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Review

The vault complex

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Abstract. Vaults are large ribonucleoprotein particles found in eukaryotic cells. They are composed of multiple copies of a M_r 100,000 major vault protein and two minor vault proteins of M_r 193,000 and 240,000, as well as small untranslated RNAs of 86–141 bases. The vault components are arranged into a highly characteristic hollow barrel-like structure of 35×65 nm in size. Vaults are predominantly localized in the cytoplasm where they may associate with cytoskeletal elements. A small fraction of vaults are found to be associated with the nucleus. As of yet, the precise cellular function of the vault complex is unknown. However, their distinct morphology and intracellular distribution suggest a role in intracellular transport processes. Here we review the current knowledge on the vault complex, its structure, components and possible functions.

Key words. Vaults; MVP; VPARP; TEP1; vRNA.

Discovery of the vault complex

Vault particles were first observed in 1986 as contaminants in preparations of clathrin-coated vesicles from rat liver [1]. The ovoid vault particles displayed highly regular dimensions and possessed a complex barrel-shaped morphology. The structures were named vaults, a term that describes the morphology of the particles that contain multiple arches reminiscent of the vaulted ceilings in cathedrals. The vault particles are identified as 13-MDa ribonucleoprotein complexes with dimensions of approximately 35×65 nm. In fact, vaults are the largest ribonucleoprotein particles described to date [2, 3] and are approximately 3-fold larger than eukaryotic ribosomes and 10-fold larger than signal recognition particles (SRPs) or small nuclear ribonucleoproteins (snRNPs) [4]. An intriguing question is how these large particles remained undetected for so long. The reason is a technical one, as pointed out by Rome et al.

[5]. The commonly used stains for electron microscopy, the heavy metal salts osmium tetroxide and uranyl acetate, have a high affinity for charged components of membranes and nucleic acids, but particles like vaults with a high protein content and a relatively low amount of RNA are stained poorly. This results in nearly invisible vault particles when cells are examined by transmission electron microscopy using these positive stains. Vaults could only be visualized after purification and a negative staining procedure (fig. 1A). Structures with similar dimensions and morphology have been detected in numerous eukaryotic species as diverse as mammals, avians, amphibians, fish, echinoderms, mollusks, the slime mold *Dictyostelium discoideum* and protozoa [2, 5]. Nevertheless, vaults are probably not an essential and integral part of the eukaryotic cell in general, as they were not detected in *Saccharomyces cerevisiae* [6], *Caenorhabditis elegans*, *Drosophila melanogaster* and the plant *Arabidopsis thaliana*. That is, no clear vault protein orthologs could be detected in the genomes of these organisms.
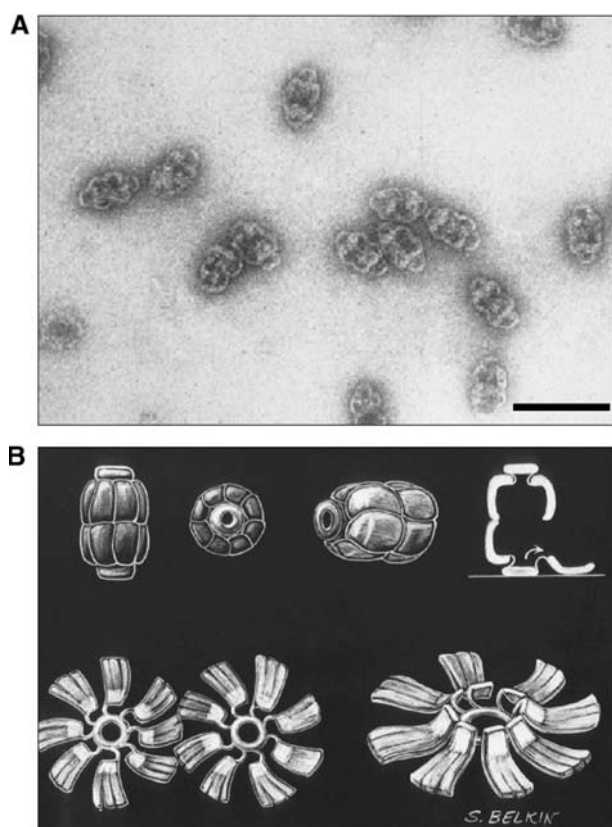
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Figure 1. Electron micrograph of vault particles and early vault model. (*A*) Electron micrograph of purified rat liver vault particles negatively stained with uranyl acetate. Bar corresponds to 100 nm. (*B*) Early schematic model of the vault complex showing its barrel-like structure, which can fold open into two 8-petaled flowerlike structures. The proposed stochiometry predicts each petal to be composed of six MVP molecules. The caps of the vault particle, in this model depicted as the ring holding the MVP petals together, were suggested to consist of the minor vault proteins and vRNA. Figures reproduced from the *Journal of Cell Biology* (1986) **103**, 699–709 and *Journal of Cell Biology* (1991) **112**, 225–235 by copyright permission of the Rockefeller University Press.

Vault components

Analysis of vaults from mammals revealed that the complex contains at least four distinct components [1], three high molecular weight proteins and several small untranslated RNA molecules. A M_r 100,000 protein, designated major vault protein (MVP), dominates the structure and constitutes over 70% of the molecular mass of the complex. Two additional proteins of M_r 193,000 and 240,000 comprise the minor vault proteins. The M_r 193,000 subunit was found to contain poly(ADP-ribose) polymerase (PARP) activity and was therefore named vault PARP (VPARP or p193) [7]. The M_r 240,000 subunit appeared to be identical to the telomerase-associated protein 1 (TEP1 or p240) [8]. This vault subunit is shared with at least one other ribonucleoprotein complex, the telomerase complex. The vault RNA (vRNA) accounts

for about 5% of the mass of vaults [1]. A stoichiometric model has been proposed where each vault particle is composed of 96 MVP molecules, 8 molecules of VPARP, 2 molecules of TEP1 and at least 6 molecules of vault RNA [9]. It was noted that the high molar frequency of MVP relative to the minor vault proteins is unlike the composition of other ribonucleoproteins, but is reminiscent of coated vesicle composition or the molecular redundancy observed in cytoskeletal structures, like microtubules and stress fibers, or in certain viruses. An interesting hypothesis – although as yet unsubstantiated – is that vaults may have originated from a viral endosymbiont [10].

Major vault protein

Following the cloning of the *MVP* complementary DNA (cDNA) from *Dictyostelium discoideum* and rat [11, 12], MVP orthologs were identified in numerous species [13–15]. The primary sequence of the various MVPs reveals a high degree of conservation, with an overall identity of ~90% between mammalian MVPs and a considerable identity (~60%) of mammalian MVPs with MVPs from most lower eukaryotes. Comparison of the murine and human *MVP* genes indicated that the genomic organization, including promoter elements, is also highly conserved [15]. The human gene for MVP, located on chromosome 16p13.1-p11.2 [16], is differentially expressed. This results in high MVP levels in lung, liver and intestines and relatively low levels in skeletal muscle and brain. Two alternative human splice variants have been described. An alternative splice acceptor site at the 3′ end of the first intron results in a longer MVP transcript with an additional open reading frame upstream of the regular initiation codon. The extra open reading frame may repress MVP translation and regulate MVP expression [17, 18]. However, such an alternative splice event does not seem to occur in mice [15].

Several distinct structural domains were identified within the human MVP (fig. 2). First, a long α-helical domain near its COOH-terminus (amino acids 652–800) forms a coiled-coil structure [19, 20]. In a yeast-based two-hybrid system, the coiled coil domain was found to be responsible for the interaction between MVP molecules and therefore essential for vault formation [20]. Deletion or partial deletion of the coiled coil completely abolished the interaction. A coiled-coil domain is present in all MVPs known, stressing the importance of this domain. Second, the N-terminal half of MVP contains at least five degenerated 45–50 amino acid repeats. Within this repeat structure two, and possibly three, calcium-binding EF-hands could be distinguished (amino acids 118–283). EF-hands are composed of two α helices separated by a loop structure that consists of about 12 amino acids, which are involved in binding calcium [21]. In vitro the
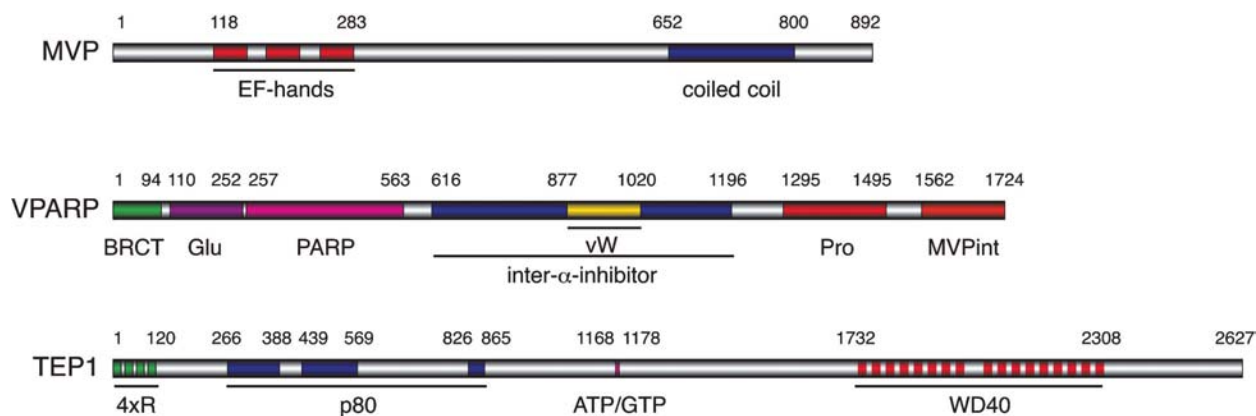
Figure 2. Architecture of the human vault proteins. Schematic representation of the three vault proteins: the major vault protein (MVP); the vault poly(ADP-ribose) polymerase (VPARP) and the telomerase-associated protein (TEP1). Indicated in different colors are protein domains with a putative functional and/or structural significance. MVP contains a degenerated repeat structure consisting of five stretches of ~50 amino acids. In at least three repeats putative calcium-binding EF-hands could be distinguished. A conserved coiled-coil domain is present in the C-terminal half of all MVPs described to date. In VPARP, a BRCT, PARP, von Willebrand (vW), inter-α-inhibitor domain and a region that interacts with MVP are indicated. In addition, a glutamate acid (Glu) and a proline (Pro)-rich domain could be identified. TEP1 contains a small domain consisting of four 30-amino acid repeats (4 × R) at its N terminus. In its C-terminal half a WD40 repeat structure is present. Furthermore, there is a region homologous to the *Tetrahymena* p80 telomerase protein (p80) and an ATP/GTP binding motif. The numbers correspond to the amino acid residues marking the start and end of the various domains.

MVP was able to bind calcium, in particular its N-terminal half [20]. Preliminary experiments indicated that calcium was necessary for the folding and assembly of MVP molecules into complete vault particles. Very recently, the tumor suppressor PTEN was found to interact with MVP and intact vaults [22]. Analysis of the interaction of MVP with PTEN in a two-hybrid setting mapped the interaction domain to amino acids 113–171 of MVP, which encompassed two EF-hands. Interestingly, in vitro binding experiments revealed that the association between MVP and PTEN required calcium.

Vault poly(ADP-ribose) polymerase
The M_r 193,000 minor vault protein VPARP was originally identified through its interaction with minor vault protein in a yeast-based two-hybrid system [7] and comprises an integral part of the vault complex. However, its subcellular distribution only partly overlaps with that of MVP, as shown in biochemical fractionation and immunofluorescence experiments [7, 23]. A non-vault associated fraction of VPARP is present in the cytoplasm and nucleus. In mitotic cells, VPARP was found associated with the mitotic spindle [7]. It is not yet clear whether the non-vault associated VPARP fulfills a completely separate function unrelated to vaults or whether there is a functional linkage.
The gene for VPARP is located on human chromosome 13q11 [24] and is heterogeneously expressed in human tissues, with the highest level of VPARP transcripts detectable in kidney. Human VPARP, also referred to as PH5P [25] and ADPRTL1 [24], contains a region of about 300 amino acids (amino acids 257–563) that exhibits a

28% sequence identity with the catalytic domain of poly(ADP-ribose) polymerase (PARP1). It was demonstrated that VPARP catalyzes the ADP ribosylation of itself and to a lesser extent of MVP [7]. Although the significance of VPARP activity for vault function is not yet known, it is interesting to note that assembled vault particles retain this enzymatic activity. Poly (ADP-ribosyl)ation of proteins is a reversible posttranslational modification that plays a significant role in the maintenance of genomic DNA stability (for review see Smith et al. [26]). So far, seven additional proteins with PARP activity have been described [27]. The prototype of this family is PARP1, which is a nuclear protein that binds to single- or double-strand DNA breaks. Upon binding, PARP1 activates its catalytic domain that transfers ADP-ribose groups from NAD$^+$ to itself and to proteins involved in maintenance of chromatin structure and DNA metabolism. The resulting delay in DNA replication permits the cell to recruit DNA repair enzymes to the site of the DNA break [28, 29]. Other members of the PARP family are also involved in DNA repair (PARP-2), whereas others act as telomere length regulators, like tankyrase 1 and 2. The identification and characterization of additional VPARP substrates may shed light on VPARP and/or vault function.
Several protein domains can be distinguished in human VPARP that relate to its PARP activity and interaction with other vault components (fig. 2). A region located at the N-terminal side of the catalytic PARP domain is rich in glutamic acid residues (amino acids 110–252), and may serve as an automodification site, analogous to sequences in PARP1. The presence of a BRCT (BRCA1 C-terminus) domain (amino acids 1–94) at the NH$_2$-termi-

nus of VPARP is another similarity between VPARP and PARP1. The BRCT domain [30, 31] in PARP1 is separated from the catalytic domain by ~145 amino acids, similar to the distance that separates these two domains in VPARP [7, 32]. Notably, the BRCT domain was first discovered in the BRCA1 genes and was found to define a superfamily of cell cycle checkpoint-DNA damage-response proteins. The BRCT domain is considered to be important for protein-protein interactions [33]. Nevertheless, it does not seem to play a role in the assembly of vault particles, since in a yeast two-hybrid system no interactions were found between the BRCT domain and the other vault proteins [20]. Instead, a domain at its COOH-terminus (amino acids 1562–1724) was found to mediate the association of VPARP with the N-terminal half of MVP [7, 20]. The proline-rich region (amino acids 1295–1495) at the N-terminal side of the MVP-interaction domain may serve as a flexible joint between the interacting domain and the enzymatically active part of VPARP. VPARP also contains a domain (amino acids 616–1196) that is common in a plasma glycoprotein family, the inter-α-inhibitor family [25]. Most inter-α-inhibitor family members are made up of so-called heavy chains, with one bikunin chain that harbors two protease inhibitory domains of the Kunitz type [34]. They all harbor a domain of about 160 residues in length, which is similar to a von Willebrand type A domain [32, 35]. Von Willebrand type A domains (amino acids 877–1020 of VPARP) are widespread in adhesive proteins and receptors [35]. This suggests a heterophillic binding capacity of VPARP for a polypeptide target.

Telomerase-associated protein 1

The M_r 240,000 minor vault protein was shown to be identical to the mammalian telomerase-associated protein 1 (TEP1). The role of TEP1 within the telomerase complex is not yet clear; however, it was demonstrated that TEP1 specifically interacts with the telomerase RNA [36, 37]. Telomerase activity is not dependent on TEP1, as telomerase activity can be reconstituted in vitro with just the catalytic protein subunit TERT and the telomerase RNA [38–40]. Furthermore, the disruption of the murine gene encoding *TEP1* did not result in a changed telomere length and telomerase activity [41]. The human *TEP1* gene was mapped by immunofluorescence in situ hybridization (FISH) to chromosome 14q11.2 [42]. Using a yeast-based three-hybrid system, it was shown that amino acids 1–871 of the murine TEP1 specifically associate with human vRNAs [8], analogous to the binding of telomerase RNA by this stretch of amino acids. No telomerase RNA could be detected within the vault complex, and vaults do not contain telomerase activity [8]. Possibly, the binding of either vRNA or telomerase RNA determines whether TEP1 associates with vaults or with the telomerase complex.

In the C-terminal part of TEP1 an extensive WD40 repeat structure is found (amino acids 1732–2308) encompassing 16 WD40 repeats (fig. 2). Such repeats are known for their ability to form β-propeller structures [43, 44], which are versatile protein-protein interaction domains. Molecular modeling of the WD40 repeats of TEP1 into a β-propeller resulted in a model that fitted the eightfold symmetry of the vault complex [9]. It was hypothesized that the TEP1 WD40 repeats imposed the observed symmetry on the vault complex. This theory was rejected when eightfold symmetric vault particles were isolated from *TEP1* knockout tissues. The NH$_2$-terminus of TEP1 contains four repeats of 30 amino acids whose function is still unknown. Furthermore, an ATP/GTP binding motif (WALKER A domain) was identified at amino acids 1168–1178, implying that TEP1 function requires energy. A detailed two-hybrid analysis was unable to map amino acid stretches in TEP1 that mediate the binding to the other vault proteins. Even co-expression of vRNA (hvg1), whose association with TEP1 may modulate its structure and interacting capabilities, failed to identify an association. Possibly several interacting vault proteins, complete vault structures and/or other proteins are necessary for TEP1 to bind vaults.

Vault RNA

The vault RNA constitutes less than 5% of the mass of the complex and is believed to be a functional rather than a structural component, as degradation of the vRNA did not affect vault morphology [1, 9, 10, 45]. Vault RNA has a species-specific length ranging from 86 to 141 bases, and the number of vRNAs expressed in various organisms differs. Rats and mice only express a single vRNA of 141 bases, whereas bullfrogs (*Rana catesbeiana*) express two vRNA species of 89 and 94 bases. Interestingly, humans harbor three related vRNAs (hvg1, hvg2 and hvg3) of 98, 88 and 88 bases, respectively. The hvg genes are arranged in a triple-repeat structure on chromosome 5, a situation that probably arose through gene duplication. The exact reason for the existence of multiple vRNAs in some species is unknown. One may speculate, however, that the functional range of the relatively long rodent vRNA is covered by multiple smaller vRNAs in other species. In all vRNAs the typical internal polymerase III promoter elements are highly conserved. Furthermore, all vRNAs are predicted to fold into similar stem-loop structures [45]. An intriguing observation, with respect to the function of the vRNAs, is the association of the vRNAs with the vault complex in several human cancer cell lines. It was shown that all three human vRNAs are bound to the vault complex, but not in a ratio that reflects their expression levels. Apparently, the individual human vRNAs have different affinities for TEP1. The bulk of vRNA associated with vaults was hvg1, and

only small amounts of hvg2 and hvg3 could be detected. Interestingly, in at least three multidrug-resistant cell lines consistently more hvg3 was found associated with the vaults compared with their drug-sensitive counterparts [46]. This suggests that the ratio in which vRNA species are associated to vaults may be of functional significance.

Vault structure

The structure of the vault complex was examined by various electron microscopical techniques [3, 9, 10]. Quantitative scanning transmission electron microscopy showed that vault particles contain two centers of mass [10]. Open and closed forms of the vault complex were observed using freeze etch techniques. The open forms resemble flowerlike structures in which eight rectangular petals are joined to a central ring. These flowerlike structures were usually seen in pairs, suggesting that an intact vault particle consists of two folded flowers (fig. 1B). It is unclear whether vaults can unfold and refold in this fashion in vivo. A more detailed structural vault model was generated using a cryoelectron microscopy image reconstruction technique (reviewed by Stewart et al. [47]). Basically, isolated vault particles were quickly frozen in liquid ethane and subsequently viewed by transmission electron microscopy. Images of individual vaults in various orientations were captured, and by combining multiple images, a high-resolution three-dimensional model could be generated (fig. 3). Striking features of the 31-Å resolution vault model are the smooth surface of the complex, the barrel-shaped mid-section and the two protruding caps [3, 9]. The barrel has an invaginated waist of 380 Å in diameter, and the two caps have a maximum diameter of 240 Å. The thickness of the walls of the hollow complex is about 20–52 Å, and the cavity ($\sim 5 \times 10^7$ Å^3) is spacious enough to enclose particles as large as ribosomes. In fact, often the cryoelectron micrographs showed extra density within the central barrel-shaped cavity of the vaults, implying the presence of a cargo inside the vault [3]. Note that in order to internalize or release large macromolecular cargoes, vaults would have to open up.

The particle reconstruction technique was also used to map the location of individual vault components within the complex. When RNase-treated and untreated vaults were compared, it was shown that the vRNAs are located in the tip of the cap structures [9, 10]. Analysis of vault particles isolated from *TEP1* knockout livers initially showed normal vault particles [48] displaying the characteristic eightfold symmetry. However, close examination showed a reduced electron density at the extreme ends of both cap structures, similar to that observed in RNase-treated vaults. This is in agreement with a role for TEP1
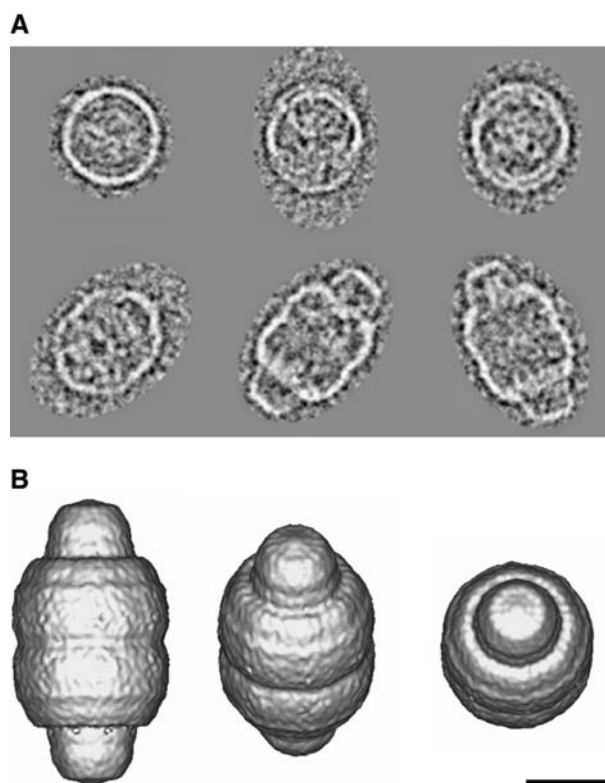

Figure 3. Reconstruction of the vault complex at a 31-Å resolution. (*A*) Cryoelectron microscopy images showing individual vault particles in different orientations. (*B*) Single-particle reconstruction techniques were used to generate a three-dimensional model of the vault complex. The combination of more than 1300 images resulted in the depicted vault model at 31-Å resolution. Bar corresponds to 100 Å. Figures reproduced from Kong et al. (1999) Structure **7**, 371–379 with permission from Elsevier Science.

in vRNA binding. The precise position of VPARP within the vault complex is currently under investigation, but it is thought to be located in the caps as well.

The initial idea that the barrel-shaped midsection consisted of MVP molecules and that the caps were completely composed of the minor vault proteins and vRNAs was abandoned when rat MVP was expressed in insect cells (Sf9), which do not contain endogenous vault particles or vault proteins [49]. Surprisingly, the expression of MVP alone resulted in the formation of particles that had the biochemical characteristics of normal vaults. They display the distinct vault-like morphology, including the protruding caps. The caps were only a bit misshapen and distorted.

Although the vault model implies a static structure, little or no information is available on the rigidity and dynamics of the vault particles and its components in vivo. For example, do vaults exchange subunits or are they present in a disassembled state in the cells ready to be assembled when needed? There are some observations reported in the literature suggesting that vaults in vivo are less rigid than the vaults isolated by differential and velocity su-

crose gradient centrifugation. First, vaults isolated from *Dictyostelium* amoebae seem to display more variability in shape and integrity [6]. Second, in purified vault preparations from rat liver the vRNA component is usually well protected against degradation by RNases, whereas in a crude microsmal extract containing vaults, vRNA is easily degraded. [6].

Intracellular localization of vaults

The number of vaults per cell has been estimated to be as many as 10,000–100,000 copies [50]. The majority of these reside in the cytoplasm where they may interact with cytoskeletal elements. Colocalization of vaults with the ends of actin stress fibers was reported in stationary rat fibroblasts [51] and in the tips of differentiated rat phaeochromocytoma (PC12) cells [52]. Likewise, vaults were found in close association with microtubules in PC12, chinese hamster ovary (CHO) and non-small-cell lung carcinoma cells [52–54]. Moreover, in sea urchin eggs vaults were in vitro copurified with microtubules and ribosomes through several cycles of polymerization and depolymerization [53]. Next to the cytoskeleton association, several groups reported an association of vaults with the nucleus, in particular the nucleoli, the nuclear membrane and/or the nuclear pore complex [53, 55, 56]. However, in general no more than 5% of the total vault fraction is found associated with the nucleus in mammalian cells.

The function of vaults

Despite the characterization of individual vault components and the development of a detailed structural model in recent years, the cellular function of vaults has still not been elucidated. Based on subcellular localization and the typical hollow barrel-like structure of the complex, a role in intracellular transport has been proposed by several investigators. The partial colocalization of vaults with cytoskeletal elements may indicate that vaults are transported along the cytoskeleton or that vaults are involved in cytoskeletal maintenance. Supporting evidence for vault-mediated cytoskeletal transport comes from studies in the electric ray *Torpedo* in which vaults were shown to be transported within axons between soma and nerve terminals [57, 58]. Immunoelectron microscopical analysis revealed a close association of vaults and synaptic vesicles in the nerve terminals of the electric organ. The authors hypothesized that material that has to be secreted associates with vaults and is transported via microtubules to the synaptic vesicles [19]. Cytoskeletal mediated transport would enable vaults to directionally shuttle cargo from and to specific cellular locations. However,

to convincingly demonstrate the existence of such a transport system, additional studies are necessary addressing vault dynamics, for instance by investigating the effects of actin- and microtubule-affecting drugs, the energy dependence of transport and the involvement of molecular motors.

The idea of vaults taking part in a nucleocytoplasmic transport route was based on observations in rat fibroblasts in which part of the vaults were found to be associated with the nuclear membrane often in close proximity to the nuclear pore complex [55]. The initial suggestion that vaults were in fact the elusive central plugs is probably not correct. The central plug is now generally regarded as material in transit through the nuclear pore rather than as a separate physical entity (see for example Stoffler et al. [59]). The observations made by Chugani et al. [55] may represent vaults docking at the cytoplasmic site of the nuclear pore complex in order to take up or give off cargo. A possible cargo might be ribosomes, since MVP was copurified with ribosomes in developing sea urchin embryos. Furthermore, Abbondanza et al. [56] found that MVP coimmunoprecipitated with the human estrogen receptor and that treatment of cells with estradiol increases the level of MVP associated with estrogen receptor in nuclear extracts. The putative vault-cargoes do not necessarily have to be inside the vault complex, but may also be stuck to the outside of the complex.

Vaults and multidrug resistance

The molecular identification of the lung resistance-related protein (LRP) as the human MVP linked the vault complex to multidrug resistance (MDR) [60]. LRP was originally found to be overexpressed in a non-small-cell lung cancer cell line selected for doxorubicin resistance that did not overexpress P-glycoprotein [61]. In subsequent studies it was found that MVP/vaults were overexpressed in many human tumor cell lines characterized by a MDR phenotype [23, 50, 62–64]. Moreover, MVP expression closely reflected known chemoresistant characteristics in a broad panel of unselected tumor cell lines and untreated clinical cancers of different histogenetic origins [61, 63, 64]. A number of clinical studies identified MVP as an independent prognostic factor for poor response to chemotherapy in various malignancies (see for review Scheffer et al. [65]). In favor of a role for vaults in cellular detoxification is the fact that MVP is highly expressed in tissues that are chronically exposed to elevated levels of xenobiotics (e.g. bronchus epithelium), metabolic active tissue (e.g. adrenal cortex) and macrophages [63].

Based on its putative transport function as well as the drug handling and cellular distribution of fluorescent anthracyclines in vault-expressing cell lines, it was postulated that vaults act by transporting drugs away from their

subcellular targets. Evidence supporting a role for vaults in the extrusion of anthracyclines from the nuclei of resistant cells came from the group of Akiyama [66–68]. Treatment of the colon carcinoma cell line (SW620) with sodium butyrate induced vault expression and resulted in cells resistant to various cytostatic drugs. Expression of MVP-specific ribozymes led to the reversion of this drug resistance phenotype [67, 68]. Furthermore, isolated nuclei incubated with doxorubicin in the presence of anti-(MVP) antibodies retained more drug compared with the nuclei that were not treated with the antibodies. These experiments suggest that MVP/vaults are directly involved in drug transport and as such contribute to the MDR phenotype. In contrast, expression of MVP stably transfected in the ovarian carcinoma cell line A2780 led to increased numbers of vault particles, but failed to confer drug resistance to etoposide, doxorubicin and vincristine [60, 62]. Siva et al. conclude that vaults may be necessary, but are not sufficient for drug resistance.

Knockout models

Several researchers attempted to determine the significance of vaults for cellular homeostasis and development by generating knockout models. In *Dictyostelium*, unlike the situation found in other organisms, three different but related *MVP* genes are present that code for MVPA, MVPB and MVPC of M_r 94,000, 92,000 and 92,000, respectively [11, 13]. Disruption of two (*MVPA* and *MVPB*) of the three *MVP* genes impedes growth under nutritional stress, suggesting a role for vaults in fundamental processes such as proliferation and cell survival. In mammals, the situation is different, as single genes code for the vault proteins. Up till now two knockout models have been generated in which either *MVP* or *TEP1* has been disrupted [41, 48, 69]. In both instances the mice were viable and healthy, breed normally and did not display obvious abnormalities. No distinguishable vault particles could be detected in *MVP* knockout tissues. In addition, the levels of the remaining vault components are dramatically reduced by the absence of MVP [M. H. Mossink et al., unpublished results]. In contrast, vault particles were still present in *TEP1* knockout tissues, but it was demonstrated that these were not only devoid of TEP1 but also of vRNA. It was found that TEP1 is required for a stable association of the vault RNA with the vault complex [48]. Recently, the generation of a *VPARP* knockout model was announced at a meeting [27]. Although the characterization of this model has not yet been reported, the mice are viable.

The *MVP* knockout has been exploited to test some of the proposed vault functions, in particular their involvement in multidrug resistance and in the development and/or function of dendritic cells. The sensitivity of MVP-defi-

cient cells to a panel of cytostatic agents was determined. It was found that both embryonic stem cells as well as bone marrow cells did not show increased sensitivity to these drugs when compared with wild-type cells [69]. It was shown that the activities of the multidrug resistance-related transporters P-glycoprotein, MRP1 and BCRP1 were not altered in vault-deficient cells, ruling out the possibility that these proteins compensate for the loss of vaults. The in vivo toxicity of doxorubicin in *MVP* knockout mice was also examined. Both knockout and control mice responded similarly to the drug treatment. It was concluded that – at least in mice – vaults are not directly involved in drug resistance. Recently, it was reported that MVP is upregulated during the development of human dendritic cells [70]. Moreover, the presence of MVP-specific antibodies, presumably interfering with the function of MVP or vaults, resulted in reduced expression levels of dendritic cell markers and costimulatory molecules and a decreased capacity to induce T-cell proliferative and interferon γ (IFN-γ)-releasing responses. However, in the *MVP* knockout mice, the development and function of dendritic cells, derived from mononuclear bone marrow cells, appeared normal [M. H. Mossink et al., unpublished results]. In addition, in vivo immunization assays showed that neither the T-cell-mediated immune response nor the T-cell-dependent humoral response were affected by the disruption of *MVP*, indicating intact antigen-presenting and migration capacities of the dendritic cells.

Future directions

Although our knowledge about the vault complex increased considerably over the years, we still lack insight into its cellular functions (see for a recent review Suprenant [71]). For future studies the developed knockout models will be highly instrumental in revealing the full significance of vaults. They provide investigators with model systems in which the effects of the absence of vaults or individual vault proteins on cellular and organ functioning can be directly studied. It might be worthwhile to intercross the various knockout mice available in order to generate a knockout in which all vault components are absent. At least such a mouse model would resolve the discussion about possible active remnants of the vault complex in various single knockouts. In this respect one could even think of disrupting the vRNA gene as well.

Another important line for future research is the identification of vault-interacting proteins. The vault components that have been identified and that are present in fixed stoichiometric amounts in highly purified vault preparations can be considered the core of the complex. In vivo, additional proteins may associate with the vault complex in a stable or transient manner depending on the

cell type and/or specific conditions. These interactions may be weak, and some of them may be lost during vault purification. Two examples of such vault-associated proteins have recently been described: the La RNA-binding protein [72] and the tumor suppressor PTEN [22]. Particularly, the significance of the vault-PTEN interaction is not yet clear. It is proposed that interaction with MVP might modulate PTEN activity. Clearly, vaults are not essential for PTEN function, as MVP-deficient animals are viable and show no apparent abnormalities, whereas PTEN disruption leads to embryonic lethality [73]. The activation of phosphoinositide 3-kinase kinase/Akt signaling pathway or tumorigenesis can be studied in the available knockout models. With regard to the relation of vaults and drug resistance, the major question is whether vaults are merely a marker of a drug-resistance phenotype or are directly involved in drug transport. Up till now few studies have addressed this question, with conflicting results. In particular, more research is necessary to verify the hypothesis that vaults mediate the efflux of drugs from the nucleus.

If vaults truly function as intracellular transport modules, then the identification of their cargo and the characterization of additional VPARP substrates may help to pinpoint its function. Equally interesting are more detailed studies into possible cross-talk and cooperation between vaults and the telomerase complex, particularly since it was recently reported that both VPARP and vRNA are also associated with the telomerase complex [27].

In coming years, as vault research progresses fully exploiting the various knockout models that have been developed, vaults will continue to surprise and to offer novel cell biological insights. After all, one can hardly imagine an organelle as unique as the vault complex having a solely ornamental role.

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