Cell. Mol. Life Sci. 66 (2009) 43 – 61
1420-682X/09/010043-19
DOI 10.1007/s00018-008-8364-z

Cellular and Molecular Life Sciences

Review

Vaults and the major vault protein: Novel roles in signal pathway regulation and immunity

W. Berger*, E. Steiner, M. Grusch, L. Elbling and M. Micksche

Medical University Vienna, Department of Medicine I, Institute of Cancer Research, Borschkegasse 8a, 1090 Vienna (Austria), Fax: +43-1-427765196; e-mail: walter.berger@meduniwien.ac.at

Received 27 June 2008; received after revision 25 July 2008; accepted 30 July 2008
Online First 19 September 2008

Abstract. The unique and evolutionary highly conserved major vault protein (MVP) is the main component of ubiquitous, large cellular ribonucleoparticles termed vaults. The 100 kDa MVP represents more than 70 % of the vault mass which contains two additional proteins, the vault poly (ADP-ribose) polymerase (vPARP) and the telomerase-associated protein 1 (TEP1), as well as several short untranslated RNAs (vRNA). Vaults are almost ubiquitously expressed and, besides chemotherapy resistance, have been implicated in the regulation of several cellular processes including transport mechanisms, signal transmissions and immune responses. Despite a growing amount of data from diverse species and systems, the definition of precise vault functions is still highly complex and challenging. Here we review the current knowledge on MVP and vaults with focus on regulatory functions in intracellular signal transduction and immune defence.

Keywords. Vault particles, major vault protein, signal pathway regulation, immunity, drug resistance.

Vaults and Their Components

Vault Particles. Despite their widespread expression in human and animal tissues, vaults were not discovered until 1986 by Kedersha and Rome [1]. This late appearance can be explained by the lack of vault visibility in standard electron microscopy (EM) preparations using stains which depict mainly membranes and nucleic acids [2]. However, the protein-rich vaults were readily recognizable in a negative stain EM as contaminants in clathrin-coated vesicle preparations from rat liver tissues. Based on the symmetric, barrel-shaped morphology resembling the ceilings of gothic cathedrals, the particles were termed "vaults" [3]. During subsequent years, vaults were characterised as the largest ribonucleoprotein particles (RNP) ever described. With an impressive mass of about 12.9 MDa and a dimension of around 41 x 41 x 71.5 nm, vaults leave far behind in size not only other RNPs but also well-known cellular particles like ribosomes. So what are the molecular components allowing the formation of such a large and fascinating cellular structure? In consecutive studies the group of Leonard Rome demonstrated that in mammals vaults are composed of three proteins: 1) the major vault protein (MVP, 100 kDa) constituting most of the particle mass; 2) the telomerase-associated protein (TEP1, relative MW 240 kDa); and 3) the vault poly(ADP)ribose polymerase (vPARP, relative MW 193 kDa). Additionally, vault preparations were demonstrated to contain several small untranslated RNAs (vRNA, 88–141 bases) [2, 4–6] (Table 1).

* Corresponding author.

Table 1. Vault Components and Interaction Partners.

Molecule	Characteristics	Locus	Vault position/ Interaction partner	Vault-related functions	References (selected)
Vault Components					
MVP	96 copies constitute the vault outer shell; completely assembled into vaults; no vault-unrelated functions known	16p11.2	vault barrel and cap - no partner necessary	structural; mediates binding to signal proteins	[10, 14, 27, 55, 109]
vPARP	weak poly(ADP) ribose polymerase; only partly associated with vaults	13q11	vault cap - direct binding to MVP N-terminal part	catalytic; poly-(ADP)-ribosylation of MVP and vPARP	[5, 77, 78]
TEP1	telomerase- and vault-binding protein; no essential role in telomerase	14q11.2	vault cap - direct binding to MVP p80 homology domain(?)	catalytic (?); assembly: targets vRNA to vaults	[6, 82]
vRNA	hvg1–3, hvg4 (not expressed); only partly associated with vaults	5q33.1 Xp11.22	vault cap - binding to TEP1	non structural; function not defined	[4, 90]
Vault interaction partners					
PTEN	tumor suppressor gene often mutated/deleted in cancer; major inhibitory phosphatase of PI3K pathway; main substrate: PIP3	10q23.3	MVP - N-terminus of MVP, Ca^{2+}-dependent	nuclear targeting of PTEN, enhanced nuclear functions	[28, 105, 106, 134]
SHP-2	protein tyrosine phosphatase; activator of RTK-mediated growth and survival signals	3q13.13	MVP - phosphorylation-dependent	promoting the EGFR-mediated MAPK-activity	[68]
Erk2	mitogen-activated tyrosine kinase; major downstream transmitter of RTK-mediated proliferation signals	22q11.21	MVP - phosphorylation-dependent	promoting the EGFR-mediated MAPK-activity	[68]
Src	(proto)oncogene; tyrosine kinase	20q11.2	MVP - phosphorylation-dependent	suppression of EGF-mediated MAPK activation	[67]
COP1	E3 ubiquitin ligase; depresses light signaling in plants; degrades c-Jun and p53 in vertebrate cells	1q25.1.-1q25.2	MVP - reduced following UV-induced MVP phosphorylation	binding modifies COP1 to enhance c-Jun degradation	[63]
Estrogen receptor	nuclear hormone receptor; induces ligand-dependent transcription of target genes	6q25.1	MVP - hormone-dependent	nuclear import and activation of the estrogen receptor (?)	[40]
La RNA-binding protein	binds and protects 3-prime UUU(OH) elements of newly RNA-polymerase III-transcripts	2q31.1	vRNAs	protection of vRNA	[95]

(?) Anticipated but not precisely defined.

Using techniques such as scanning transmission EM [7], cryo-EM [8, 9] and nuclear magnetic resonance (NMR) [10], the vault particle and the interaction of its main components were characterised (Table 1) depicting a barrel-like, natural "nanocapsule" with protruding caps, invaginating waist and covered by a distinct protein shell [8] (Fig. 1A). Ninety-six copies of the main vault component MVP are predicted to form the outer shell of the hollow particle. The copy numbers of the other components may vary and estimations range from 2–4 copies for TEP1 and 4–16 copies of vPARP with both proteins residing in the internal space of the barrel. Based on EM data, two and eight molecules of TEP1 and vPARP per vault, respectively, are most likely [11]. The symmetry of the whole vault particle is not entirely clear yet. Eight-fold symmetry imposing in cryo-EM suggested that the vault complex has an eight-fold dihedral symmetry [8]. Reconstitution of a recombinant vault with a cysteine-rich tag revealed a 48-fold rotational symmetry [9]. However, very recently rat liver vaults in a crystalline state were reported to be in 39-fold dihedral symmetry [12].

Vault particles have been identified from a wide array of species including, in addition to diverse mammals, evolutionary widely separated entities such as protozoa (e.g. slime mold *Dictyostelium discoideum*), molluscs and echinoderms (e.g. sea urchin *Strongylocentrotus pupuratus*), but also amphibians (e.g. *Rana catesbeiana*, *Xenopus laevis*), avians (e.g. *Gallus gallus*) and fish (e.g. electric ray *Torpedo marmorata*) [13–18]. A recent detailed sequence comparison extended this list to flatworms and trypanosomatides [19]. Surprisingly, vaults are missing in several other species popular in molecular research such as *Saccharomyces cerevisiae*, *Caenorhabditis elegans* and *Drosophila melanogaster* as well as in plants [17, 20, 21]. The high evolutionary conservation suggests a fundamental function linked to the nanocapsule structure of vaults; however, this assumption leaves open the question of similarities between *Saccharomyces, Drosophila* and *Caenorhabditis* cells apparently not needing vaults for life.

The Major Vault Protein MVP. Increasing evidence has accumulated that the responsible architect in charge of construction of the vault particles is one unique protein likewise highly conserved in evolution, namely the major vault protein (MVP) [8, 9, 20, 22, 23]. This notion is supported by the fact that practically all MVP molecules spontaneously form vaults without the need for other vault components [24]. MVP is capable of generating the minimal vault structure and the entire outer shell of the vault barrel [25]. Recently, the crystal structure of the recombinant vault shell at a solution of 9-A was generated, consequently placing it amongst the largest non-icosahedral particles ever crystallized [22]. The derived data are in agreement with those from isolated vaults suggesting that the outer shell of the vault particle is entirely made of MVP and represents a rather closed, smooth surface without any larger gaps or windows. Based on these observations, a cellular role of MVP distinct from that transmitted in the form of vaults is rather unlikely and there is currently no evidence suggesting such a function. Within the vault shell, the N-terminal region of MVP forms the particle waist with a part extending into the interior, while the C-terminus builds the cap as well as the cap/barrel junction. Consequently, fusion proteins or tags engineered to the N-terminus protrude into the inner particle space [26] and parts of the N-terminus are thought to account for the non-covalent interface between the identical particle halves [9].

The human MVP gene contains 15 exons coding for 893 aa and is localised to chromosome 16p11.2. MVP represents a unique 100–110 kDa protein with widely lacking homologies to other proteins but distinct conservation during evolution (e.g. around 90% homology within mammals and around 40 to 60% with lower organisms) [14, 16]. Several domains within the MVP molecule have been described (Fig. 1B), their functions analysed and a model of intra-and intermolecular interactions underlying vault formation proposed [27]. Most importantly, the highly conserved α-helical domain close to the C-terminus, functioning as a coiled coil domain, mediates interaction between the MVP molecule and thus vault formation [27]. Mutation of this sequence results in loss of vaults and distinctly altered subcellular localisation of MVP. Secondly, the N-terminal part of MVP was reported to contain at least two Ca^{2+}-binding EF hands. Indeed, MVP is able to bind Ca^{2+} predominantly via its N-terminus which may be necessary for correct folding and subsequently particle formation. Interactions with other proteins such as Phosphatase and Tensin Homolog Deleted On Chromosome 10 (PTEN) is mediated via the proposed EF hand domain and modulated by calcium [28]. However, recent substructure determinations by NMR did not confirm these EF hands and suggested alternative Ca^{2+}-binding mechanisms such as coordination by the large number of acidic residues in the long β-strand loops of multiple MVP domains [10].

Recently, Suprenant at al. have suggested – based on extensive sequence and structure comparisons – that MVP is related to the toxic anion resistance protein (TelA) family of prokaryotes based on a shared fold consisting of a three-stranded antiparallel β-sheet [19]. A similar structure builds up the vault wall and

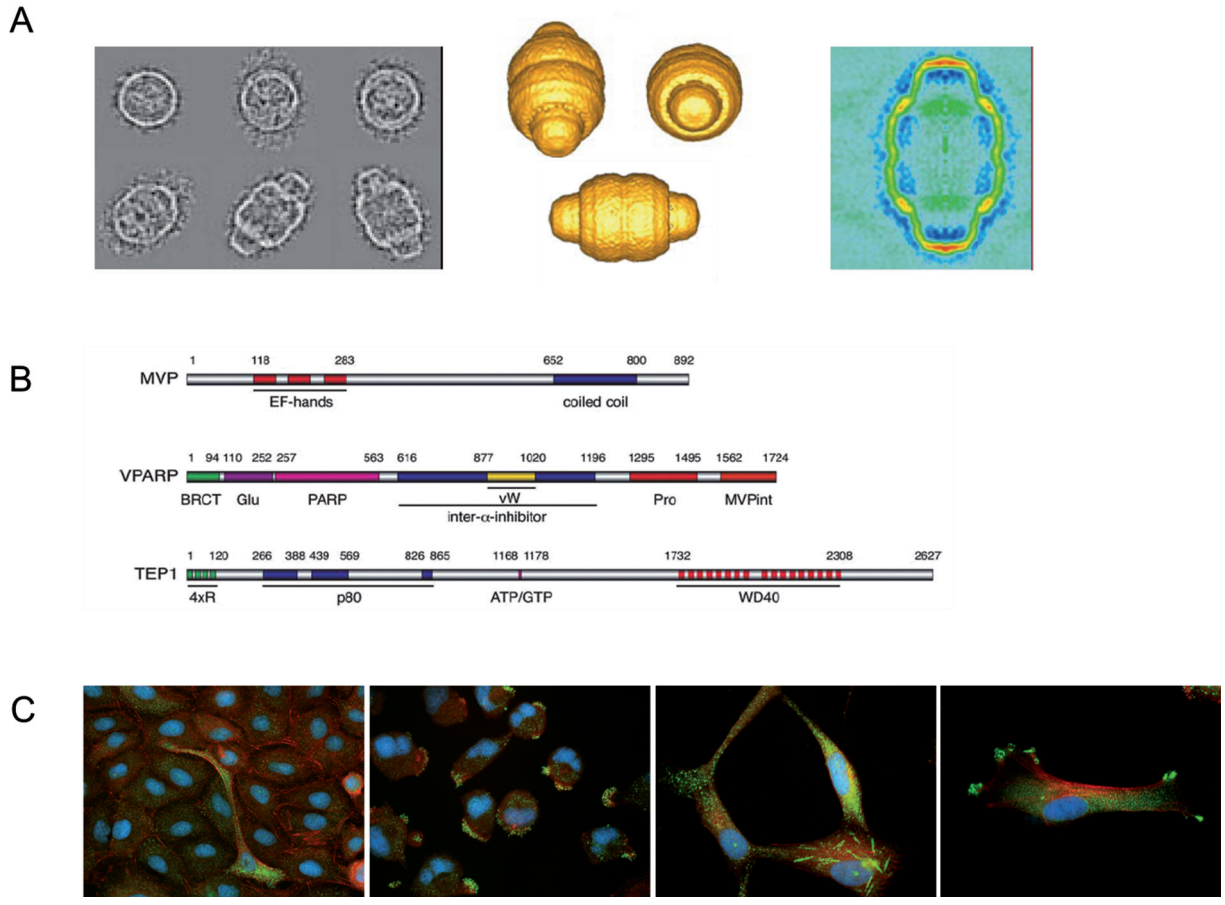

Figure 1. The wonderful vault world: (*A*) Cryo-EM images of vault particles in various orientations (*left*), three dimensional reconstruction with imposed cyclic eightfold symmetry from different perspectives (*middle*) and a central slice from the intact vault reconstruction indicating a central mass within the particle where the cap meets the barrel (*right*). All parts in (*A*) are reprinted from [8] with permission from Elsevier Science; (*B*) The three human vault proteins and their structural and functional important protein domains. The figure is taken from reference [21] with permission. For explanations of the respective domains *see text;* (*C*) Immunofluorescence staining of vaults (*green*) in the lung cancer cell line A549 either untreated (*first from left*) or treated with the differentiation-inducing agent phorbol 12-myristate-13-acetate for 12 h (*second from left*). Note the intense relocation of vaults to defined membrane loci. MVP immunofluorescence staining of a glioblastoma cell line treated with daunomycin for 24 h (*third from left*) demonstrates the appearance of "vault tubes" in one of the cells. The *right panel* shows the cellular distribution of vaults after expression of an MVP-GFP construct in a human glioblastoma cell. In all images DNA is stained by DAPI (*blue*) and actin filaments by TRITC-phalloidin (*red*).

the central barrel as revealed by the solution NMR structure of the respective conserved MVP two-repeat domain [10]. Both, the MVP repeat and the putative TelA homolog display reasonable similarity with S-layer paracrystalline bacterial surface coatings, suggesting a common ancestry [19].

MVP is an abundant protein with expression levels allowing formation of up to 10^5 vaults per mammalian cell [29] and 10^7 per mature sea urchin egg [30]. Despite the widespread expression, MVP levels seem to be strictly regulated. Diverse conditions characterised by upregulated MVP expression have been described including chemotherapy resistance [16, 29, 31–34], malignant transformation [35–37], senescence/aging [38], hyperthermia [39], estradiol treatment [40], as well as short-term exposure to diverse antineoplastic and/or differentiation-inducing drugs [41–46]. MVP expression is also stimulated in response to certain cytokines like interferons [47, 48] while others, including TNFα, potently suppress MVP expression [49]. Consequently, several research groups have addressed the mechanisms underlying the expression regulation of MVP at the level of gene dose, promoter activation, as well as mRNA and protein stability.

Overexpression of genes mediating multidrug resistance (MDR) like the ABC drug transporters ABCB1 (mdr1) and ABCG2 (breast cancer resistance protein) is often mediated by gene amplification, especially following *in vitro* drug selection [50–52]. So far, comparable data for the MVP gene have only been reported in one fibrosarcoma cell line selected against doxorubicin [53]. In contrast, MVP gene amplification was missing in several other MVP-overexpressing

MDR cell models even when harbouring ABCC1 (MRP1) amplification in close vicinity at chromosome 16p [42, 53–55]. Additionally, MVP expression is reduced but not activated in myxoid liposarcomas containing a specific chromosome 16p11 break close to the MVP locus [56]. These data suggest that the MVP gene is not prone to be amplified during drug selection, or at least not to a comparable extent as ABC drug transporter genes.

The human MVP gene promoter, like the murine one [15], is TATA-less and lacks other core promoter elements [44, 57]. Deletion analyses revealed an activating core sequence close to the transcription start point and additional upstream inhibitory regions. The core promoter sequence contains several putative transcription factor binding sites including consensus sequences for p53 and STAT1, an inverted CCAAT box, a GATA box, an E box and a GC box element [44, 57]. Several observations suggest that activation of transcription via usage of these binding sites might be central to MVP expression stimulation. Thus we have shown that SP1 transcription factors are involved in basal and histone deacetylase (HDAC) inhibitor-induced MVP expression [44]. A STAT1-binding site (GAS element) in the human promoter was demonstrated to be responsible for interferon γ-activated MVP expression [48]. Stein et al. have shown that activation of MVP expression by the anticancer drug 5-FU is based on enhanced binding of the Y-box-binding protein 1 (YB-1) to the inverted CCAAT box [43]. In this study a strong correlation between YB-1 and MVP expression in colon cancer was also described. This is corroborated by a significant correlation between nuclear YB-1 and MVP expression recently reported for breast [58] and ovarian cancer [59]. In the case of p53 we have found distinctly differing impacts of wild-type and mutant protein on MVP promoter activity, suggesting a functional p53-binding site in the MVP promoter (unpublished results). In summary these data suggest that MVP transcription is controlled at least in part by several transcription factors involved in cell development and differentiation but also malignant transformation. Based on the fact that several HDAC inhibitors activate MVP transcription, Emre et al. additionally assumed a general role of chromatin remodelling in MVP expression regulation [42].

Several studies suggested that the transcriptional activation of the MVP promoter is not sufficient to explain the upregulation of MVP expression in response to diverse stimuli [48, 54]. Thus it was postulated that posttranscriptional regulation of MVP expression, for example via stabilisation of the MVP mRNA [54], may support protein expression. Additionally, we have shown that MVP mRNA is spliced into two transcripts differing only in the 5-prime untranslated region [60]. Interestingly, the longer variant contains a short upstream open reading frame completely repressing translation of MVP. Thus changes in alternative splicing events can additionally regulate MVP expression.

Generally, vault-incorporated MVP is a long-living protein with a half-life of at least three days [61]. This might explain why a relatively minor increase in transcription can lead to significantly enhanced protein levels. Additionally, pulse-chase analyses in interferon-treated cells suggested that protein stability varies under different cellular conditions [48]. Moreover, the maximum level of stimulated MVP expression seems to be controlled as MVP upregulation by diverse stimuli including interferon [48], phorbol esters [42], and drug-selection [29] could only be observed in cells with low to moderate endogenous MVP expression. The molecular factors underlying these regulatory events are completely unexplored as yet.

Knowledge on protein modifications regulating MVP stability is limited. An active degradation of MVP via the proteasome has been suggested based on the vault stabilizing effects of the proteasome inhibitors MG132 in porcine zygotes [62]. Accordingly, another ALLN inhibitor led to vault accumulation/aggregation in human HeLa cells [19]. Moreover, MVP was demonstrated to be contained in protein complexes with the E3 ligase Constitutively Photomorphogenic 1 (COP1) [63] known to regulate p53 and c-jun stability [64]. However, ubiquitination of MVP or other vault components has not been directly demonstrated so far and expression of COP1 dramatically increases rather than decreases MVP expression in HEK293 cells [63]. MVP is a phosphoprotein since it is tyrosine-phosphorylated, for example, by protein kinase C and casein kinase II [65, 66] as well as Src kinase [67]. Phosphorylation of MVP, which is assumed to be important for its cell signal regulating functions (*compare below*), has been demonstrated following UV-radiation [63] and EGFR-mediated signals [63, 67, 68]. Moreover, MVP is a substrate for dephosphorylation by the phosphatase SHP2 [68] and poly-(ADP)-ribosylation by vPARP [5]. However, the precise impacts of these protein modifications on MVP stability and/or assembly of the vault particle have not been conclusively determined so far.

vPARP. When analysing the minor protein components of the vault particle by a yeast two-hybrid approach, Kickhoefer et al. in 1999 identified the 193 kDa vault component as a novel poly-(ADP)-ribose polymerase (PARP) [5]. The vault PARP (vPARP) is number four of at least seven family members in

humans [69] with PARP1 being by far the best characterised protein [70]. PARPs are an ancient family of predominantly nuclear enzymes catalysing the covalent transfer of ADP-riboses from nicotinamide dinucleotide (NAD) to glutamic and aspartic residues on themselves and other protein substrates. Moreover, PARPs catalyse polymerisation to long branches of poly-(ADP)-ribose through glycosidic bonds. The resulting poly-(ADP)-ribosylation changes the interaction of the modified substrates with other proteins and DNA [71]. Poly-(ADP)-ribosylation has been implicated in the pathogenesis of cancer and inflammatory as well as neurodegenerative disorders by regulating, amongst others, genomic stability, transcriptional fidelity, energy metabolism and cell death [70]. Interestingly, outside of their catalytical domains, PARP family members lack strong homologies and contain multiple, different localisation and interaction domains again suggesting involvement of poly-(ADP)-ribosylation reactions in multiple cellular functions [72]. PARP1 has recently been suggested as a promising target for anticancer therapy and to improve chemotherapeutic responses by inhibiting repair processes [73].

The vPARP gene is located on chromosome 13q11 with 34 exons encoding 1724 aa [5]. Homology search also discovered, besides the enzymatically active PARP domain, a BRCA1 C-terminal (BRCT), an inter-alpha-trypsin and a putative van Willebrand type A domain (vWA) as well as a C-terminal interaction domain binding to MVP [21, 74] (Fig. 1B). vPARP is (though rather weakly as compared to other PARPs) catalytically active both as a single molecule as well as within the vault complex and poly-(ADP)-ribosylates itself and MVP [5]. However, the consequences of these modifications have not been elucidated so far. Several PARP family members have distinct functions in nuclear processes supporting DNA stabilisation and repair as well as telomere dynamics [70, 71, 75, 76]. Interestingly, vPARP shares with PARP1 the BRCT domain characteristic for multiple proteins involved in DNA damage-mediated cell cycle arrest. In contrast to PARP1, however, vPARP does not seem to be activated by DNA damage [5] and vPARP (-/-) mice have no obvious chromosomal instability phenotype [77]. Interestingly, vPARP (-/-) mice are hypersensitive to dimethylhydrazine-induced colon cancer and to a lesser extent urethane-induced lung carcinogenesis [78]. These data suggest that vPARP or vaults are involved in the repair of DNA damage caused, for example, by alkylating agents. However, the molecular mechanisms underlying these effects and a possible contribution of MVP/vaults are undefined as of now. Considering the predominant localisation of vaults in the cytoplasm and the presence of non-vault-associated vPARP in the nucleus and at the mitotic spindle [5], it is tempting to suggest vault-independent DNA-modifying functions of vPARP. Nevertheless, it has to be mentioned that the presence of MVP strongly enhances the half life of vPARP [16, 79] suggesting at least an indirect impact of vaults as a reservoir for other vault components. Considering the high mobility of vaults (*compare below*) they might also function as shuttle allowing rapid delivery of the vault components such as, for instance, vPARP to specific subcellular locations.

TEP1. While vPARP was described for the first time in association with the vault particle [6], the second minor vault protein component (240 kDa) turned out to be identical with the already known telomerase-binding protein TEP1 [80]. Although telomerase activity is associated with TEP1 immunoprecipitates, this protein is not necessary for proper telomerase function and is not a component of the core telomerase complex [81]. Accordingly, TEP1 (-/-) mice exhibit unaltered telomere lengths and are, comparable to vPARP and MVP (-/-) mice, fertile without any signs of apparent developmental defects [77, 82]. Vaults of TEP1 (-/-) mice contain less density within the very ends of the cap regions, suggesting its localisation there [9, 82]. TEP1 has been shown to interact with both telomerase RNA (hTR) [80] and several human vault RNAs [6] in yeast RNA-protein interaction assays. However, vaults neither contain telomerase activity nor do they associate with telomeres. vPARP has been shown to associate with a vault-independent telomerase activity mediated by binding to TEP1 [77]. This is interesting as several PARPs have a telomere length-regulating function. For example tankyrases (PARP5a and 5b) are known to poly-(ADP)-ribosylate and thus inhibit the telomere-binding protein 1 (TRF1) [83], a repressor of telomere elongation [84]. However, telomere lengths are unaltered in vPARP (-/-), TEP1 (-/-), as well as in double knock-out animals [77], excluding such a role for vPARP.

Several interesting conserved domains are found in the TEP1 gene (Fig. 1B) residing on chromosome 14q11.2 and coding for a 2629 aa protein [80]. The N-terminal part of TEP1 represents a *Tetrahymena* p80 homology region and is required for binding of both telomerase RNA and vRNA [85]. The two RNA species compete for binding to TEP1, which suggests an overlapping binding site. Bateman and Kickhoefer [74] have identified, by *in silico* analysis, a TROVE module as a common ribonucleoprotein element present in p80 of *Tetrahymena*, TEP1, the Ro60 protein component of the Ro RNP complex (containing Y RNA along with the LA autoantigen) [86], and several uncharacterised bacterial sequences. It is

unclear to date why vRNA but not telomerase RNA is targeted to the vault particle and vice versa in case of the telomerase complex. Surprisingly, TEP1 is not necessary for targeting telomerase RNA to the telomerase complex while vaults in TEP(-/-) mice are devoid of vRNA [82]. Moreover, vRNA levels are reduced in multiple organs of these mice, suggesting that TEP1-binding and/or inclusion into vaults stabilises vRNA. The mechanisms underlying the distinct differences between vRNA and telomerase RNA with respect to TEP1 are currently unknown.

Coexpression experiments of all vault components in vault-less Sf9 cells demonstrated that both vPARP and TEP1 incorporate into the particle either alone or in combination [9]. However, only a vPARP but not a TEP1 binding site has been found in MVP by a yeast-two hybrid approach [27] suggesting that intact vaults but not MVP monomers are able to form the respective binding site for TEP1 [9, 85]. In addition to vRNA-binding, the p80 homology region is sufficient to target TEP1 to the vault localising the respective interaction domain to this region [85]. Moreover, the vRNA interaction is not necessary for targeting TEP1 to the vault in the Sf9 expression system, suggesting a direct interaction with MVP. Besides the TROVE module, the p80 homology domain contains solely a vWA sequence which is also present in the vPARP gene [74]. Thus, one of the two domains must be responsible for the association of TEP1 with the vault particle which is independent of RNA binding. The evolutionary conserved VWA domains are Rossmann folds consisting of a β-sheet sandwiched by multiple α-helices. They are involved in the binding of metals, contribute to multi-protein complexes and are frequently found in proteins mediating cell adhesion like integrins but also in those residing in the extracellular matrix [87]. Additionally, removal of the vWA domain had an impact on vRNA binding to the TROVE module suggesting a complex interaction platform [85]. However, the functions of the vWA domains in both minor vault proteins need to be determined.

The C-terminus of TEP1 contains a 16-fold WD40 repeat region known, in many cases, to build propeller-like structures representing a platform for reversible binding and assembly of multiprotein complexes involving e.g. histones [88] and cell signalling molecules [89]. In cryo-EM, the finding of a 16-fold density ring at the top of the cap allowed modelling the WD40 repeat of TEP1 within this density [11]. WD40 repeats were suggested to exhibit structural functions while possible protein interaction partners in the case of TEP1 are unknown so far.

vRNA. Vaults are canonical RNPs thus containing in all species – in addition to proteins – untranslated small RNAs termed vault RNAs (vRNA). Comparison of RNAse treated and untreated vaults localised vRNA molecules to the cap region [11] whereby the numbers per particle and the nature of vRNA molecules seems to be variable and species-dependent [4, 90]. Mice and rats express only one 141 bases long vRNA. While the bullfrog transcribes two shorter vRNAs with 89 and 94 bases, humans express three vRNA genes (hvg 1–3) with 88–98 bases. The respective genes are located on chromosome 5p within a region of 16 kb suggesting that they are a consequence of a tandem duplication event [90]. Additionally, the human genome contains a fourth vRNA gene (hvg4) on chromosome X which does not seem to be expressed [90]. Very recently an additional non-coding RNA closely resembling vRNA has been described which is encoded at an intergenic locus on chromosome 5 [91]. vRNAs are transcribed by RNA polymerase III and the respective internal type 2A and B box promoter elements are highly conserved [4, 92, 93]. Additionally, an external promoter element type 3 TATA box and proximal sequence elements, necessary for transcription of the rat genes [93], are present in the expressed human vRNA genes but lacking in hvg4 [90]. Little is known about factors regulating vRNA expression. Recently it has been demonstrated, however, that infection with Epstein-Barr virus (EBV) strongly induces expression of vRNAs [91, 94] suggesting a role of vRNA and/or vaults in viral defence (*compare below*).

Although the vRNA genes of different species exhibit a relatively low degree of homology, the predicted secondary structures seem to be highly conserved [4, 90]. Studies with an insect cell expression system, however, indirectly indicated that the predicted secondary structures of vRNAs may not always be assembled in living cells [85]. Thus, *in vitro* transcribed vRNA only weakly interacted with the recombinant RNA-binding domain of TEP1 targeting vRNA to the vault particle *in vivo*. RNAse H mapping suggested that this low affinity might be based on the fact that the *in vivo* configuration of vRNAs could be more open as compared to the rather closed secondary structures of the *in vitro* transcribed vRNA (suggested also by thermodynamic models). Accordingly, mutations affecting base pairing in the central loop strongly enhanced the affinity of vRNA towards TEP1 [85]. *In vivo*, longer sequences of single-stranded RNA might be caused by binding to protein components like MVP or vPARP but probably also by La RNA-binding protein [95], another vRNA-binding protein (Table1). The highly abundant La RNA-binding protein interacts with the N-termini of many newly

synthesised small RNAs based on the recognition of the sequence UUU-OH which is produced by transcription termination by RNA polymerase III [96]. Also vRNAs do not appear to be further processed and thus contain the 3′polyuridylate tail recognised by the La protein. Thus, in addition to a loose association with vaults, La RNA-binding protein together with vRNA could be part of another yet undefined RNP [95].

vRNA represents only around 5 % of the particle mass and only around 20 % of vRNA is associated with vaults while the majority is localised in the soluble fraction. However, vRNA shifts towards the vault-bound fraction in drug-resistant cell lines [29]. While hvg1 is the predominant vRNA associated with vaults, all isoforms are found associated with the particles [29, 90]. The binding of vRNA species to vaults does not exactly reflect the expression levels and especially in drug-resistant cell models the vault-bound fraction of hvg3 was disproportionately high [90]. This led to the hypothesis that, dependent on the cellular functions, different vRNAs are bound to vaults. However, the regulating factors underlying these observations need to be determined and a defined function of vaults relying on the RNA component has not been identified so far.

Conformational States, Intracellular Localisation and Movements of Vaults

Freeze-etch EM demonstrated that vaults can change to an opened state resulting in a pair of flower-like structures with eight petals [7]. Recently, opening of recombinant vaults into this open conformation was described to be triggered by low pH [97]. However, it is unclear whether this state is occurring in living cells or represents an *in vitro* artefact. In protein–mixing experiments with recombinant MVP in Sf9 cells, Poderycki et al. [25] demonstrated that other vault components could be incorporated in already preformed vaults. Accordingly, a more rapid association of newly synthesised vPARP as compared to MVP with already preformed particles was demonstrated by pulse-chase and immunoprecipitation experiments [61]. This suggests that the exterior MVP shell of the vault is not a fixed, rigid structure but allows dynamic exchange processes.

Distinct differences in the main intracellular localisation of vaults in different cell types have been described and partly contradictory observations have been published even when comparable cell types were investigated by identical detection methods. This indicates that the cellular localisation of vaults might not only be cell type- and species-dependent but also variable and subjected to distinct changes in response to external stimuli (Fig. 1C). Generally, the majority of reports agree that at least in mammalian cells MVP/vaults are predominantly (>90 %) localised in the cytoplasm. These observations were comparably described based on MVP immunostaining, fluorescence-tagged MVP, and/or biochemical cell fractionation experiments [20, 21, 34]. Additionally, a subgroup of vaults was repeatedly reported to be localised to the nuclear envelope of unstressed human cells [16, 29, 41, 98–101]. Accordingly, based on photobleaching experiments van Zon et al. demonstrated that vaults can relocate from the cytoplasm to the nuclear envelope of non-small cell lung cancer cells [100]. Comparable observations in rat, sea urchin, and *Dictyostelium* cells led to the suggestion that vaults associate with the nuclear pore complex (NPC) [102, 103]. A close association between the NPC and vaults was recently proven by FRET analysis in *Xenopus* oocytes. This association was sensitive to the depletion of cisternal Ca^{2+} stores. A comparable observation has also been made before with respect to the yet unidentified, dynamic, central mass of the NPC, which most likely represents cargo in transit [104]. These data suggest in addition to the comparable size and symmetry of vaults and NPC, that at least occasionally vaults represent a central mass within the NPC [103].

In some investigations higher amounts of MVP were found within the nuclei not only in sea urchin but also in mammalian (including human) cells. For example, in the glioblastoma cell line U373, a 5 % subpopulation of vaults localised in the nucleoplasm in association with particulate structures [99]. Furthermore, in a stomach cancer cell line immunofluorescence images indicated strong accumulation of MVP within the nucleus [67]. Based on subcellular fractionation, almost equal amounts of MVP were detected in the cytoplasm and nuclei of human fibroblasts [38]. Some groups even suggested that important roles of MVP in several cellular processes could be based on a vault-mediated shuttle function between cytoplasm and nucleus including nuclear import of the tumor-suppressor molecule PTEN [105, 106] or the nuclear hormone receptors [40], as well as export of drugs [107, 108]. Interestingly, sea urchin vaults change their localisation during embryogenesis with increasing amounts in the nucleus from about the blastulation/gastrulation stage onwards, suggesting a role in cell differentiation. In adult cells, vaults localise nuclear with accumulation around the nucleoli [30, 109]. As this is the main region of ribosome synthesis/assembly and vaults were co-purified with ribosomes, early reports suggested that vaults might function as carrier for ribosome subunits or, more general, for all RNPs from the nucleus to the cytoplasm [30]. However, even

in these studies the vault/ribosome interaction was suggested to be indirect and/or weak, as it did not persist during sucrose gradients and comparable observations in mammalian cells have never been reported. Nevertheless, considering all these data, it has to be assumed that at least under specific conditions a substantial amount of vaults may pass the cytoplasm/nucleus barrier extending the potential for functional diversity of these amazing particles (*compare below*).

Besides the association with components of the nucleus, also the localisation of vaults within the cytoplasm is highly dynamic. Using fluorescence recovery after photobleaching (FRAP) experiments, van Zon et al. [100] suggested vault movement through the cytoplasm rather by diffusion than active transport. This suggestion was mainly based on temperature-insensitivity of vault movements in these experiments [101]. However, the size of vaults militate against passive diffusion through the cytoplasm [110]. Accordingly, a rapid, probably saltatory movement of vaults with a velocity of about 10 μm/s was described by Selesina et al. based on comparable FRAP techniques and video-enhanced microscopical analysis [98], which is well in the range of the fast anterograde axonal transport of synaptic vesicles [111]. This is in accordance with earlier ligature experiments of *Torpedo marmorata* electric nerves demonstrating that vaults accumulate proximal and distal to the crush point, suggesting axonal anterograde and retrograde transports [112]. Moreover, in multiple situations vaults were shown to rapidly react to diverse signals by translocation to distinct subcellular compartments including ruffling edges, neuritic tips, presynaptic compartments, lipid rafts and others [14, 35, 99, 113–115]. Additionally, no migration or diffusion front was obvious during reappearance of GFP-tagged vaults to photobleached regions of neuritic tips of PC12 cells [98].

The rapid transport of vaults over longer distances implicates the involvement of molecular motors and the cytoskeleton, as also known from the transport of synaptic vesicles. Indeed, several studies suggested that vaults interact with cytoskeletal components. For example, MVP immunolocalised in the close proximity to actin fibers at ruffling edges and in cell adhesion sites [23] as well as cholinergic nerve terminals of electric ray [114]. However, a direct interaction between actin and vault components has never been shown. In contrast, vaults were repeatedly demonstrated to colocalise and interact with tubulin respectively microtubules in several cell types. Both cellular components co-purify from sea urchin cells [30], colocalise to the tips of PC12 neurits [113], and co-immunoprecipitate (with MVP) in non-small cell lung cancer cells [100]. A direct association of a subgroup of vaults with microtubules predominantly via the particle caps was demonstrated with 5 to 6 vaults binding each micron filament [116]. As in this study no vault-mediated crosslinking of microtubules was seen, the authors hypothesised that only one of the vault caps might be able to bind to the filament. However, this is to our knowledge the only hypothesis so far published that the two vault halves could be different. Mammalian vaults remained bound to tubulin di- and oligomers even after filament destruction by nocodazole [116], and tubulin was enhanced in the MVP immunoprecipitates after microtubule stabilisation with taxol [100]. However, the intracytoplasmic movement of vaults was only reduced but not completely blocked by nocodazole, suggesting that vault transport, though stimulated, is not entirely dependent on intact microtubules [100].

Another fascinating aspect of vaults regulated by the cytoskeleton are the so-called "vault-tubes" described by van Zon et al. as a consequence of exposing GFP-tagged MVP-transfected cells to room temperature [101]. Under these conditions, MVP and probably additional vPARP interact to form tube-like cylindrical structures within the cytoplasm with a length of around 7 micron which disappear following a shift to 37°C. Comparable observations have been made in our lab with regard to endogenous MVP in human cancer cells treated with certain chemotherapeutic drugs (Fig. 1C, unpublished). For vault tube formation, MVP has to interact via its coiled-coil domain as already known for vault particle formation, suggesting that these structures include intact vaults. Based on the possibility of purified vaults to aggregate *in vitro* side-to-side into crystal-like structures [7], this would implicate that about 7500 vaults might polymerise to 1μm of vault-tube. From the disappearance of other vPARP containing cytoplasmic structures, so-called "vPARP rods", during vault-tube formation, an inclusion of cytoplasmic vPARP was suggested [101]. However, these assumptions rely only on immunofluorescence data and lack any further direct proof. Interestingly, FRAP experiments demonstrated that vault-tubes are dynamic structures including a particular MVP molecule or intact vault particle on average only for around 100 seconds. While depolymerisation of microtubules by nocodazole enhanced vault tube formation, stabilisation by taxol almost completely blocked this process, suggesting a fundamental involvement of microtubular dynamics in vault-tube formation [101].

Another form of aggregation of vaults to large (up to 7 μm in diameter) cytoplasmic structures, in this case termed "vaultosomes", was recently reported by Suprenant et al. in response to metal-containing oxyanions like tellurite [19]. Vaultosomes are dis-

tinctly different from vault-tubes as they are irregularly shaped and not generally elongated, predominantly localise to the cell margins, are preferentially built at 37°C, and their formation seems to be independent of microtubule dynamics. Co-immunostaining indicated that vaultosomes are different from known forms of aggresomes and stress granules. However, both vaultosomes and vault-tubes are reversible, dynamic structures and precise functions are unknown so far.

Cellular Functions of MVP/Vaults

Considering the widespread expression but also the high evolutionary conservation and the peculiar structure of vaults, the identification of precise functions of the vault particle turned out to be astoundingly complicated. Generally, several of the functions ascribed to this unique cellular organelle are not irrevocably defined as yet. Diverse hypotheses have been developed on possible functions of vaults mainly based on their unique barrel-like structure (implicating transport of cargo) in a "form ever follows function" approach [17]. Additionally, the molecular characteristics of the minor vault components as a telomerase protein, a poly-(ADP)-ribose polymerase and as untranslated RNAs have fuelled the imaginations of vault researchers [16, 17, 20]. However, several of these assumptions have not been substantiated by experimental evidence. Nevertheless, during the last years the fog is clearing somewhat and more precise concepts regarding the cellular functions of vaults are emerging (Table 1).

Vaults and Drug Resistance: an Intricate Liaison

With the discovery that MVP is identical with the human lung resistance protein LRP [55], known to be overexpressed in multiple chemotherapy resistance models [31], literature boomed concerning a role of vaults in chemotherapeutic drug resistance. Today an extended body of literature exists concerning the relation between vaults/MVP/LRP expression and drug resistance in clinical oncology. As the respective literature has been summarized in several quite recent reviews [16, 17, 34], we decided to discuss in this paper only the very current and still extremely controversial literature on this issue.

To say it in a nutshell, the question as to whether vaults are involved in drug resistance and, if so, based on what molecular mechanisms has still not been answered. However, several recent observations and arising questions need to be discussed and implemented before shelving the issue of vaults and drug resistance.

What argues for a decisive role of vaults in chemoprotection? Vaults are frequently expressed in tissues chronically exposed to the environment and consequently prone to get in contact with xenobiotics, including the epithelia of lung and digestive tract as well as early stages of embryonic development [62, 117, 118]. In addition to the distinct correlation of intrinsic drug resistance with MVP expression in several human malignancies *in vitro* and *in vivo* [16, 34], MVP is almost generally overexpressed in drug resistant human cancer cells selected against a wide array of chemotherapeutic drugs [29, 33, 55, 119]. Besides the mere description of this phenomenon, several research groups have indicated that MVP is causally involved in the associated resistance phenotype. For example, a series of earlier studies demonstrated that in a human colon cancer cell line sodium butyrate treatment induced an MDR phenotype that was based on the nuclear exclusion of drugs like doxorubicin [108]. Transfection with an MVP-targeting ribozyme or incubation with the pyridine analogue PAK-104P, a putative MVP inhibitor, restored chemosensitivity and nuclear drug accumulation [107, 108]. Comparable effects were seen when isolated nuclei were incubating with an anti-MVP antibody [108]. Considering the fact that vaults were suggested as a central plug of the NPC [102], these data support the model that vaults export drugs from the nucleus leading to sequestration in cytosolic vesicles. Accordingly, Meschini et al. [120] colocalised MVP and doxorubicin in cytoplasmic vesicles in the intrinsically drug-resistant and MVP-overexpressing non-small lung cancer cell line A549. Very recently, Herlevsen et al. [121] demonstrated that MVP knock-down by siRNA in human bladder cancer cells inhibited cytosolic doxorubicin sequestration in perinuclear lysosomes. This effect was accompanied by enhanced nuclear drug accumulation and cytotoxicity but also by a general disruption of the lysosomal compartment, suggesting a fundamental role of MVP in lysosomal maintenance. This is in agreement with the partial colocalisation of vaults with the lysosomal marker CD63 in human dendritic cells [122]. As the endosomal network which includes the lysosomes is strongly dependent on microtubules, one could assume that MVP depletion might impact on this cytoskeletal component. However, no obvious disruption of the microtubular network by knock-down of MVP expression was observed [121].

In a very recent study, Ryu et al. suggested an essential involvement of MVP in the enhanced expression of the anti-apoptotic protein bcl-2 in senescent human fibroblasts [38]. The corresponding resistance against apoptosis induction by H_2O_2, staurosporine, or thapsigargin was reversed by siRNA-mediated down-

modulation of MVP. As upregulated MVP levels were found in several aged organs, these data indicate that MVP might be a mediator of general cell-death resistance in different cell compartments and tissues. Whether comparable interactions exist in malignant tissues and thus might support chemotherapy resistance needs to be determined.

A completely different aspect of the issue on vaults and drug resistance was reported by Gopinath et al. [123] demonstrating that the recombinant vRNAs hvg1 and hvg2 (but not hvg3) directly bind the antineoplastic drug mitoxantrone but do not interact with doxorubicin. Generally, RNA can bind to an array of small molecules including antibiotics and metabolites. Whether the *in vitro* binding of chemotherapeutics to vRNA also takes place in the living cells and how this would be influenced by interaction with the vault particle is unknown.

Several other observations, however, exclude a direct role of MVP in drug resistance. Mere upregulation of vaults was not sufficient to induce an MDR phenotype [124]. Most importantly, MVP (-/-) and thus vault-less mice and derived stem and bone marrow cell cultures were not hypersensitive to diverse antineoplastic drugs [79]. Additionally, intracellular daunomycin distribution and nuclear export were changed neither in MVP(-/-) as compared to wild-type mouse embryonic fibroblasts (MEFs) nor in human lung cancer cells SW1573 overexpressing GFP-tagged MVP [125]. Restoration of MVP expression in MVP (-/-) MEFs did not change daunomycin handling despite leading to formation of intact vaults. Accordingly, knockdown of MVP in the doxorubicin-selected lung cancer cell line SW1573–2R120 restored neither chemosensitivity nor nuclear accumulation of doxorubicin [126]. The fact that cell proliferation, survival, and sensitivity against heat-shock were found unaltered in this cell model led the authors to the conclusion that vaults are not involved in cell growth, stress response, or chemoresistance.

So how can all these contradictory data be explained? The simple and honest answer is that we do not know! When considering all data published so far it appears relatively certain that vaults do not cause an MDR phenotype comparable to the one mediated by ABC transporter molecules [127]. However, it has to be kept in mind that even this MDR phenotype is mainly induced by *in vitro* drug selection and data on the clinical situation are far less clear. Another novel aspect is that recently MVP and vaults were found to have a modulating function on several cellular signal pathways involved in cell survival and proliferation (*compare next chapter*). MEFs derived from MVP(-/-) mice were hypersensitive against the induction of apoptosis by serum starvation [68]. Accordingly, disruption of the two MVP genes in the slime mold *Dictyostelium discoideum* led to growth and morphological defects under nutritional stress [128]. This implicates that the upregulation of MVP in drug resistant cells may cause only subtle changes in survival signals which may become essential during certain stress conditions.

Another exciting finding is the relation of MVP and the TelA family of prokaryotes like *Rhodobacter sphaeroides* which mediates resistance against tellurite by a yet undefined mechanism [129]. Interestingly, contact with tellurite and other redox/thiol-active (metal) compounds like arsenate, vanadate and selenate induced relocation of vaults into vaultosomes at the cell surface [19]. Consequently, the authors suggested that vaults might play a role in oxyanion detoxification probably be internalisation of telluroproteins or components of the thiol-redox system. In this respect it is interesting that several cell models selected in our lab for resistance against novel redox-active anticancer metal drugs harbour enhanced levels of MVP (unpublished data). However, the precise role of vaults in these resistance phenotypes needs to be established.

With regard to the MVP knock-out mouse and the lack of enhanced chemosensitivity, it has to be kept in mind that several observations on MVP expression regulation in human cells could not be reproduced in the mouse. This includes, e.g., stimulations of the MVP promoter by HDAC inhibitors [42]. Moreover, the mouse promoter lacks a DNA stretch of the human sequence containing lentiviral elements and the inverted CCAAT box and thus cannot bind YB-1 [15]. This substantial difference between human and mouse promoter may explain why – to our knowledge – no murine MDR cell model with enhanced MVP expression has been reported, in contrast to a wide array of human examples. Consequently, the question arises whether the mouse model is generally suitable to analyse the functions of vaults in drug resistance.

Vaults as Multifaceted Signal Regulation and Transport Platform

The hollow structure, the rapid movements, the distinct subcellular localisation (as for example at the nuclear membrane), and the *in vitro* and clinical correlations with drug resistance in several types of human cancers led to the hypothesis that vaults might represent rather promiscuous transport vehicles [3, 17, 20, 34]. This was supported by the occasional occurrence of a mass in the inner hollow cavity of vaults [8]. However, to cut a long story short, after more than 20 years of research the nature of this cargo and the role of vaults in transport processes are still widely speculative. Several proteins have been identified as

being bound and some of them also as being transported by MVP (*compare below* and Table 1); however, whether they are encapsulated in the interior of vaults needs to be determined. Several groups utilising mainly yeast two hybrid systems or immunoprecipitation of intracellular signalling complexes accidentally came across MVP and/or vaults [28, 63, 67, 68]. These findings have recently opened a broad research field suggesting MVP to be an important regulator of intracellular signalling pathways. Whether these functions are executed by MVP or need the contribution of the other vault components is, to date, unclear and further investigations on this issue are urgently needed.

PTEN. The first hint that MVP might regulate intracellular signals came from Yu et al. [28] demonstrating that MVP binds via the proposed EF hands to the C2 domain of the tumor-suppressor phosphatase PTEN in a Ca^{2+}-dependent manner. These data led to the hypothesis that MVP regulates PTEN function probably by changing its intracellular localisation. This is interesting, as in addition to mutation and/or deletion of PTEN, other forms of inactivation in malignant tissues exist, including blocked expression activation (e.g. following p53 mutation), altered translation and degradation, as well as changed intracellular localisation [130–133]. Indeed, in a series of papers the group of Charis Eng indicated that MVP can mediate nuclear import of PTEN [105, 106, 134]. This process was dependent on the presence of Ca^{2+}, antagonised by high levels of Mg^{2+}, and independent of MVP tyrosine phosphorylation. PTEN lacks canonical nuclear localisation sequences (NLS); however, four putative non-traditional NLS were found, each of which, when mutated, did not alter nuclear import of PTEN [106]. However, combined mutation of at least two NLS attenuated both binding to MVP and transport into the nucleus [134]. Nuclear PTEN tends to be high in normal cells but decreases with neoplastic progression [135]. The main function of cytoplasmic PTEN is to inhibit the phosphoinositol-3-kinase (PI3K)/AKT pathway by dephosphorylation of phosphatidylinositol-3,4,5-trisphosphate (PIP3) to PIP2, thus directly antagonizing the activity of PI3K. In contrast, nuclear PTEN is believed to inhibit predominantly phosphorylation of MAPK, reduce expression of cyclin D, and induce G0/G1 arrest [105]. These data implicate MVP in facilitating the nuclear tumor-suppressing function of PTEN. However, it has to be kept in mind that MVP is rather overexpressed in diverse malignant as compared to normal tissues [35–37, 136] while nuclear PTEN levels tend to the opposite [132]. Moreover, one might hypothesise that – by sequestration of PTEN into the nucleus – MVP prevents proper inhibition of the PI3K pathway at the cell membrane, thus supporting malignant progression of tumors overexpressing MVP as, for example, astocytomas [35] or colon cancer [36]. Recently, an essential role of nuclear PTEN in maintenance of chromosomal stability has been indicated [137]. This would imply that MVP counteracts drug-induced DNA damage by delivering nuclear import of PTEN and thus indirectly contributing to drug resistance. However, these hypotheses must be carefully investigated in a normal and a tumor background.

EGFR/SHP2/MAPK/Src. The first indication that MVP might regulate the epidermal growth factor (EGFR)-induced MAPK pathway was reported by Kolli et al. [68] demonstrating that tyrosyl-phosphorylated MVP binds to several effectors of these cascades, namely at least the activated MAPK Erk and the tyrosine phosphatase SHP-2. SHP-2 is widely expressed and genetic studies have demonstrated that the activity of SHP-2 positively affects the transmission of RTK-mediated signals [138]. Disruption of PTPN11, the gene encoding SHP-2, results in early embryonic lethality in mice and generates profound developmental defects in lower organisms. This mimics the loss of essential RTKs [139]. Accordingly, fibroblasts of SHP-2 (-/-) mice are defective in MAPK but also in PI3K pathway activation by growth factors [140, 141]. MVP is phosphorylated in response to EGFR stimulation and dephosphorylated by SHP-2 [68]. This interaction with SHP-2 was essential for full stimulation of the MAPK pathway measured by activation of EGF-induced Erk phosphorylation as well as Elk1-mediated transcription as downstream signals in MVP wild-type and (-/-) MEFs. Moreover, a dominant-active Ras molecule was unable to rescue the MAPK activation defect of MVP knock-out cells, demonstrating that MVP functions at the level of Ras or even downstream [68]. These data suggest that the binding of SHP-2 to phosphorylated MVP promotes the supporting activity of SHP-2 in MAPK activation, thus resembling other so called “scaffolding proteins” like IRS, Gab and FRS families [142]. These proteins foster SHP-2-mediated pathway activation by both direct activation via the SH2 domain and by mediating colocalisation with other pathway mediators. The fact that activated Erk was also found bound to MVP suggests that vaults may serve a more general signal scaffolding function for the MAPK pathway [68].
SHP-2 binds to MVP via its Src homology 2 domain (SH2) as does the original name-giving Src kinase [67]. Src, the first oncogene and tyrosine kinase ever discovered, participates in multiple signalling pathways that regulate cell proliferation, differentiation, motility, adhesion, and immune functions [143]. Based

on its contribution to malignant transformation and chemotherapy resistance, Src inhibitors are promising candidates as anticancer drugs [144]. Using a pull-down assay with the SH2 domain of Src, MVP was identified as a Src binding partner [67]. Again EGF stimulation resulted in MVP phosphorylation and, surprisingly, translocation from the nucleus to the cytoplasm to colocalise with Src. Purified MVP in this study inhibited the kinase activity of Src, and MVP overexpression reduced Erk activation in Src-overexpressing cells. These data are somewhat contradictory to the observed enhancement of MAPK pathway activity by MVP when interacting with SHP-2 and/or activated Erk [68] and suggest that the balance between different SH2 domain-containing, MVP-binding proteins might define whether MVP supports or inhibits activation of the MAPK pathway.

COP1/c-Jun/AP-1. Constitutive Photomorphogenic 1 (COP1) is an E3 ubiquitin ligase originally described to be essential for plant development. COP1 is required to suppress light-signalling in the dark based on degradation of several light-inducible transcription factors [145]. However, COP1 is highly conserved in evolution and homologues have been detected up to humans [64]. Also in these organisms COP1 functions as ubiquitin ligase and is involved in the degradation of important cancer-related proteins such as p53 [146] and c-Jun [147]. As in plants, mammalian COP1 can shuttle between the cytoplasm and the nucleus and forms large cellular protein complexes. Yi et al. used an affinity purification approach to define novel COP1 binding partners [148]. These experiments have uncovered that MVP binds cytoplasmic COP1 and that this interaction is essential for full COP1 activity in terms of c-Jun degradation. The molecular alterations leading to this COP1 activation are unclear so far, but may be executed by other vault components such as vPARP, which also copurifies with COP1 [63]. Upon UV radiation, MVP is tyrosine phosphorylated resulting in reduced interaction with COP1. This leads to attenuation of COP1-mediated degradation of c-Jun and consequently enhanced AP-1 transcriptional activity as characteristic for MVP (-/-) cells. Whether MVP-binding also impacts on the p53-degrading activity of COP1 needs to be determined in further experiments. Interestingly, MVP and COP1 are missing in exactly the same eukaryotic species, namely *Saccharomyces cerevisiae*, *Caenorhabditis elegans* and *Drosophila melanogaster,* which might be pure coincidence or indicate a co-evolutionary process. In plants, however, where COP1 has a major function in regulating photomorphogenic development [64], vaults are missing [17]. Very recently, it was shown that age-related upregulation of MVP contributes to the apoptosis resistance of senescent human fibroblasts based on the interaction with c-jun-mediated down-modulation of bcl-2 [38]. Whether COP1 is also involved in this c-Jun-mediated MVP effect has not been reported.

Estrogen receptor. In a single study, Abbondanza et al. demonstrated that both MVP and vRNA co-immunoprecipitated with the estrogen (and to a lesser extent progesterone and the glucocorticoid) receptor(s) from cytosolic and nuclear extracts of the human breast cancer cell line MCF7 [40]. Accordingly, a small amount of the estrogen receptor co-migrated with intact vaults in sucrose gradients. The estrogen receptor/vault binding was stimulated by estradiol and virtually exclusive with that of the chaperon heat shock protein 90 (Hsp90). MVP interacted with that part of the receptor containing several proto-NLS responsible for hormone-independent nuclear import [149]. In unstimulated cells, Hsp90 binds to the estrogen receptor, thus keeping it in an inactive form, while hormone-binding leads to dissociation of Hsp90 and a subsequent conformational change of the receptor to the DNA-binding, active form [150]. Interestingly, sodium molybdate, which prevents Hsp90 dissociation and receptor activation, inhibited the interaction with vaults [40]. Together these data suggest that vaults may serve an important role in estrogen receptor nuclear import and/or activation.

Interferon/JAK/STAT. One of the cytokines activating the MVP promoter and consequently gene expression is interferon γ [48]. Interferons are a family of structurally related cytokines with a hallmark function of antiviral activity and are only found in vertebrates [151]. Interferon-binding to cell surface receptors activates the associated Janus kinases (JAK) which, in turn, phosphorylate Signal Transducers and Activators of Transcription (STAT) on a specific tyrosine residue Y701. Tyrosine phosphorylation functions as a switch to induce a conformational change generating STAT dimers via reciprocal phosphotyrosine and SH2 domain interaction [152]. STAT homo- and heterodimers are able to enter the nucleus and bind to consensus sequences in the promoter regions of target genes, including MVP [48]. Interestingly, we found that overexpression of MVP led to a distinct reduction of STAT1 phosphorylation and, consequently, nuclear localisation. Accordingly, the activation of interferon γ-induced genes including ICAM-1 was reduced by MVP overexpression but enhanced by knock-down of endogenous MVP by shRNA. Although the molecular basis of this MVP-mediated STAT inhibition has

not been elucidated, these data indicate that vaults are part of a negative feedback loop involved in fine-tuning the interferon γ-induced JAK/STAT signal pathway [48].

Vaults and Immunity

Besides the activation of MVP by interferon, several other observations indicate that MVP might be involved in immunological responses. For example, a dramatic induction of vRNA expression in response to infection with Epstein-Barr virus (EBV) has been reported recently [91, 94]. Interestingly, vRNA induction paralleled that of micro-RNA (miRNA) 146a which, comparable to vaults, has been suggested as playing important roles in innate immunity [153] but probably also malignant transformation [154]. The response of vRNA expression to EBV expression was specific, as no comparable changes were observed in response to HIV [91]. While the induction level of several miRNAs reached up to about 30-fold, the ones of vRNA were more pronounced with hvg1 upregulated to >1000-fold [91]. Fluorescence in situ hybridization experiments employing antisense probes directed against hvg1 demonstrated a localisation close to the nucleus in EBV-infected B cells. This led the authors to speculate that vRNA/vaults could be involved in anti-viral defence by nucleo-cytoplasmic transport mechanisms.

Both macrophages and dendritic cells belong to those cells expressing the highest levels of vaults [118, 122, 155]. A distinct upregulation of MVP/vault expression was observed during maturation of dendritic cells from blood-derived monocytes, $CD34^+$ mononuclear cells or chronic myeloid leukaemia cells [122]. In this study, application of anti-MVP antibodies led to reduced viability of LPS- and TNFα-matured dendritic cells and blocked the expression of critical differentiation/maturation markers as well as the ability to induce antigen-specific T cell proliferation and interferon γ release. These findings indicate a central role of vaults in supporting dendritic cell maturation and consequently immune responses. Surprisingly, none of these observations could be recapitulated in MVP (-/-) mice [156], suggesting distinct interspecies differences in dendritic cell-mediated immune responses.

An important contribution of vaults to innate immunity, in contrast to the dendritic cell data, was also reproducible in MVP knock-out mice. Very recently, it has been demonstrated that MVP/vaults are essential for host resistance against lung infection by *Pseudomonas aeruginosa* based on the effective internalisation and clearance of the pathogen [115]. The respective MVP-mediated mechanism of pathogen clearance is based on a rapid recruitment of vaults to lipid rafts and depends on cooperation with the cystic fibrosis transmembrane conductance regulator (CFTR), a member of the ABC transporter family. From the typical cellular responses to bacterial infection in respiratory epithelial cells only bacterial internalisation but not NF-κB activation, interleukin 8 secretion, or apoptosis induction were significantly reduced following knock-down of MVP expression by siRNA [115]. Accordingly, intranasal infection of MVP (-/-) mice with *Pseudomonas aeruginosa* uncovered reduced bacterial internalisation, enhanced bacterial burden in the lung and consequently increased mortality by a factor of almost 3. The molecular mechanisms underlying this important role of MVP in innate immunity are unclear so far but the authors suggest that either cytoskeletal regulatory functions necessary for lipid raft formation or impacts on signal pathways like the MAPK might be involved.

Conclusions and Perspectives

Twenty-two years have passed since the first description of vaults by the group of Leonard Rome [1, 2] as one of the most outstanding cellular particles ever discovered. During all these years the history of vault research remained characterised by both surprises and disappointments. Surprises included the fascinating finding that MVP is identical with the drug resistance protein LRP [55] and the recent discovery that vaults play essential roles in innate immunity to infection with lung pathogens [115]. In contrast, disappointments enclosed the unexpected lack of an apparent phenotype of MVP knock-out mice, especially when considering the widespread expression and high conservation of MVP/vaults in eukaryotes. Moreover, given the fact that vaults are so frequently upregulated in drug resistant cells and tissues, the convincing evidence that MVP/vaults are not directly involved in chemoprotection was completely unexpected.

Recent discoveries, like the involvement of MVP in the regulation of several and in some cases overlapping cellular signal transduction pathways, indicate that the vault story may become much more complicated than anticipated. Thus, for example, stimulation and blockade of MAPK activation in response to EGF was demonstrated dependent on the interaction with SHP-2/ERK or Src kinase, respectively [67, 68]. This implies that the balance between the multiple interaction partners of MVP in different cells, tissues, and/or species might lead to even opposite net outcomes when manipulating vaults. Additionally, it must be mentioned that several proteins encoded by very important (proto)oncogenes (EGF/EGFR, Src, c-Jun), but also tumor-suppressor genes (e.g. PTEN,

p53, STAT1), are bound/regulated directly and/or indirectly by MVP. This implicates vaults in possibly having important roles in cell survival and malignant progression and explains why several tumors are either characterised by upregulated MVP/vault levels [35, 36, 136] or maintain the high MVP expression already present in the respective normal tissues [117, 118, 157]. Accordingly, in some tumor types MVP levels correlated with a worse prognosis not related to chemotherapy failure [37]. This corresponds to the diminished survival of cells lacking MVP under nutritional stress [68] resembling conditions found within solid tumor nodules. These findings call for a re-evaluation of vaults/MVP in carcinogenesis without focussing only on therapy responses.

Taking into consideration e.g. the comparable observations on growth defects under nutritional stress conditions in species widely separated in evolution like mice [68] and the slime mold [128] together with the highly specialised role of MVP in lipid raft formation and innate immunity [115], it is tempting to speculate that vaults represent a versatile regulation platform for diverse cellular signal and transport processes adapted by the process of evolution to the respective actual needs. These considerations also shed new light on the distinct differences observed in some aspects between humans and mice. The inconsistencies between MVP expression regulation at the transcriptional level due to an altered promoter sequence have already been discussed. This suggests a high variability at the MVP gene locus and also that several vault functions might have adapted rather late in evolution and consequently in a more or less species-specific fashion. This would mean that obviously no vital functions for vaults have been developed (or has been lost) in those species missing vaults. Consequently, vaults do not seem to be an essential basic component of eukaryotic cell physiology and thus the respective functions might have to be established at a more or less species-specific level. In that respect the use of model organisms (e.g. knock-out models) to evaluate and identify all vault functions might have its limitations.

To put all these considerations into perspective for vault researchers: The amazing vault story is not finished and ready to be shelved yet but, quite the contrary, it is just extending for several exiting new chapters which need to be translated, read, and understood.

1 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.

2 Kedersha, N. L., Hill, D. F., Kronquist, K. E., and Rome, L. H. (1986) Subpopulations of liver coated vesicles resolved by preparative agarose gel electrophoresis. J. Cell Biol. 103, 287–297.

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

4 Kickhoefer, V. A., Searles, 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 III. J. Biol. Chem. 268, 7868–7873.

5 Kickhoefer, V. A., Siva, A. C., Kedersha, N. L., Inman, E. M., Ruland, C., Streuli, M., and Rome, L. H. (1999) The 193-kD vault protein, VPARP, is a novel poly(ADP-ribose) polymerase. J. Cell Biol. 146, 917–928.

6 Kickhoefer, V. A., Stephen, A. G., Harrington, L., Robinson, M. O., and Rome, L. H. (1999) Vaults and telomerase share a common subunit, TEP1. J. Biol. Chem. 274, 32712–32717.

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

8 Kong, L. B., Siva, A. C., Rome, L. H., and Stewart, P. L. (1999) Structure of the vault, a ubiquitous celular component. Structure Fold Des. 7, 371–379.

9 Mikyas, Y., Makabi, M., Raval-Fernandes, S., Harrington, L., Kickhoefer, V. A., Rome, L. H., and Stewart, P. L. (2004) Cryoelectron microscopy imaging of recombinant and tissue derived vaults: localization of the MVP N termini and VPARP. J. Mol. Biol. 344, 91–105.

10 Kozlov, G., Vavelyuk, O., Minailiuc, O., Banville, D., Gehring, K., and Ekiel, I. (2006) Solution structure of a two-repeat fragment of major vault protein. J. Mol. Biol. 356, 444–452.

11 Kong, L. B., Siva, A. C., Kickhoefer, V. A., Rome, L. H., and Stewart, P. L. (2000) RNA location and modeling of a WD40 repeat domain within the vault. Rna 6, 890–900.

12 Kato, K., Tanaka, H., Sumizawa, T., Yoshimura, M., Yamashita, E., Iwasaki, K., and Tsukihara, T. (2008) A vault ribonucleoprotein particle exhibiting 39-fold dihedral symmetry. Acta Crystallogr. D. Biol. Crystallogr. 64, 525–531.

13 Herrmann, C., Zimmermann, H., and Volknandt, W. (1997) Analysis of a cDNA encoding the major vault protein from the electric ray Discopyge ommata. Gene 188, 85–90.

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

15 Mossink, M., van Zon, A., Franzel-Luiten, E., Schoester, M., Scheffer, G. L., Scheper, R. J., Sonneveld, P., and Wiemer, E. A. (2002) The genomic sequence of the murine major vault protein and its promoter. Gene 294, 225–232.

16 Mossink, M. H., van Zon, A., Scheper, R. J., Sonneveld, P., Wiemer, E. A., Schoester, M., Houtsmuller, A. B., Scheffer, G. L., Franzel-Luiten, E., Kickhoefer, V. A., Mossink, M., Poderycki, M. J., Chan, E. K., and Rome, L. H. (2003) Vaults: a ribonucleoprotein particle involved in drug resistance? Oncogene 22, 7458–7467.

17 Suprenant, K. A. (2002) Vault ribonucleoprotein particles: sarcophagi, gondolas, or safety deposit boxes? Biochemistry 41, 14447–14454.

18 Vasu, S. K., Kedersha, N. L., and Rome, L. H. (1993) cDNA cloning and disruption of the major vault protein alpha gene (mvpA) in Dictyostelium discoideum. J. Biol. Chem. 268, 15356–15360.

19 Suprenant, K. A., Bloom, N., Fang, J., and Lushington, G. (2007) The major vault protein is related to the toxic anion resistance protein (TelA) family. J. Exp. Biol. 210, 946–955.

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

21 van Zon, A., Mossink, M. H., Scheper, R. J., Sonneveld, P., and Wiemer, E. A. (2003) The vault complex. Cell. Mol. Life Sci. 60, 1828–1837.

22 Anderson, D. H., Kickhoefer, V. A., Sievers, S. A., Rome, L. H., and Eisenberg, D. (2007) Draft crystal structure of the vault shell at 9-A resolution. PLoS Biol. 5, e318.
23 Kedersha, N. L., and Rome, L. H. (1990) Vaults: large cytoplasmic RNP's that associate with cytoskeletal elements. Mol. Biol. Rep. 14, 121–122.
24 Stephen, A. G., Raval-Fernandes, S., Huynh, T., Torres, M., Kickhoefer, V. A., and Rome, L. H. (2001) Assembly of vault-like particles in insect cells expressing only the major vault protein. J. Biol. Chem. 276, 23217–23220.
25 Poderycki, M. J., Kickhoefer, V. A., Kaddis, C. S., Raval-Fernandes, S., Johansson, E., Zink, J. I., Loo, J. A., and Rome, L. H. (2006) The vault exterior shell is a dynamic structure that allows incorporation of vault-associated proteins into its interior. Biochemistry 45, 12184–12193.
26 Kickhoefer, V. A., Garcia, Y., Mikyas, Y., Johansson, E., Zhou, J. C., Raval-Fernandes, S., Minoofar, P., Zink, J. I., Dunn, B., Stewart, P. L., and Rome, L. H. (2005) Engineering of vault nanocapsules with enzymatic and fluorescent properties. Proc. Natl. Acad. Sci. U S A 7, 7.
27 van Zon, A., Mossink, M. H., Schoester, M., Scheffer, G. L., Scheper, R. J., Sonneveld, P., and Wiemer, E. A. (2002) Structural domains of vault proteins: a role for the coiled coil domain in vault assembly. Biochem. Biophys. Res. Commun. 291, 535–541.
28 Yu, Z., Fotouhi-Ardakani, N., Wu, L., Maoui, M., Wang, S., Banville, D., and Shen, S. H. (2002) PTEN associates with the vault particles in HeLa cells. J. Biol. Chem. 277, 40247–40252.
29 Kickhoefer, V. A., Rajavel, K. S., Scheffer, G. L., Dalton, W. S., Scheper, R. J., and Rome, L. H. (1998) Vaults are up-regulated in multidrug-resistant cancer cell lines. J. Biol. Chem. 273, 8971–8974.
30 Hamill, D. R., and Suprenant, K. A. (1997) Characterization of the sea urchin major vault protein: a possible role for vault ribonucleoprotein particles in nucleocytoplasmic transport. Dev. Biol. 190, 117–128.
31 Izquierdo, M. A., Scheffer, G. L., Flens, M. J., Shoemaker, R. H., Rome, L. H., and Scheper, R. J. (1996) Relationship of LRP-human major vault protein to in vitro and clinical resistance to anticancer drugs. Cytotechnology 19, 191–197.
32 Scheffer, G. L., Schroeijers, A. B., Izquierdo, M. A., Wiemer, E. A., and Scheper, R. J. (2000) Lung resistance-related protein/major vault protein and vaults in multidrug-resistant cancer. Curr. Opin. Oncol. 12, 550–556.
33 Scheper, R. J., Broxterman, H. J., Scheffer, G. L., Kaaijk, P., Dalton, W. S., van Heijningen, T. H., van Kalken, C. K., Slovak, M. L., de Vries, E. G., van der Valk, P., and et al. (1993) Overexpression of a M(r) 110,000 vesicular protein in non-P-glycoprotein-mediated multidrug resistance. Cancer Res. 53, 1475–1479.
34 Steiner, E., Holzmann, K., Elbling, L., Micksche, M., and Berger, W. (2006) Cellular functions of vaults and their involvement in multidrug resistance. Curr. Drug Targets 7, 923–934.
35 Berger, W., Spiegl-Kreinecker, S., Buchroithner, J., Elbling, L., Pirker, C., Fischer, J., and Micksche, M. (2001) Over-expression of the human major vault protein in astrocytic brain tumor cells. Int. J. Cancer 94, 377–382.
36 Meijer, G. A., Schroeijers, A. B., Flens, M. J., Meuwissen, S. G., van der Valk, P., Baak, J. P., and Scheper, R. J. (1999) Increased expression of multidrug resistance related proteins Pgp, MRP1, and LRP/MVP occurs early in colorectal carcinogenesis. J. Clin. Pathol. 52, 450–454.
37 Zurita, A. J., Diestra, J. E., Condom, E., Garcia Del Muro, X., Scheffer, G. L., Scheper, R. J., Perez, J., Germa-Lluch, J. R., and Izquierdo, M. A. (2003) Lung resistance-related protein as a predictor of clinical outcome in advanced testicular germ-cell tumours. Br. J. Cancer 88, 879–886.
38 Ryu, S. J., An, H. J., Oh, Y. S., Choi, H. R., Ha, M. K., and Park, S. C. (2008) On the role of major vault protein in the resistance of senescent human diploid fibroblasts to apoptosis. Cell Death Differ. doi: 10.1038/cdd.2008.96.
39 Stein, U., Jurchott, K., Schlafke, M., and Hohenberger, P. (2002) Expression of multidrug resistance genes MVP, MDR1, and MRP1 determined sequentially before, during, and after hyperthermic isolated limb perfusion of soft tissue sarcoma and melanoma patients. J. Clin. Oncol. 20, 3282–3292.
40 Abbondanza, C., Rossi, V., Roscigno, A., Gallo, L., Belsito, A., Piluso, G., Medici, N., Nigro, V., Molinari, A. M., Moncharmont, B., and Puca, G. A. (1998) Interaction of vault particles with estrogen receptor in the MCF-7 breast cancer cell. J. Cell Biol. 141, 1301–1310.
41 Berger, W., Elbling, L., and Micksche, M. (2000) Expression of the major vault protein LRP in human non-small-cell lung cancer cells: activation by short-term exposure to antineoplastic drugs. Int. J. Cancer 88, 293–300.
42 Emre, N., Raval-Fernandes, S., Kickhoefer, V. A., and Rome, L. H. (2004) Analysis of MVP and VPARP promoters indicates a role for chromatin remodeling in the regulation of MVP. Biochim. Biophys. Acta 1678, 33–46.
43 Stein, U., Bergmann, S., Scheffer, G. L., Scheper, R. J., Royer, H. D., Schlag, P. M., and Walther, W. (2005) YB-1 facilitates basal and 5-fluorouracil-inducible expression of the human major vault protein (MVP) gene. Oncogene 7, 7.
44 Steiner, E., Holzmann, K., Pirker, C., Elbling, L., Micksche, M., and Berger, W. (2004) SP-transcription factors are involved in basal MVP promoter activity and its stimulation by HDAC inhibitors. Biochem. Biophys. Res. Commun. 317, 235–243.
45 Shimamoto, Y., Sumizawa, T., Haraguchi, M., Gotanda, T., Jueng, H. C., Furukawa, T., Sakata, R., and Akiyama, S. (2006) Direct activation of the human major vault protein gene by DNA-damaging agents. Oncol. Rep. 15, 645–652.
46 Hu, Y., Stephen, A. G., Cao, J., Tanzer, L. R., Slapak, C. A., Harrison, S. D., Devanarayan, V., Dantzig, A. H., Starling, J. J., Rome, L. H., and Moore, R. E. (2002) A very early induction of major vault protein accompanied by increased drug resistance in U-937 cells. Int. J. Cancer 97, 149–156.
47 Miracco, C., Maellaro, E., Pacenti, L., Del Bello, B., Valentini, M. A., Rubegni, P., Pirtoli, L., Volpi, C., Santopietro, R., and Tosi, P. (2003) Evaluation of MDR1, LRP, MRP, and topoisomerase IIalpha gene mRNA transcripts before and after interferon-alpha, and correlation with the mRNA expression level of the telomerase subunits hTERT and TEP1 in five unselected human melanoma cell lines. Int. J. Oncol. 23, 213–220.
48 Steiner, E., Holzmann, K., Pirker, C., Elbling, L., Micksche, M., Sutterluty, H., and Berger, W. (2006) The major vault protein is responsive to and interferes with interferon-gamma-mediated STAT1 signals. J. Cell Sci. 119, 459–469.
49 Stein, U., Walther, W., Laurencot, C. M., Scheffer, G. L., Scheper, R. J., and Shoemaker, R. H. (1997) Tumor necrosis factor-alpha and expression of the multidrug resistance-associated genes LRP and MRP. J. Natl. Cancer Inst. 89, 807–813.
50 Knutsen, T., Rao, V. K., Ried, T., Mickley, L., Schneider, E., Miyake, K., Ghadimi, B. M., Padilla-Nash, H., Pack, S., Greenberger, L., Cowan, K., Dean, M., Fojo, T., and Bates, S. (2000) Amplification of 4q21-q22 and the MXR gene in independently derived mitoxantrone-resistant cell lines. Genes Chromosomes Cancer 27, 110–116.
51 Rao, V. K., Wangsa, D., Robey, R. W., Huff, L., Honjo, Y., Hung, J., Knutsen, T., Ried, T., and Bates, S. E. (2005) Characterization of ABCG2 gene amplification manifesting as extrachromosomal DNA in mitoxantrone-selected SF295 human glioblastoma cells. Cancer Genet. Cytogenet. 160, 126–133.
52 Van der Bliek, A. M., Baas, F., Van der Velde-Koerts, T., Biedler, J. L., Meyers, M. B., Ozols, R. F., Hamilton, T. C., Joenje, H., and Borst, P. (1988) Genes amplified and overexpressed in human multidrug-resistant cell lines. Cancer Res. 48, 5927–5932.

53 Slovak, M. L., Ho, J. P., Cole, S. P., Deeley, R. G., Greenberger, L., de Vries, E. G., Broxterman, H. J., Scheffer, G. L., and Scheper, R. J. (1995) The LRP gene encoding a major vault protein associated with drug resistance maps proximal to MRP on chromosome 16: evidence that chromosome breakage plays a key role in MRP or LRP gene amplification. Cancer Res. 55, 4214–4219.

54 Laurencot, C. M., Scheffer, G. L., Scheper, R. J., and Shoemaker, R. H. (1997) Increased LRP mRNA expression is associated with the MDR phenotype in intrinsically resistant human cancer cell lines. Int. J. Cancer 72, 1021–1026.

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

56 Plaat, B. E., Molenaar, W. M., Sagrudny, J., Bohle, R. M., Mastik, M. F., Hoekstra, H. J., Van der Graaf, W. T., Hollema, H., and van den Berg, E. (2000) The 16p11 breakpoint in myxoid liposarcomas might affect the expression of the LRP gene on 16p11.2 encoding the multidrug resistance associated major vault protein. Eur. J. Clin. Invest. 30, 447–453.

57 Lange, C., Walther, W., Schwabe, H., and Stein, U. (2000) Cloning and initial analysis of the human multidrug resistance-related MVP/LRP gene promoter. Biochem. Biophys. Res. Commun. 278, 125–133.

58 Fujii, T., Kawahara, A., Basaki, Y., Hattori, S., Nakashima, K., Nakano, K., Shirouzu, K., Kohno, K., Yanagawa, T., Yamana, H., Nishio, K., Ono, M., Kuwano, M., and Kage, M. (2008) Expression of HER2 and estrogen receptor alpha depends upon nuclear localization of Y-box binding protein-1 in human breast cancers. Cancer Res. 68, 1504–1512.

59 Oda, Y., Ohishi, Y., Basaki, Y., Kobayashi, H., Hirakawa, T., Wake, N., Ono, M., Nishio, K., Kuwano, M., and Tsuneyoshi, M. (2007) Prognostic implications of the nuclear localization of Y-box-binding protein-1 and CXCR4 expression in ovarian cancer: their correlation with activated Akt, LRP/MVP and P-glycoprotein expression. Cancer Sci. 98, 1020–1026.

60 Holzmann, K., Ambrosch, I., Elbling, L., Micksche, M., and Berger, W. (2001) A small upstream open reading frame causes inhibition of human major vault protein expression from a ubiquitous mRNA splice variant. FEBS Lett. 494, 99–104.

61 Zheng, C. L., Sumizawa, T., Che, X. F., Tsuyama, S., Furukawa, T., Haraguchi, M., Gao, H., Gotanda, T., Jueng, H. C., Murata, F., and Akiyama, S. (2004) Characterization of MVP and VPARP assembly into vault ribonucleoprotein complexes. Biochem. Biophys. Res. Commun. 326, 100–107.

62 Sutovsky, P., Manandhar, G., Laurincik, J., Letko, J., Caamano, J. N., Day, B. N., Lai, L., Prather, R. S., Sharpe-Timms, K. L., Zimmer, R., and Sutovsky, M. (2005) Expression and proteasomal degradation of the major vault protein (MVP) in mammalian oocytes and zygotes. Reproduction 129, 269–282.

63 Yi, C., Li, S., Chen, X., Wiemer, E. A., Wang, J., Wei, N., and Deng, X. W. (2005) Major vault protein, in concert with constitutively photomorphogenic 1, negatively regulates c-Jun-mediated activator protein 1 transcription in mammalian cells. Cancer Res. 65, 5835–5840.

64 Yi, C., and Deng, X. W. (2005) COP1 – from plant photomorphogenesis to mammalian tumorigenesis. Trends Cell. Biol. 15, 618–625.

65 Ehrnsperger, C., and Volknandt, W. (2001) Major vault protein is a substrate of endogenous protein kinases in CHO and PC12 cells. Biol. Chem. 382, 1463–1471.

66 Herrmann, C., Kellner, R., and Volknandt, W. (1998) Major vault protein of electric ray is a phosphoprotein. Neurochem. Res. 23, 39–46.

67 Kim, E., Lee, S., Mian, M. F., Yun, S. U., Song, M., Yi, K. S., Ryu, S. H., and Suh, P. G. (2006) Crosstalk between Src and major vault protein in epidermal growth factor-dependent cell signalling. Febs J. 273, 793–804.

68 Kolli, S., Zito, C. I., Mossink, M. H., Wiemer, E. A., and Bennett, A. M. (2004) The major vault protein is a novel substrate for the tyrosine phosphatase SHP-2 and scaffold protein in epidermal growth factor signaling. J. Biol. Chem. 279, 29374–29385.

69 Ame, J. C., Spenlehauer, C., and de Murcia, G. (2004) The PARP superfamily. Bioessays 26, 882–893.

70 Hassa, P. O., and Hottiger, M. O. (2008) The diverse biological roles of mammalian PARPS, a small but powerful family of poly-ADP-ribose polymerases. Front. Biosci. 13, 3046–3082.

71 Schreiber, V., Dantzer, F., Ame, J. C., and de Murcia, G. (2006) Poly(ADP-ribose): novel functions for an old molecule. Nat. Rev. Mol. Cell Biol. 7, 517–528.

72 Smith, S. (2001) The world according to PARP. Trends Biochem. Sci. 26, 174–179.

73 Lewis, C., and Low, J. A. (2007) Clinical poly(ADP-ribose) polymerase inhibitors for the treatment of cancer. Curr. Opin. Investig. Drugs 8, 1051–1056.

74 Bateman, A., and Kickhoefer, V. (2003) The TROVE module: A common element in Telomerase, Ro and Vault ribonucleoproteins. BMC Bioinformatics 4, 49.

75 Ame, J. C., Spenlehauer, C., and de Murcia, G. (2004) The PARP superfamily. Bioessays 26, 882–893.

76 Smith, S., and de Lange, T. (2000) Tankyrase promotes telomere elongation in human cells. Curr. Biol. 10, 1299–1302.

77 Liu, Y., Snow, B. E., Kickhoefer, V. A., Erdmann, N., Zhou, W., Wakeham, A., Gomez, M., Rome, L. H., and Harrington, L. (2004) Vault poly(ADP-ribose) polymerase is associated with mammalian telomerase and is dispensable for telomerase function and vault structure in vivo. Mol. Cell Biol. 24, 5314–5323.

78 Raval-Fernandes, S., Kickhoefer, V. A., Kitchen, C., and Rome, L. H. (2005) Increased susceptibility of vault poly(-ADP-ribose) polymerase-deficient mice to carcinogen-induced tumorigenesis. Cancer Res. 65, 8846–8852.

79 Mossink, M. H., van Zon, A., Franzel-Luiten, E., Schoester, M., Kickhoefer, V. A., Scheffer, G. L., Scheper, R. J., Sonneveld, P., and Wiemer, E. A. (2002) Disruption of the murine major vault protein (MVP/LRP) gene does not induce hypersensitivity to cytostatics. Cancer Res. 62, 7298–7304.

80 Harrington, L., McPhail, T., Mar, V., Zhou, W., Oulton, R., Bass, M. B., Arruda, I., and Robinson, M. O. (1997) A mammalian telomerase-associated protein. Science 275, 973–977.

81 Weinrich, S. L., Pruzan, R., Ma, L., Ouellette, M., Tesmer, V. M., Holt, S. E., Bodnar, A. G., Lichtsteiner, S., Kim, N. W., Trager, J. B., Taylor, R. D., Carlos, R., Andrews, W. H., Wright, W. E., Shay, J. W., Harley, C. B., and Morin, G. B. (1997) Reconstitution of human telomerase with the template RNA component hTR and the catalytic protein subunit hTRT. Nat. Genet. 17, 498–502.

82 Kickhoefer, V. A., Liu, Y., Kong, L. B., Snow, B. E., Stewart, P. L., Harrington, L., and Rome, L. H. (2001) The Telomerase/vault-associated protein TEP1 is required for vault RNA stability and its association with the vault particle. J. Cell Biol. 152, 157–164.

83 Smith, S., and de Lange, T. (2000) Tankyrase promotes telomere elongation in human cells. Curr. Biol. 10, 1299–1302.

84 van Steensel, B., and de Lange, T. (1997) Control of telomere length by the human telomeric protein TRF1. Nature 385, 740–743.

85 Poderycki, M. J., Rome, L. H., Harrington, L., and Kickhoefer, V. A. (2005) The p80 homology region of TEP1 is sufficient for its association with the telomerase and vault RNAs, and the vault particle. Nucleic Acids Res. 33, 893–902.

86 Deutscher, S. L., Harley, J. B., and Keene, J. D. (1988) Molecular analysis of the 60-kDa human Ro ribonucleoprotein. Proc. Natl. Acad. Sci. U S A 85, 9479–9483.

87 Whittaker, C. A., and Hynes, R. O. (2002) Distribution and evolution of von Willebrand/integrin A domains: widely dispersed domains with roles in cell adhesion and elsewhere. Mol. Biol. Cell 13, 3369–3387.

88 Suganuma, T., Pattenden, S. G., and Workman, J. L. (2008) Diverse functions of WD40 repeat proteins in histone recognition. Genes Dev. 22, 1265–1268.
89 Lambright, D. G., Sondek, J., Bohm, A., Skiba, N. P., Hamm, H. E., and Sigler, P. B. (1996) The 2.0 A crystal structure of a heterotrimeric G protein. Nature 379, 311–319.
90 van Zon, A., Mossink, M. H., Schoester, M., Scheffer, G. L., Scheper, R. J., Sonneveld, P., and Wiemer, E. A. (2001) Multiple human vault RNAs. Expression and association with the vault complex. J. Biol. Chem. 276, 37715–37721.
91 Mrazek, J., Kreutmayer, S. B., Grasser, F. A., Polacek, N., and Huttenhofer, A. (2007) Subtractive hybridization identifies novel differentially expressed ncRNA species in EBV-infected human B cells. Nucleic Acids Res. 35, e73.
92 Kickhoefer, V. A., Emre, N., Stephen, A. G., Poderycki, M. J., and Rome, L. H. (2003) Identification of conserved vault RNA expression elements and a non-expressed mouse vault RNA gene. Gene 309, 65–70.
93 Vilalta, A., Kickhoefer, V. A., Rome, L. H., and Johnson, D. L. (1994) The rat vault RNA gene contains a unique RNA polymerase III promoter composed of both external and internal elements that function synergistically. J. Biol. Chem. 269, 29752–29759.
94 Motsch, N., Pfuhl, T., Mrazek, J., Barth, S., and Grasser, F. A. (2007) Epstein-Barr Virus-encoded latent membrane protein 1 (LMP1) induces the expression of the cellular microRNA miR-146a. RNA Biol. 4, 131–137.
95 Kickhoefer, V. A., Poderycki, M. J., Chan, E. K., and Rome, L. H. (2002) The La RNA-binding protein interacts with the vault RNA and is a vault-associated protein. J. Biol. Chem. 277, 41282–41286.
96 Maraia, R. J., and Bayfield, M. A. (2006) The La protein-RNA complex surfaces. Mol Cell 21, 149–152.
97 Goldsmith, L. E., Yu, M., Rome, L. H., and Monbouquette, H. G. (2007) Vault nanocapsule dissociation into halves triggered at low pH. Biochemistry 46, 2865–2875.
98 Slesina, M., Inman, E. M., Moore, A. E., Goldhaber, J. I., Rome, L. H., and Volknandt, W. (2006) Movement of vault particles visualized by GFP-tagged major vault protein. Cell Tissue Res. 324, 403–410.
99 Slesina, M., Inman, E. M., Rome, L. H., and Volknandt, W. (2005) Nuclear localization of the major vault protein in U373 cells. Cell Tissue Res. 321, 97–104.
100 van Zon, A., Mossink, M. H., Houtsmuller, A. B., Schoester, M., Scheffer, G. L., Scheper, R. J., Sonneveld, P., and Wiemer, E. A. (2006) Vault mobility depends in part on microtubules and vaults can be recruited to the nuclear envelope. Exp. Cell Res. 312, 245–255.
101 van Zon, A., Mossink, M. H., Schoester, M., Houtsmuller, A. B., Scheffer, G. L., Scheper, R. J., Sonneveld, P., and Wiemer, E. A. (2003) The formation of vault-tubes: a dynamic interaction between vaults and vault PARP. J. Cell Sci. 116, 4391–4400.
102 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.
103 Dickenson, N. E., Moore, D., Suprenant, K. A., and Dunn, R. C. (2007) Vault ribonucleoprotein particles and the central mass of the nuclear pore complex. Photochem. Photobiol. 83, 686–691.
104 Beck, M., Forster, F., Ecke, M., Plitzko, J. M., Melchior, F., Gerisch, G., Baumeister, W., and Medalia, O. (2004) Nuclear pore complex structure and dynamics revealed by cryoelectron tomography. Science 306, 1387–1390.
105 Chung, J. H., and Eng, C. (2005) Nuclear-cytoplasmic partitioning of phosphatase and tensin homologue deleted on chromosome 10 (PTEN) differentially regulates the cell cycle and apoptosis. Cancer Res. 65, 8096–8100.
106 Chung, J. H., Ginn-Pease, M. E., and Eng, C. (2005) Phosphatase and tensin homologue deleted on chromosome 10 (PTEN) has nuclear localization signal-like sequences for nuclear import mediated by major vault protein. Cancer Res. 65, 4108–4116.
107 Kitazono, M., Okumura, H., Ikeda, R., Sumizawa, T., Furukawa, T., Nagayama, S., Seto, K., Aikou, T., and Akiyama, S. (2001) Reversal of LRP-associated drug resistance in colon carcinoma SW-620 cells. Int. J. Cancer 91, 126–131.
108 Kitazono, M., Sumizawa, T., Takebayashi, Y., Chen, Z. S., Furukawa, T., Nagayama, S., Tani, A., Takao, S., Aikou, T., and Akiyama, S. (1999) Multidrug resistance and the lung resistance-related protein in human colon carcinoma SW-620 cells. J. Natl. Cancer Inst. 91, 1647–1653.
109 Stewart, P. L., Makabi, M., Lang, J., Dickey-Sims, C., Robertson, A. J., Coffman, J. A., and Suprenant, K. A. (2005) Sea urchin vault structure, composition, and differential localization during development. BMC Dev. Biol. 5, 3.
110 Luby-Phelps, K., Castle, P. E., Taylor, D. L., and Lanni, F. (1987) Hindered diffusion of inert tracer particles in the cytoplasm of mouse 3T3 cells. Proc. Natl. Acad. Sci. U S A 84, 4910–4913.
111 Kaether, C., Skehel, P., and Dotti, C. G. (2000) Axonal membrane proteins are transported in distinct carriers: a two-color video microscopy study in cultured hippocampal neurons. Mol. Biol. Cell 11, 1213–1224.
112 Li, J. Y., Volknandt, W., Dahlstrom, A., Herrmann, C., Blasi, J., Das, B., and Zimmermann, H. (1999) Axonal transport of ribonucleoprotein particles (vaults). Neuroscience 91, 1055–1065.
113 Herrmann, C., Golkaramnay, E., Inman, E., Rome, L., and Volknandt, W. (1999) Recombinant major vault protein is targeted to neuritic tips of PC12 cells. J. Cell Biol. 144, 1163–1172.
114 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.
115 Kowalski, M. P., Dubouix-Bourandy, A., Bajmoczi, M., Golan, D. E., Zaidi, T., Coutinho-Sledge, Y. S., Gygi, M. P., Gygi, S. P., Wiemer, E. A., and Pier, G. B. (2007) Host resistance to lung infection mediated by major vault protein in epithelial cells. Science 317, 130–132.
116 Eichenmuller, B., Kedersha, N., Solovyeva, E., Everley, P., Lang, J., Himes, R. H., and Suprenant, K. A. (2003) Vaults bind directly to microtubules via their caps and not their barrels. Cell. Motil. Cytoskeleton 56, 225–236.
117 Bouhamyia, L., Chantot-Bastaraud, S., Zaidi, S., Roynard, P., Prengel, C., Bernaudin, J. F., and Fleury-Feith, J. (2007) Immunolocalization and cell expression of lung resistance-related protein (LRP) in normal and tumoral human respiratory cells. J. Histochem. Cytochem. 55, 773–782.
118 Izquierdo, M. A., Scheffer, G. L., Flens, M. J., Giaccone, G., Broxterman, H. J., Meijer, C. J., van der Valk, P., and Scheper, R. J. (1996) Broad distribution of the multidrug resistance-related vault lung resistance protein in normal human tissues and tumors. Am. J. Pathol. 148, 877–887.
119 Schroeijers, A. B., Siva, A. C., Scheffer, G. L., de Jong, M. C., Bolick, S. C., Dukers, D. F., Slootstra, J. W., Meloen, R. H., Wiemer, E., Kickhoefer, V. A., Rome, L. H., and Scheper, R. J. (2000) The Mr 193,000 vault protein is up-regulated in multidrug-resistant cancer cell lines. Cancer Res. 60, 1104–1110.
120 Meschini, S., Marra, M., Calcabrini, A., Monti, E., Gariboldi, M., Dolfini, E., and Arancia, G. (2002) Role of the lung resistance-related protein (LRP) in the drug sensitivity of cultured tumor cells. Toxicol. In Vitro 16, 389–398.
121 Herlevsen, M., Oxford, G., Owens, C. R., Conaway, M., and Theodorescu, D. (2007) Depletion of major vault protein increases doxorubicin sensitivity and nuclear accumulation and disrupts its sequestration in lysosomes. Mol. Cancer Ther. 6, 1804–1813.
122 Schroeijers, A. B., Reurs, A. W., Scheffer, G. L., Stam, A. G., de Jong, M. C., Rustemeyer, T., Wiemer, E. A., de Gruijl, T.

D., and Scheper, R. J. (2002) Up-regulation of drug resistance-related vaults during dendritic cell development. J. Immunol. 168, 1572–1578.
123 Gopinath, S. C., Matsugami, A., Katahira, M., and Kumar, P. K. (2005) Human vault-associated non-coding RNAs bind to mitoxantrone, a chemotherapeutic compound. Nucleic Acids Res. 33, 4874–4881.
124 Siva, A. C., Raval-Fernandes, S., Stephen, A. G., LaFemina, M. J., Scheper, R. J., Kickhoefer, V. A., and Rome, L. H. (2001) Up-regulation of vaults may be necessary but not sufficient for multidrug resistance. Int. J. Cancer 92, 195–202.
125 van Zon, A., Mossink, M. H., Schoester, M., Scheper, R. J., Sonneveld, P., and Wiemer, E. A. (2004) Efflux kinetics and intracellular distribution of daunorubicin are not affected by major vault protein/lung resistance-related protein (vault) expression. Cancer Res. 64, 4887–4892.
126 Huffman, K. E., and Corey, D. R. (2005) Major vault protein does not play a role in chemoresistance or drug localization in a non-small cell lung cancer cell line. Biochemistry 44, 2253–2261.
127 Gottesman, M. M., Fojo, T., and Bates, S. E. (2002) Multidrug resistance in cancer: role of ATP-dependent transporters. Nat. Rev. Cancer 2, 48–58.
128 Vasu, S. K., and Rome, L. H. (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.
129 O'Gara, J. P., Gomelsky, M., and Kaplan, S. (1997) Identification and molecular genetic analysis of multiple loci contributing to high-level tellurite resistance in Rhodobacter sphaeroides 2.4.1. Appl. Environ. Microbiol. 63, 4713–4720.
130 Teresi, R. E., Zbuk, K. M., Pezzolesi, M. G., Waite, K. A., and Eng, C. (2007) Cowden syndrome-affected patients with PTEN promoter mutations demonstrate abnormal protein translation. Am. J. Hum. Genet. 81, 756–767.
131 Cully, M., You, H., Levine, A. J., and Mak, T. W. (2006) Beyond PTEN mutations: the PI3K pathway as an integrator of multiple inputs during tumorigenesis. Nat. Rev. Cancer 6, 184–192.
132 Planchon, S. M., Waite, K. A., and Eng, C. (2008) The nuclear affairs of PTEN. J. Cell Sci. 121, 249–253.
133 Tamguney, T., and Stokoe, D. (2007) New insights into PTEN. J. Cell Sci. 120, 4071–4079.
134 Minaguchi, T., Waite, K. A., and Eng, C. (2006) Nuclear localization of PTEN is regulated by Ca(2+) through a tyrosil phosphorylation-independent conformational modification in major vault protein. Cancer Res. 66, 11677–11682.
135 Eng, C. (2003) PTEN: one gene, many syndromes. Hum. Mutat. 22, 183–198.
136 Aronica, E., Gorter, J. A., Van Vliet, E. A., Spliet, W. G., Van Veelen, C. W., Van Rijen, P. C., Leenstra, S., Ramkema, M. D., Scheffer, G. L., Scheper, R. J., Sisodiya, S. M., and Troost, D. (2003) Overexpression of the Human Major Vault Protein in Gangliogliomas. Epilepsia 44, 1166–1175.
137 Shen, W. H., Balajee, A. S., Wang, J., Wu, H., Eng, C., Pandolfi, P. P., and Yin, Y. (2007) Essential role for nuclear PTEN in maintaining chromosomal integrity. Cell 128, 157–170.
138 Van Vactor, D., O'Reilly, A. M., and Neel, B. G. (1998) Genetic analysis of protein tyrosine phosphatases. Curr. Opin. Genet. Dev. 8, 112–126.
139 Edouard, T., Montagner, A., Dance, M., Conte, F., Yart, A., Parfait, B., Tauber, M., Salles, J. P., and Raynal, P. (2007) How do Shp2 mutations that oppositely influence its biochemical activity result in syndromes with overlapping symptoms? Cell. Mol. Life Sci. 64, 1585–1590.
140 Shi, Z. Q., Yu, D. H., Park, M., Marshall, M., and Feng, G. S. (2000) Molecular mechanism for the Shp-2 tyrosine phosphatase function in promoting growth factor stimulation of Erk activity. Mol. Cell. Biol. 20, 1526–1536.
141 Wu, C. J., O'Rourke, D. M., Feng, G. S., Johnson, G. R., Wang, Q., and Greene, M. I. (2001) The tyrosine phosphatase SHP-2 is required for mediating phosphatidylinositol 3-kinase/Akt activation by growth factors. Oncogene 20, 6018–6025.
142 McKay, M. M., and Morrison, D. K. (2007) Integrating signals from RTKs to ERK/MAPK. Oncogene 26, 3113–3121.
143 Yeatman, T. J. (2004) A renaissance for SRC. Nat. Rev. Cancer 4, 470–480.
144 Kopetz, S., Shah, A. N., and Gallick, G. E. (2007) Src continues aging: current and future clinical directions. Clin. Cancer. Res. 13, 7232–7236.
145 Deng, X. W., and Quail, P. H. (1999) Signalling in light-controlled development. Semin. Cell. Dev. Biol. 10, 121–129.
146 Dornan, D., Wertz, I., Shimizu, H., Arnott, D., Frantz, G. D., Dowd, P., O'Rourke, K., Koeppen, H., and Dixit, V. M. (2004) The ubiquitin ligase COP1 is a critical negative regulator of p53. Nature 429, 86–92.
147 Wertz, I. E., O'Rourke, K. M., Zhang, Z., Dornan, D., Arnott, D., Deshaies, R. J., and Dixit, V. M. (2004) Human De-etiolated-1 regulates c-Jun by assembling a CUL4A ubiquitin ligase. Science 303, 1371–1374.
148 Yi, C., Li, S., Wang, J., Wei, N., and Deng, X. W. (2006) Affinity purification reveals the association of WD40 protein constitutive photomorphogenic 1 with the hetero-oligomeric TCP-1 chaperonin complex in mammalian cells. Int. J. Biochem. Cell. Biol. 38, 1076–1083.
149 Ylikomi, T., Bocquel, M. T., Berry, M., Gronemeyer, H., and Chambon, P. (1992) Cooperation of proto-signals for nuclear accumulation of estrogen and progesterone receptors. Embo. J. 11, 3681–3694.
150 Beato, M., and Klug, J. (2000) Steroid hormone receptors: an update. Hum. Reprod. Update 6, 225–236.
151 Pestka, S., Krause, C. D., and Walter, M. R. (2004) Interferons, interferon-like cytokines, and their receptors. Immunol. Rev. 202, 8–32.
152 Reich, N. C. (2007) STAT dynamics. Cytokine Growth Factor Rev. 18, 511–518.
153 Taganov, K. D., Boldin, M. P., Chang, K. J., and Baltimore, D. (2006) NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. Proc. Natl. Acad. Sci. U S A 103, 12481–12486.
154 Volinia, S., Calin, G. A., Liu, C. G., Ambs, S., Cimmino, A., Petrocca, F., Visone, R., Iorio, M., Roldo, C., Ferracin, M., Prueitt, R. L., Yanaihara, N., Lanza, G., Scarpa, A., Vecchione, A., Negrini, M., Harris, C. C., and Croce, C. M. (2006) A microRNA expression signature of human solid tumors defines cancer gene targets. Proc. Natl. Acad. Sci U S A. 103, 2257–2261.
155 Sunnaram, B. L., Gandemer, V., Sebillot, M., Grandgirard, N., Amiot, L., Leray, E., and Goasguen, J. E. (2003) LRP overexpression in monocytic lineage. Leuk. Res. 27, 755–759.
156 Mossink, M. H., De Groot, J., Van Zon, A., Franzel-Luiten, E., Schoester, M., Scheffer, G. L., Sonneveld, P., Scheper, R. J., and Wiemer, E. A. (2003) Unimpaired dendritic cell functions in MVP/LRP knockout mice. Immunology 110, 58–65.
157 Raidl, M., Berger, W., Schulte-Hermann, R., Kandioler-Eckersberger, D., Kappel, S., Wrba, F., Micksche, M., and Grasl-Kraupp, B. (2002) Expression of the lung resistance-related protein in human and rat hepatocarcinogenesis. Am. J. Physiol. Gastrointest. Liver Physiol. 283, G1117–1124.