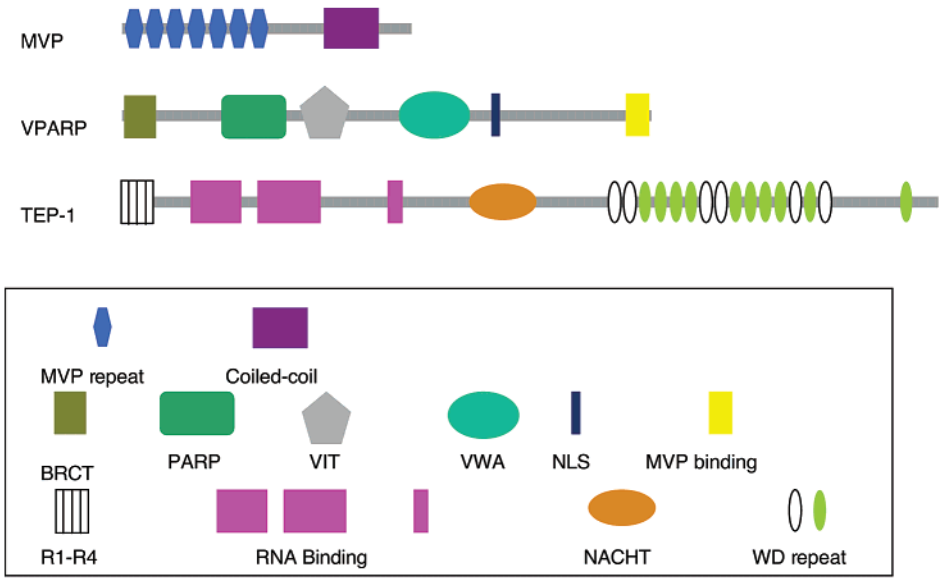

FIGURE 2: Schematic diagram of the major vault protein (MVP), vault-associated poly(ADP-ribosyl)ating polymerase (VPARP), and telomerase-associated protein-1 (TEP1). Several types of domains are found in these proteins: major vault protein (MVP) repeat; two-stranded coiled-coil domain; BRCT domain; poly(ADPribose)polymerase domain (PARP); vault protein inter-α trypsin domain (VIT); von Willebrand factor type A domain (VWA); nuclear localization signal (NLS); MVP-binding domain; N-terminal repeats (R1−R4); RNA-binding domain; NACHT-NTP domain; WD-repeat domain. Regions with a high probability of folding into a WD-repeat domain are shown in green. The coiled-coil domain was predicted by the COILS program using the MTK matrix derived from the sequences of myosin, tropomysosin, and keratin (*99*). Other domains were identified by the *p*rotein *fam*ily (PFAM) (*100*), simple *m*odular *a*rchitecture *r*esearch *t*ool (SMART) (*101*), and PROSITE (*102*) databases.

PARP family is PARP-1, a nuclear protein that detects DNA strand breaks that result from genotoxic stress (*45*). Currently, there is considerable interest in the PARP family because of connections between genome surveillance and several human diseases including cancer and aging. Similar to nuclear PARP-1, the VPARP catalytic domain retains poly-(ADP-ribosyl)ation activity and ribosylates itself as well as the MVP (*43*). The function of this unusual posttranslational modification of vaults is unknown; however, the electrostatic repulsion that results from the addition of a negatively charged ADP ribose polymer may control access to the vault interior through the vault caps.

Similar to PARP-1, a BRCT domain is present at the extreme amino terminus of the 1724 aa long VPARP (Figure 2). The BRCT motif was first identified in the breast cancer suppressor protein BRCA1 and is commonly found in proteins involved in cell cycle checkpoints that respond to DNA damage (*46*). The crystal structure of the human DNA repair protein, XRCC1, reveals that the BRCT domain can dimerize with itself or form a heterodimer with the BRCT domain in DNA ligase III (*47*). This allows for the possibility that VPARP may dimerize with other BRCT-containing polypeptides.

VPARP also contains a vault inter-α trypsin domain (VIT), a von Willebrand factor (VWF) type A domain, and a nuclear localization signal. The function of the VIT domain is unknown but is found in the heavy chains of the inter-α trypsin inhibitor protein family as well as a novel glycoprotein (*48*). VWF domains mediate protein binding through a metal ion-dependent adhesion site and occur predominantly in extracellular proteins, although VWF domains have been found in intracellular proteins as well as prokaryotes (*49*). The MVP binding domain is located at the carboxyl terminus of VPARP (*43*).

Telomerase-Associated Protein-1 (TEP1). A peptide sequence was used to identify the vault-associated p240 as the telomerase-associated protein TEP1 (TP1/TLP1), a polypeptide related to *Tetrahymena* telomerase-associated protein, p80 (*30*, *50*). TEP1 has an unusual structure with a large WD-repeat domain postulated to be involved in protein−protein interactions. Of the 16 candidate WD repeats identified in TEP1, 10 have a high probability of folding into a 4-stranded twisted β-sheet diagnostic of a WD repeat (Figure 2). TEP1 also contains an unusual NACHT-NTPase domain (*51*). The acronym NACHT was named after the five proteins used to define this domain: the neuronal apoptosis inhibitor protein (*NA*IP), MHC class II transcription activator (*C*IITA), Podosporina anserina incompatibility locus protein (*H*ET-E), and the telomerase-associated protein (*T*EP1/TP1).

TEP1 copurifies with vaults, and a yeast three-hybrid assay has shown that the vault RNA interacts with TEP1 in a sequence-specific manner (*30*). Cryo-EM has confirmed that a significant fraction of TEP1 is associated with the vault caps where it associates with vault RNA (*25*). It is likely that TEP1 performs a catalytic rather than structural function, since vault structure in TEP1-deficient mice is largely unchanged (*25*). For example, the NTPase activity of TEP1 may regulate access to the vault interior.

Mammalian TEP1 also interacts with the catalytic telomerase reverse transcriptase (hTERT) as well as telomerase RNA; however, TEP1 is not required for telomerase activity or maintenance of telomere length (*25*, *52*−*57*). Similarly, vaults do not appear to have telomerase activity (*25*). The function of TEP1 is not known; however, it is intriguing that this polypeptide is shared between two ribonucleoprotein complexes, vaults and telomerase.

Vault RNA. In addition to the proteins MVP, TEP1, and VPARP, vaults contain one or more small RNAs transcribed

by RNA polymerase III (*4*, *29*). In rats, a single vault RNA (rVR) of 141 nt copurifies with the vault particle (*29*), and in humans, four vault RNA genes have been characterized (*30*, *58*). Human genes (*hVR1*, *hVR2*, *hVR3)* are arranged in a tandem repeat on the long arm of chromosome 5 (5q33.1), and the pseudogene, *hVR4*, is located on the X chromosome at position Xp11.2. In all cases, vault RNA genes have internal polymerase III type 2 A and B box promoter elements. With the exception of *hVR4*, vault RNA genes have, in addition, an external type 3 TATA box and external proximal sequence elements (*58*). In rats, both the external and internal promoter sequences are necessary for transcription (*59*). hVR1, hVR2, and hVR3 are all coexpressed, albeit at different ratios, in cell lines derived from a variety of cells and tissues (*58*).

Each vault RNA (hVR1, hVR2, and hVR3) is found associated with the vault particle, although it is not known whether an individual vault particle contains all three RNAs or whether there are subsets of vaults with only a single species of vault RNA. Among vault particles, hVR1 predominates, indicating that vault RNAs are not equivalent (*58*). In addition, not all of the vault RNA is vault-associated. Each of the expressed vault RNA is detected in a soluble cytosolic fraction as well as the particulate vault fraction (*60*). These results indicate that vault RNA may have additional partners and cellular functions that are not vault-related (*25*, *60*).

Recently, Kickhoefer and colleagues (*60*) showed that a portion of the vault RNA in vivo and in vitro can be UV-cross-linked with the La/SS-B autoantigen. A significant amount of the La polypeptide is also found in purified vault fractions (*4*, *60*). La is an ~50 kDa polypeptide that is involved in the processing and assembly of untranslated RNAs into RNPs (*61*, *62*). In addition, La is associated with the RNA component of the human telomerase RNP complex (*63*). Antibodies against La immunoprecipitate the telomerase complex, and overexpression of La in immortalized cells results in telomere shortening (*63*).

The function of the vault RNA is a mystery. Overall, RNase-treated vaults are very similar in structure to intact vaults (*11*, *26*). A cryo-EM single particle reconstruction and difference mapping between intact and RNase-treated vaults reveal that vault RNA is located predominantly at the ends of the caps (Figure 1) (*26*). Vault RNA association with the caps is dependent upon the presence of TEP1. In a TEP1-deficient mouse, purified vaults have reduced mass at the caps, and vault RNA is no longer stably associated with vaults (*25*). Furthermore, the half-life of the vault RNA is dramatically reduced in the TEP1-deficient mice, indicating that TEP1 may protect vault RNA from degradation through either direct binding or an association with the vault particle. Surprisingly, the steady-state telomerase RNA levels and the association of telomerase RNA with the telomerase complex are unaffected in the TEP1-deficient mouse (*25*).

VAULTS BY DESIGN

The beauty of the vault design lies in how well it performs its function. But what is its function? The complex localization pattern of vault components suggests that vaults may function in both the nucleus and cytosol. The major vault protein (MVP) is located predominantly in the cytoplasm of most eukaryotic cells (*8*, *9*, *13*, *23*, *43*, *64*). A large fraction of VPARP colocalizes with MVP in the cytosol (*43*). However, antibodies against VPARP also reveal immunoreactive speckles within the nucleus that are not obvious with anti-MVP antibodies (*43*). During mitosis, a fraction of VPARP is concentrated at the mitotic apparatus, in the region of the central spindle and in some cases the astral arrays. MVP staining is largely excluded from the mitotic apparatus. It appears that VPARP and MVP have overlapping but distinct intracellular distributions. Importantly, VPARP staining is distinct from nuclear PARP-1 staining, and VPARP does not appear to relocate to the nucleus in response to UV-induced DNA damage (*43*). These results indicate that the in vivo function of VPARP is separable from nuclear PARP-1 (*44*, *45*).

Nucleocytoplasmic Transport of Ribonucleoprotein Particles. Immunofluorescence studies of rat fibroblasts have shown that a fraction (5%) of the MVP is associated with the cytoplasmic surface of the nucleus. Immunogold electron microscopy of isolated rat liver nuclei shows that the immunoreactivity is concentrated at the nuclear pore complex (NPC) (*65*). While the molecular architechture of the central plug of the NPC has yet to be determined, it is intriguing that the central plug is similar in size, symmetry, and overall structure to the vault particle (*27*, *66*, *67*). These similarities suggest that vaults may be able to dock with the NPC or displace the central plug to gain entrance to the nucleus. Whether intact vaults can enter the nucleus is unknown; however, the MVP is detected in isolated nuclei by immunoblotting (*9*, *65*, *68*). Moreover, within the nucleus the MVP appears to be concentrated in the nucleolus, raising the possibility that vaults may be transported to or assembled within the nucleolus (*9*). A general picture emerges where vaults are involved in some aspect of nucleocytoplasmic transport (*9*, *65*).

Kedersha and Rome (1986) (*4*) first suggested that the large size of a vault portended a complex function, and the association of a small vault RNA indicated that nucleic acids would likely be involved. The largest macromolecular complexes to exit the nucleus are the messenger ribonucleoprotein particles (mRNP) and ribosomal subunits. Importantly, the interior of the vault is of sufficient size to sequester two 80S ribosomes (*27*). Transport of RNPs from the nucleus to the cytosol is a complicated process involving a dynamic association and restructuring of many different proteins during transcription, splicing, nuclear pore transport, and polysome assembly (*69*, *70*). In many cells, specific mRNA complexes are transported over relatively large distances in the cytosol by microtubules and actin filaments (*71*−*74*). In light of these results, it is intriguing that sea urchin vaults copurify with a microtubule-associated complex of mRNAs, poly(A)-binding proteins, and polyribosomes (*9*, *75*). The colocalization of MVP to the nucleolus and the cofractionation of vaults with ribosomes led these authors to speculate that vaults are involved in transport and/or assembly of ribosomes or other RNPs.

Nucleocytoplasmic Efflux of Drugs. The lung resistance-related protein (LRP) was first identified as a protein that was overexpressed in non-P-glycoprotein multidrug-resistant tumor cells (*76*). Surprisingly, molecular cloning revealed that LRP was the human orthologue of the rat MVP (*14*). It was subsequently found that vault protein and RNA levels

are elevated in multidrug-resistant (MDR) cells (*12*, *14*, *19*, *20*, *77*−*83*). In addition, the level of vault organelles is dramatically increased in some MDR cells (*12*, *19*, *20*) and decreased in revertant cell lines (*12*). Overexpression of MVP is correlated with a poor response to chemotherapy, as well as reduced survival in selected human cancers (*15*, *84*−*87*). Moreover, reducing vault expression with hammerhead ribozymes produces colorectal carcinoma cells that are sensitized to a variety of cytotoxic agents (*16*, *17*). These observations led to a compelling hypothesis that vaults are an unrealized component of the multidrug resistance pathway (*14*, *15*).

A physical model has predicted that chemotherapeutic drugs are sequestered within the vault cavity or bound tightly to a vault component (*16*, *17*, *78*, *88*). For example, in drug-sensitive cells, the intrinsically fluorescent chemotherapeutic agent doxorubicin (adriamycin) accumulates in the nucleus. However, in MDR cells that overexpress MVP, doxorubicin is more abundant in the cytosol (*78*). In the case of mitozantrone-resistant cells, mitozantrone appears to accumulate in cytosolic vesicles of MVP-expressing cells (*88*). Together, these results have led to the idea that vaults may be involved in the nucleocytoplasmic or vesicular efflux of drugs.

It should be pointed out that not all studies support the tenet that vault overexpression predicts a poor response to chemotherapy. For example, the advance of acute myeloid leukemia is correlated with the expression of the breast cancer resistant gene (BCRP/ABCG2) but not the MVP gene (*89*). In addition, MVP expression may not be predictive for a response to chemotherapy in lung cancer patients (*90*). Clearly, there is much more to learn regarding vault expression in clinical samples and cancer patients.

Trafficking of Vaults through the Cytoplasm. Vaults are too large to freely diffuse within the cytosol (*91*); therefore, it is likely that vaults are transported through the cytoplasm through an association with the cytoskeleton. Immunofluorescent studies have shown that vaults are concentrated at cellular adhesion sites rich in actin filaments (*8*) and are highly enriched in the actin-rich cholinergic nerve terminals of electric rays (*92*). In addition to an association with actin filaments, vaults copurify with microtubules through repeated cycles of assembly and disassembly (*9*, *93*), and recombinant MVP colocalizes with microtubules in neuritic tips of PC12 cells (*64*). Furthermore, vaults appear to be transported bidirectionally within the axons of the electromotor neurons of the electric ray *Torpedo marmorata* (*94*). The initial rate of anterograde vault transport is estimated to be slower than the fast transport of synaptic vesicles but faster than slow axonal transport. It is not known whether vaults interact directly with a molecular transport motor or whether they are carried along the axon through an association with a vesicular structure. It is significant that MVP transport within the axon is associated with granular structures much larger than the size of a single vault particle, suggesting that vaults are either clustered together in the axon or associated with larger organelles or granules (*94*). It will be important to determine whether vaults are transported down the axon with specific mRNP particles.

CONCLUDING REMARKS

Although form and function are elaborately entangled, the vault structure is intriguing unto itself. The unusual size, shape, and stability of the vault particle invite speculation. For example, vaults may act as gondolas to carry macromolecular passengers to and from their cytosolic destinations. Instead of being suspended from a cable, as in a ski lift, these gondolas may motor along cytoskeletal railways, either through their own power or through that of a molecular motor such as kinesin, dynein, or myosin (*95*, *96*). In the absence of motor activities, cellular commodities within Venetian-style vaults may be pushed through aqueous channels by collisions with elongating cytoskeletal filaments (*97*, *98*).

The large size of the vault interior indicates that the cargo can be a large complex of macromolecules (*27*). The vault contents are unknown, although it has been suggested since their discovery that they are involved in some aspect of RNA biology (*4*) such as the nucleocytoplasmic transport (*65*) of RNPs (*9*). Besides nucleic acids, vaults could also accommodate complexes of regulatory kinases/phosphatases or transcription factors that need to be shuttled between the cytoplasm and nucleus.

Once inside the vault particle, are the macromolecules safe or in peril? For example, do vaults function as a Greek sarcophagus in which the limestone coffin was thought to consume the flesh of the corpses within? Why are vaults essentially empty as isolated? Have they gruesomely consumed their contents or have they lost their quarry? In contrast are vaults, as their name portends, safety deposit boxes where macromolecules can be stockpiled or hidden from sight?

The conservation of an abundant, predominantly cytosolic, vaulted organelle from slime molds to humans underscores their importance, and yet their function is illusive. For insight, we currently have to rely upon what we know of vault structure, the vault neighborhood, and the company that vaults keep. Certainly, some of these qualitative correspondences will prove misleading. Each vault-associated component (MVP, VPARP, TEP1, La, PTEN, and vault RNA) is intriguing and provides new pathways for discovery. Moreover, the overexpression of vaults in multidrug-resistant tumor cells and LPS response cells of the immune system and the association of vault components with the telomerase RNP are all indicators of an important function. In this regard, vaults may act to integrate the normal cellular pathways of growth and proliferation with the stress responses evoked by chemotherapeutic agents or endotoxins.

ACKNOWLEDGMENT

Many thanks to Drs. Valerie Kickhoefer, Lenny Rome, Phoebe Stewart, James Coffman, and Richard Himes for providing helpful comments regarding the manuscript.

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BI026747E