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Structure, regulation and cellular functions of Rab geranylgeranyl transferase and its cellular partner Rab Escort Protein

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Abstract

Rab geranylgeranyl transferase is an enzyme responsible for double geranylgeranylation of Rab proteins in all eukaryotic cells. In the present article we would like to focus on new findings concerning the holoenzyme structure and mechanism of catalytic activity, its mode of regulation and consequences of RGGT deficiency in different eucaryotic model organisms and patients.

Keywords: *Rab geranylgeranyl transferase, Rab Escort Protein, choroideremia*

Introduction

Protein prenylation is the post translational modification leading to an attachment of a 15-carbon farnesyl or 20-carbon geranylgeranyl isoprenoid chain to a cysteine residue in a protein by a tioether bond. Three enzymes catalyse this modification: Farnesyl transferase (FT), Geranylgeranyl transferase I (GGT I) or Rab geranylgeranyl transferase (RGGT syn. GGT II). The cysteine is localized in a conserved amino acid motif at the C-terminus of the polypeptide and the recognised sequence is specific for each of the enzymes. FT and GGT I recognize the -CAAX motif (cysteine, two aliphatic amino acids, any amino acid) and RGGT AQ4 recognizes the group of motifs -CXCX, -CCXX or –XXCC. RGGT is an enzyme responsible exclusively for prenylation of proteins belonging to the Rab GTPase family. The RGGT complex is built of two subunits: α and β forming a catalytic core and an accessory, substrate presenting protein – Rab Escort Protein (REP). The double geranylgeranylation enables the proper localization of Rab proteins in the cell membranes. Unmodified Rabs localize to the cytoplasm where they are unable to perform their normal function in vesicle budding, transport and fusion.

The RGGT activity was discovered in 1980s and since then has been the subject of increasing attention. Many interesting aspects of the enzyme structure, mode of substrate recognition and involvement in pathophysiology of human disease have been thoroughly reviewed in earlier articles by Alory and Balch (2001), Leung et al. (2006), Hutagalung and Novick (2011), Nguyen et al. (2010) and Coussa and Traboulsi (2011).

In the present article we would like to focus on new findings concerning the holoenzyme structure and mechanism of catalytic activity, its mode of regulation and consequences of RGGT deficiency in different eucaryotic model organisms and patients.

The structure of RGGT

Crystallographic studies on the structure of Rab Geranylgeranyl Transferase (RGGT) have started more than a decade ago with the release of the structure of the heterodimer of the α and β subunits of the rat enzyme (Zhang et al. 2000). So far, efforts to obtain the structure of the whole complex of RGGT αβ heterodimer together with Rab Escort Protein (REP) bound to Rab protein and prenyl substrate (geranylgeranyl diphosphate GGPP) or prenylated product have not been successful due to the low diffraction of a crystal of such a multidomain complex (Rak et al. 2001, Wu et al. 2009). Structures of distinct functional modules of the complex (αβ heterodimer of RGGT (Zhang et al. 2000), REP/monoprenylated Rab7 (Rak et al. 2004), REP with αβ heterodimer (Pylypenko et al. 2003) and structure of a ternary complex with truncated α subunit (Guo et al. 2008)

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have been solved experimentally leading to a detailed computational model of the whole Rab geranylgeranylation machinery from mammals (Wu et al. 2009). No structures of the enzymatic complex from other organisms have been solved so far. Literature data on the structure of the RGGT holoenzyme and the catalytic mechanism of the RGGT enzymatic complex are summarized in a following chapter.

α subunit of RGGT

The rat α subunit of RGGT (RGGTA) is very similar to the corresponding α subunit of farnesylprotein transferase (FT), containing 15 α-helices arranged in a crescent-shaped, double layered right-handed superhelix, enveloping the beta-subunit (Zhang et al. 2000). Structurally it can be classified as a tetratricopeptide repeat (TPR) superfamily protein. Phylogenetic analysis showed that the one and only duplication event of CAAX prenyltransferase α subunit leading to RGGTA subunit must have happened and the diversification preceded the split of the eukaryotic main groups (Rasteiro and Pereira-Leal 2007).

RGGTA subunit interacts with REP on one surface and with β subunit of RGGT (RGGTB) on the opposite surface of the protein (Pylypenko et al. 2003). The contact side with REP covers a surprisingly small area, compared with the large interaction surface of both subunits of the αβ heterodimer. Binding of REP causes only small rearrangements in overall structure of the RGGTAB heterodimer, mainly in the phosphoisoprenoid binding pocket in the RGGTB subunit and on the REP binding surface of the RGGTA. The RGGTA/REP interaction is allosterically regulated by the binding of phosphoisoprenoid by the means of a long range trans-domain transduction (Pylypenko et al. 2003).

Rat RGGTA displays a globular domain inserted in the middle of the TPR domain. The function of this insertion is unclear but it is not involved in contacts either with REP or Rabs (Pylypenko et al. 2003) as was suggested earlier (Zhang et al. 2000). This domain belongs to a class of C2-like domains which are involved in signalling, vesicular transport and modification of lipids (Nalefski and Falke 1996). C2s function in establishing phospholipid complexes; sometimes they mediate protein-protein interactions by direct binding to phosphotyrosine (Benes et al. 2005). This domain is not present in the yeast orthologue and interestingly in a related α subunit of the FT another domain, 3_{10} helix, is inserted in the same place. Only metazoa, plants and alveolata display insertions of an IgG-like domain in this region of RGGT. The domains in different groups of organisms result from independent insertion or expansion events. They are similar within taxonomical groups, but different beyond recognition across taxa (Rasteiro and Pereira-Leal 2007). Multiple insertions in the same site suggest that this site is capable of accommodating structural variations more easily than others. Whether the structural diversity of this region between animals and plants is mirrored by the diversity of functions remains unknown.

At the C-terminus of the rat RGGTA subunit a Leucine Rich Repeat domain is present, not found in the related FT. This domain is a right handed βα superhelix. LRRs are involved in the establishment of complexes with other proteins (Kobe and Kajava 2001). LRR in RGGTA is not universal, it is found in some animals, angiosperms and alveolata. The phylogenetic data point to multiple losses of this insert during evolution (Rasteiro and Pereira-Leal 2007). RGGTA lacking the LRR and IgG domains is stable in a dimer with RGGTB subunit and shows prenylation activity comparable to a wt enzyme (Guo et al. 2008). The arrangement of the TPR domain of RGGTA subunit with the RGGTB subunit in the truncated enzyme is nearly identical to the structure of the intact complex.

β subunit of RGGT

The β subunit of RGGT (RGGTB) of rat is an α-α barrel composed of 12 α-helices, resembling the fold of the β subunit of farnesyltransferase (FT) and geranylgeranyltransferase I (GGT I) (Zhang et al. 2000). Generally, the β subunits are more conserved than the α ones. The isoprenoid (geranylgeranyl diphosphate [GGPP]) is held in the hydrophobic binding cleft buried in the barrel that is formed by the conserved aromatic residues. The phosphate moiety binds in a positively charged cleft that is located near the subunit interface and is close to the catalytic zinc ion. Binding of GGPP causes minor changes in the structure, mostly in the hydrophobic pocket. The region of binding of the phosphate group and carbons 1–12 is very similar to GGT I but the bottom of the cavity is expanded, which makes RGGT more tolerant to the substitution of the distal part of the isoprenoid chain than other CAAX prenyltransferases (Nguyen et al. 2009, 2010).

Farnesyl diphosphate (FPP) functions as an efficient lipid donor for Rab prenylation *in vitro*, but farnesylated Rabs have not been found *in vivo*. This is likely due to the fact that RGGT binds with 100-fold greater preference GGPP over FPP, providing a thermodynamically driven selection of the appropriate lipid substrate. Crystallization of the enzyme with mono- and di-prenylated peptides showed that the lipid binding cavity is occupied by the isoprenoid, but the electron density for the peptide part was very poor, indicating weak interactions

within the peptide-binding site. In contrast to the other prenyltransferases, the RGGT does not possess the exit groove for the product. This is also an indirect indication that the affinity of the prenylated intermediate or product for RGGT is low. Mono- and di- prenylated peptides bind with comparable low micromolar affinities. The only strong interaction is by the lipid binding site, and the second lipidation does not positively contribute to the affinity of the interaction of the enzyme and the product, di-geranylgeranylated Rab (Guo et al. 2008).

Rab Escort Protein (REP)

The Rab Escort Protein (REP) belongs to the same family as Rab GDP dissociation inhibitor (RabGDI) (Waldherr et al. 1993, Ragnini et al. 1994). Its structure is composed of two subdomains. The crystal structures of the REP protein in complex with the mono-prenylated or C-terminally truncated Rab revealed that Rab interacts with the Rab-binding platform of REP via an extended interface involving the switch 1 and 2 regions (Rak et al. 2004). The C-terminus of the REP molecule acts as a mobile lid covering a conserved hydrophobic patch on the surface of REP that in the complex coordinates the C-terminus of Rab proteins. Several functional motifs such as the Rab-binding platform and the mobile effector loop are highly conserved between REP and RabGDI. The RGGT-binding site in domain II is unique for REP. The C-terminal binding region (CBR) binds the C-terminus of Rab and directs it towards a lipid binding site located on domain II.

Mammalian REPs display an insertion between domains I and II that is absent in RabGDIs. This insert is not involved in contacts with the RGGT subunits nor Rab substrates. Some phylogenetic groups display a longer insert in this position of REP protein, its sequence is not conserved; the inserts are similar within taxa, but different across taxa (Rasteiro and Pereira-Leal 2007). These inserts are not similar to any other protein, in the crystal structure of the rat enzyme they show no clear electron density (Rak et al. 2004). By computer analysis the inserts are predicted to have disordered structure, and probably the regions are natively unfolded (Rasteiro and Pereira-Leal 2007). They might have a regulatory function. Similarly, the very C-terminus of REP is disordered in the structure of REP and RGGT heterodimer in the absence of Rab (Pylypenko et al. 2003). It appears that the REP 36 C-terminal amino acids form a lid covering the CBR in the absence of Rab. In the structure of Rab truncated of C-terminal tail, the two REP molecules swap C-termini (Rak et al. 2004). In the structure of REP-Rab complex the C-terminus of REP interacts with the C-terminus of Rab, albeit in an inverted direction of polypeptide chain (Rak et al. 2004).

The hypervariable tail of Rab protein, consisting of amino acids C-terminal to the above mentioned motif interacting with REP and including prenylatable cysteines, do not substantially contribute to the affinity of Rab to REP (Wu et al. 2009). However, together the C-terminal hydrophobic motif (CIM) and the length of the spacer between it and prenylatable cysteines in Rab have a central function in Rab prenylation. Mutation in the CIM motif results in a 30-to 70-fold reduction in Rab-REP affinity. Deletion of the prenylation motif or even the whole 14 amino acids downstream of CIM has limited influence on the affinity of the interaction. Mutations in the CIM motif lead to underprenylation and mislocalization of Rabs in the cells (Rak et al. 2004). Extension of the C-terminus by four or five arbitrarily chosen residues after the prenylatable cysteines does not decrease prenylation efficiency suggesting that substrate recognition is truly sequence independent (Guo et al. 2008). The C-terminus binding region (CBR) of REP appears to promote prenylation by enabling the localization of the Rab C-terminal cysteines in the vicinity of the RGGT active site. Binding of GDP-bound Rab to REP structuralizes the Rab Switch I and Switch II regions and strengthens the interaction, slowing the rate of GDP to GTP exchange. In the Rab GTP bound form, the structure of Switch II has a different conformation that would lead to a steric clash with REP.

Out of 32 residues forming contacts on the REP-Rab interface only six are specific for REP and not GDI. Three of them are engaged in contacts with the C-terminus of Rab. The specific residues are located at the edges of the interaction interface (Rak et al. 2004). REP protein may be mutagenized to perform both REP and GDI-like functions but not *vice versa* (Alory and Balch 2003).

Prenylation of Rab proteins

The following data led to a proposal of a model of Rab prenylation (Guo et al. 2008): The Rab GTPase domain is recognized by Rab-binding platform (RBP) of REP. Next the complex is tightened by the interaction of C-terminal hydrophobic motif on Rab (CIM) with the C-terminal Binding Region (CBR) on REP. This complex binds with high affinity to the RGGTA by an interaction of domain II of REP. The affinity is further strengthened by the interaction of the very C-terminus of Rab with the active site of RGGTB subunit. From this perspective one can view CIM as being analogous to the AAX motif of CAAX-type protein prenyltransferases working from a remote location.

The lack of a well-defined substrate binding mode also explains the variation in the observed sequence of isoprenoid addition in the Rab geranylgeranylation reaction. The order of prenylation of the two cysteines is random, but the mono-prenylated product of the first reaction does not dissociate from the enzyme. The lack of precise positioning and high affinity of the enzyme-substrate interaction may be the cause why RGGT is the slowest prenyltransferase (K1 = 0.16/s, K2 = 0.04/s). Following the second prenylation finally the high affinity binding substrate GGPP dislodges the bulky product from the active site. The di-prenylated Rab molecule C-terminus consequently associates with the lipid-binding site on REP. This induces REP conformational change and liberates the RGGTAB heterodimer from Rab-REP complex.

It is possible to predict the influence of a mutation in a particular site of interaction on Rab affinity towards REP (Rak et al. 2004, Guo et al. 2008, Wu et al. 2009). However, so far it is still impossible to state arbitrarily which Rab will have a higher affinity for REP *in vitro*, since the binding surface is composed of many separated interactions, as described above. An even more complicated situation is encountered *in vivo*, where both the affinity of particular Rabs towards REP and the number of molecular species competing for the interaction must be considered. Therefore the influence of REP (or RGGT) mutation on deficiency of Rab proteins prenylation (all Rab species present in particular cell/tissue) must be considered separately. Some examples of cell or tissue specific influence of the geranylgeranylation machinery are described in later sections of this article.

Regulatory aspects of RGGT

Apart from the well documented function of the RGGT complex machinery in Rab protein prenylation, some unexpected regulatory links of protein transport to other cellular processes have been found. The best documented results come from yeast genetic interaction screens, however, the precise mechanism of how the Rab geranylgeranylation activity may be related to other cellular processes has not always been proposed. Nevertheless, vesicular transport facilitated by Rabs seem to be coupled to pre-mRNA splicing and nutrient sensing (Fujimura et al. 1994, Jacoby et al. 1998, Bialek-Wyrzykowska et al. 2000, Singh and Tyers 2009) in yeast *Saccharomyces cerevisiae* as is described in later sections. On the other hand, the whole process of Rab geranylgeranylation in yeast is strictly dependent on the GGPP substrate availability (Miaczynska et al. 2001) and the genes responsible for GGPP synthesis are often found in genetic screens together with the RGGT complex (Newman and Ferro-Novick 1987, Vincent et al. 2003, Singh and Tyers 2009). The coupling of isoprenoid phosphate level in a cell and Rab geranylgeranylation may be different in higher Eukaryotes.

RGGT regulation by GGPP in yeast, mammals and plants

Binding of the Rab-REP complex to mammalian RGGT was found to be strongly modulated by GGPP (Thoma et al. 2001a). The affinity of RGGT for the Rab-REP complex increases from 120–2 nM in the presence of GGPP. Affinity of doubly prenylated Rab-REP complex to RGGT was 2 and 18 nM in the absence and presence of the GGPP, respectively. Binding of the new isoprenoid substrate molecule facilitates the diprenylated product release by RGGT. As was shown on the basis of crystal structures, RGGTA subunit changes conformation upon GGPP binding to RGGTB subunit by means of long distance allosteric interaction (Pylypenko et al. 2003). This conformational change in the RGGT-GGPP complex increases its affinity for REP and REP-Rab complex. GGPP plays three different roles in the catalytic cycle of the RGGT: as an allosteric activator, phosphoisoprenoid donor and substrate release trigger (Thoma et al. 2001b). In yeast enzyme the Km values for the prenyl diphosphates are approximately an order of magnitude larger than for their mammalian counterparts (Witter and Poulter 1996). This phenomenon may reflect differences in the concentrations of isoprenoid metabolites in mammalian and fungal cells. In the case of yeast RGGT Km for GGPP is 40 nM and for prenylated Rab-REP or unprenylated Rab-REP both Km are 200 nM, GGPP does not influence the affinity of REP to RGGT, in contrast to the mammalian enzyme (Dursina et al. 2002), so it does not serve as an activator. Similarly to the mammalian enzyme the binding of a new molecule of lipid substrate enables product release. As it has been mentioned above, the synthesis and demand for GGPP as a substrate for geranylgeranylation of proteins in yeast are coupled. Despite differences in affinity of RGGT to GGPP, the mode of product release in mammalian RGGT resembles its yeast counterpart. In both cases there exists only one gene coding for the GGPP synthase. Moreover, human REP may substitute for its yeast counterpart in yeast *mrs6*$^{-/-}$ strain.

In plants where several genes coding for GGPP synthases are present the situation might be strikingly different. Additionally, it should be taken into consideration that in plants isoprenoid precursors are derived from two biosynthetic pathways, unlike in yeast or mammals. The GGPP moieties used for geranylgeranylation of proteins are synthesized mainly by a

plastidial methylerythritol phosphate (MEP) pathway (Gerber et al. 2009); however, the metabolite exchange of the intermediates between the classical cytosolic mevalonate (MVA) pathway and the plastidial MEP pathway cannot be excluded (Skorupinska-Tudek et al. 2008). Therefore, the availability of the metabolites for geranylgeranylation must be completely different than in yeast and animals. Interestingly, the conserved amino acid residue involved in REP binding to RGGTA subunit is changed in the whole plant phylogenetic lineage. It is known that plant REP cannot substitute for yeast REP *in vivo* due to this single amino acid change. This situation may reflect a different mode of regulation of plant RGGT by isoprenoid donor than in the case of mammalian and yeast enzymes (Hala et al. 2005, Wojtas et al. 2007).

Genetic interactions of RGGT subunits in yeast

The first interesting, but not fully understood aspect of RGGT genetic interactions is a suppressor role of RGGT α and β subunits (together with other proteins engaged in vesicle transport) in pre-mRNA splicing (Vincent et al. 2003, Pandit et al. 2009).

Overexpression from *bet4* gene (block in early transport-coding for yeast RGGTA) suppresses the mutation (but not the complete loss) of clf1 spliceosome assembly factor even though both proteins seem not to interact with each other physically, since the affected strain growth defect is rescued without detectable improvement in pre-mRNA splicing efficiency (Vincent et al. 2003). The authors suggest that similarly to earlier findings (Chen et al. 1998) mutations in splicing factor genes cause the vesicular transport defect. Overexpression of genes responsible for the early stages (ER to Golgi) of sorting may rescue the splicing phenotype simply by increasing the pool of modified and active transduction molecules if some of the molecules have overlapping and redundant functions. This may be true, as a gene coding for ypt1p (yeast Rab), one of the main ER to Golgi transport molecules, modified by RGGT complex, is intronless. Interestingly, overexpression of the RGGTB coding gene, *bet2*, interfered with growth of another splicing defective strain carrying the mutation in prp38 helicase (Pandit et al. 2009).

Another well documented genetic interaction of RGGT complex subunit is *mrs6* (yeast gene coding for REP) suppression of *mrs2* mutation (mrs2 is a Mg^{2+} channel involved in mitochondrial cytochrome assembly). mrs6p overproduction rescues the respiratory deficiency in *mrs2* strain (Waldherr et al. 1993); however, the precise mechanism has never been elucidated.

In parallel to the aforementioned *mrs6* genetic interaction, the same gene (formerly called *msi4*) was discovered as a multicopy suppressor of the *ira1* mutation in yeast (*ira* codes for a GTPase activating protein for ras2p, acting competitively with cdc25p guanine exchange factor for ras2, and upstream from adenylate cyclase in a pathway regulating response to the nutrient supply). *mrs6* overexpression reverts the heat shock phenotype caused by accumulation of a high level of cAMP, stimulation of protein phosphorylation and lack of cell cycle arrest at G1 upon nutrient starvation in an *ira1* mutant strain (Fujimura et al. 1994). The *mrs6* gene must therefore act negatively on the ras/cAMP pathway, downstream of the cAMP dependent protein kinase. The precise mechanism of this genetic interaction has never been solved, however, it has been more recently confirmed by the observation that *mrs6* overexpression partially bypasses the growth defect caused by hyperactivation of the PKA pathway in $ras2^{Val19}$ strain (in which ras2p is constitutively active) (Singh and Tyers 2009).

The most interesting observations concerning mrs6p function have been published recently by two groups (Lempiainen et al. 2009, Singh and Tyers 2009). In two independent genetic screens in yeast the link between vesicular transport and TOR kinase signalling has been demonstrated. In search for a direct protein interaction, the sfp1 and mrs6p tight and stoichiometric binding was discovered by the proteomic methods (Singh and Tyers 2009). The transcription factor sfp1 couples nutrient status to cell growth rate by controlling the expression of ribosome biogenesis (Ribi) and ribosomal protein (RP) genes. sfp1 is localised to the nucleus in rich nutrients, but upon nutrient limitation or TOR pathway inhibition by rapamycin, sfp1 rapidly exits the nucleus, leading to repression of the Ribi and RP regulons. PKA signalling inhibition affects the localization status of sfp1. In a $ras2^{Val19}$ strain the nuclear localization of sfp1 is affected as well (Jorgensen et al. 2004).

mrs6 protein exhibits a nutrient sensitive interaction with sfp1. Overexpresion of mrs6p prevents nuclear localization of sfp1 in rich nutrients and loss of mrs6p causes nuclear localization of sfp1 even in poor nutrients. Unexpectedly, this effect is independent of protein kinase C (Fujimura et al. 1994). mrs6p and sfp1 interaction links the secretory pathway and TOR dependent nutrient signalling to ribosome biogenesis. *mrs6* is able to override the nutrient control of sfp1 localization. The TOR network and PKA network relay amino acid and glucose supply to the cellular machinery, but some of the targets of the kinases are different. So the regulation of sfp1 must be parallel to the PKA pathway.

The authors also localized the probable site of interaction of sfp1 and mrs6p. TOR, its activators and effectors localise to the internal membrane system. In a pull down-proteomic screen for interactors

of sfp1 the prominent band was mrs6p. mrs6p was also shown to localize in the ER/Golgi membranes (Miaczynska et al. 1997). Its localization and sensitivity to salt and detergents is very similar as for sfp1 and suggests that they may meet with TOR on the endomembranes.

The interaction of sfp1 with mrs6p engage the same surface on mrs6 protein that is occupied by Rabs. In a screen for protein interactions, ypt1p overexpression interfered with the growth of the *mrs6/sfp1* strain. The sec4p overexpression gave a weaker effect. Rapamycin-resistant alleles of *mrs6*, mimicking constitutively active TOR, are defective for sfp1 relocalization. The mutations in RGGT interactions, responsible for growth defects and G2/M arrest (Bialek-Wyrzykowska et al. 2000) are separable from the mutations responsible for sfp1 re-localization alleles. The location of the mrs6p amino acid substitutions in the Rab-binding domain suggests that the Rab and sfp1 compete for closely juxtaposed binding sites on mrs6p.

In one of the non-lethal *mrs6* mutated strains, *mrs6-2* (Bialek-Wyrzykowska et al. 2000), bi-nucleated cells are formed. A similar effect is apparent in *rho3* and *pkc1delta* strains. General secretion defects do not show the phenotype of nuclear segregation defects that may be independent of the role of mrs6p in geranylgeranylation. The *slg* supressor of *mrs6-2* codes for a protein able to activate pkc1p (Jacoby et al. 1998), a kinase that mainly transmits the signals for transcription of genes involved in cell wall biosynthesis and cell cycle progression.

It remains unknown whether there is a link of the machinery introducing geranylgeranyl groups to Rab proteins (mrs6p/REP) and therefore having a role in the intracellular transport, to one of the main regulatory proteins of cell growth in higher Eucaryotes (TOR kinase). Moreover, a tempting possibility is that the system is also regulated by the input from other important regulatory kinase pathways, PKA and PKC. In mammals a functional homologue of TORC1 and mrs6p exist and the c-myc proto-oncogene product has a similar function to sfp1. c-myc is one of the few known regulators of RP and Ribi genes, its function is linked to TOR and PKA signalling, c-myc overexpression leads to increased expression of genes encoding ribosomal proteins (Lempiainen et al. 2009). The homologues of ras, PKC and PKA are also present in mammals. Plants, similarly to animals, lack the homologue of sfp1 and its potential analogue, c-myc, but the remaining elements of the machinery (TOR, PKA, PKC, REP) are present. The potential similarities in REP function as a molecular switch in nutrient response in higher organisms cannot be ruled out; this concept, however, needs experimental proof.

RGGT complex regulation in higher Eukaryotes

While compared to yeast, the knowledge on regulation of the RGGT complex in plants and animals still remains elusive. Some aspects of regulation and phenotypic effects of RGGT or REP deficiency are described in the following section. Here attention will be paid to a few results directly concerning the aspects of RGGT regulatory roles.

As a less obvious aspect of the RGGT complex activity we have to mention the results on the human protein phosphatase PRL2 function in the RGGT activity regulation (Si et al. 2001). Protein phosphatase PRL2 was found to specifically interact with the RGGTB subunit in human HeLa cells and in yeast two-hybrid system. The protein is natively farnesylated (but never geranylgeranylated) and the modified form localizes to the early endosome while the unmodified one is found in the nucleus. The interaction is strongly dependent on an intact farnesyl moiety and residues in the C-terminus preceding the CAAX motif, since an unmodified protein or one devoid of the C-terminus does not interact with RGGTB. Binding of RGGTA and PRL2 to RGGTB is mutually exclusive. By this means PRL2 overexpression inhibits RGGT activity. This suggests a cellular mechanism by which the activities of protein prenyltransferases may be reciprocally balanced.

A very recent report (Lachance et al. 2011) states that the human RGGTA subunit interacts with a dileucine motif in the β2 adrenergic receptor to regulate its maturation and trafficking. The receptor regulates Rab prenylation by RGGTA. The β2 adrenergic receptor co-localizes with RGGTA to intracellular membrane compartments and the plasma membrane. RGGTA binds the dileucine motif in the β2 adrenergic receptor C-terminus known to be involved in the transport of the receptor from the ER to the cell surface. RGGTA has a positive role in maturation and anterograde trafficking of the receptor and the receptor modulates the geranylgeranylation of some, but not all Rabs. The geranylgeranylation function in RGGTA and the receptor maturation competence are independent.

Pathophysiological manifestations of RGGT activity impairment

The following section attempts to summarize the consequences of RGGT impairment. Its structure reflects composition of the RGGT holoenzyme. Thus literature referring to the effects of the disturbance in the cellular function of REP – the RGGT

accessory protein – is followed by the observations concerning heterodimeric enzyme subunits.

Rab Escort Protein

As mentioned earlier Rab Escort Protein is an accessory subunit of RGGT absolutely required for proper catalysis. So far at least one REP sequence has been identified in all studied species. In contrast to most species, mammals have two paralogous sequences – REP1 (CHM) and REP2 (CHM-like, CHML), sharing 75% amino acids identity (Cremers et al. 1994); duplication of REP has also been noted for *Xenopus laevis* (Rasteiro and Pereira-Leal 2007).

Humans

REP1 is a *CHM* gene product and the *CHM*/*REP1* gene is the only gene to date associated with choroideremia (CHM), it refers to the absence of the choroid. This rare inherited disease is caused by loss-of-function mutations leading to a truncated, non-functional, or rapidly degraded REP1 protein (Scriver et al. 1995). CHM is an X-linked recessive progressive retinal degeneration disease affecting males and with milder symptoms in carrier females, its incidence is 1 in 50,000. In CHM-affected males night blindness is the most common first symptom in childhood (first or second decade of life). As the disease progresses constrictions of the visual field and progressive loss of vision are noted. Most patients are legally blind by their mid-40s (MacDonald et al. 1993). In parallel to the changes of visual acuity fine pigmentary changes with focal choroidal atrophy appear around the equatorial fundus ('salt and pepper' pattern) and degeneration progresses more centrally (atrophy of the choroid and retinal pigment epithelium, RPE) (Coussa and Traboulsi 2011). Female carriers of CHM are mostly asymptomatic, except for the enlargement of the blind spot and with clinical findings resembling those of young affected males (patchy fundal pigmentation). This characteristics could be explained by the hypothesis of unbalanced X chromosome inactivation (the presence of embryologically distinct lines of photoreceptors and RPE clones expressing either the mutant or normal *REP1* allele) or by the X-autosomal translocations of Xq21 (Coussa and Traboulsi 2011, and references therein). Even though CHM is most often an isolated ophthalmic disease a few reports of associated abnormalities resulting from defects in the *REP1* adjacent loci have been reported (manifested as psychomotor retardation, birth defects, deafness, cognitive deficit) (Coussa and Traboulsi 2011, and references therein).

In humans *CHM*/*REP1* loss-of-function mutations result most often in eye disease; lack of symptoms in other tissues is explained by the functional redundancy provided by the presence of REP2, *CHML* (choroideremia-like) gene product with 75% amino acid identity to REP1. REP-2 in mammals has emerged presumably through reversed transcription of REP-1 gene message and is devoid of introns that makes it refractory to mutations at splicing sites. Both REP1 and REP2 are ubiquitously expressed in human tissues (Cremers et al. 1994). Two possible explanations of CHM background have been suggested. The first hypothesis suggests that prenylation of different Rabs by REP1 and REP2 is performed with variable efficacy. Consequently, REP2 efficiently compensates for the loss of REP1 in all tissues except the eye where a subset of Rabs, such as Rab 27a remains underprenylated (Seabra et al. 1995). According to the second hypothesis the rate of prenylation of Rab27a mediated by REP2 is only 2-fold lower than that mediated by REP1; however, the affinity of Rab27a is generally lower for both isoforms of REP. Competition among all the cellular Rabs for REP2 upon reduction of overall REP activity caused by the absence of REP1 discriminates against those Rabs of low affinity (Rak et al. 2004). In contrast to REP1, no disease resulting from the loss of REP2 has been identified so far.

Several studies have been performed aimed at identification of the molecular cause of the disease. Most of the pathogenic mutations reported so far in the human *CHM* gene result in a complete loss of REP1 protein or its function (MacDonald et al. 2004). A few selected reports are summarized below. In some CHM patients the *CHM* reading frame is maintained but the protein product lacks several amino acids belonging to the structurally conserved regions thus the disease is probably caused by the loss of function of the REP-1 protein rather than by its absence (Garcia-Hoyos et al. 2008, Esposito et al. 2011). Analysis of the functional effects of some *CHM* mutations revealed a point mutation L550P which results in an unfolded protein product that is rapidly degraded. Analysis of the structure of this and three other mutated (shortened by 150 C-terminal amino acids or devoid of internal 473 or 100 amino acids) REP1 protein variants based on homology modeling (rat and human REP1 were superimposed) explained the effects of mutations as a loss of the REP1 essential activity or protein-protein interactions (Sergeev et al. 2009).

To follow CHM pathogenesis and genotype/phenotype correlations peripheral cells of CHM patients (primary skin fibroblasts and CD14+ fraction of monocytes) have been employed (Strunnikova et al. 2009). pH was found to be increased in lysosomes of

monocytes of CHM patients and consequently significantly reduced rates of proteolytic abilities of the monocytes were noted. Elucidation of the secretion processes revealed significantly lower levels of secreted cytokine/growth factors (macrophage chemoattractant protein-1 MCP-1, pigment epithelial derived factor PEDF, tumor necrosis factor TNFα, fibroblast growth factor FGFβ and interleukin IL-8) in CHM fibroblasts. Microarray analysis revealed significant up-and-down regulation of a number of genes involved in the immune response, small GTPase regulation, secretion, the regulation of transcription, cell adhesion and the regulation of exocytosis in both CHM fibroblasts and monocytes.

It has been also suggested that CHM could result from genetically altered renewal systems (defect of phagocytosis) in photoreceptors and the retinal pigment (Rodrigues et al. 1984), however, the direct connection with the dedicated Rab(s) remains elusive.

Rodents

Studies on rat tissues have revealed ubiquitous expression of both REPs (Seabra, 1996). Analysis of the mRNA localization of *Chm* (coding for REP1) and *Chml* (coding for REP2) transcripts in the mouse retina proved their overlapping broad expression profile, however, the pattern of REP1 protein localization was found to be different from what had been reported in the human (Keiser et al. 2005).

Knock-out of the *Chm* gene results in much more severe symptoms in animals. Mice with disrupted *Chm/rep-1* gene were obtained by a gene targeting approach which produced a mutated REP1 protein with a C-terminal truncation of 274 amino acids (van den Hurk et al. 1997). In these mice *Chm* KO is lethal in hemizygous male embryos (Chm^-/Y); in heterozygous female embryos it is only lethal if the mutation is of maternal (Chm^-/Chm^+) but not paternal (Chm^+/Chm^-) origin (van den Hurk et al. 1997). Heterozygous Chm^+/Chm^- females are viable and exhibit progressive degeneration of the photoreceptors reminiscent of human CHM. The observed imprinted pattern is explained by the preferential inactivation of the paternally inherited X-chromosome in murine extra-embryonic tissues. Abnormalities in extra-embryonic mouse tissues, yolk sac and placenta (severe defects in vasculogenesis) have been observed despite the presence of the *Chml* gene. Moreover, it has been suggested that REP1 acts in maintaining proliferation and in differentiation of diploid trophoblast (Shi et al. 2004).

As mentioned above, heterozygous females are unable to transmit the Chm^{null} allele to either heterozygous females or hemizygous males. This problem has been avoided by creating a conditional model of CHM – a tissue-specific (in the retinal pigment epithelium and photoreceptors, independently) and tamoxifen-inducible knockout of the *Chm* gene. Heterozygous females show early onset and progressive retinal degeneration similar to human CHM. Cell-autonomous degeneration associated with different subsets of underprenylated Rabs in photoreceptors and retinal pigment epithelium has been observed (Tolmachova et al. 2006).

Non-mammalian vertebrates

In zebrafish and other non-mammalian vertebrates only a single *chm* gene encoding protein similar to mammalian REP is present. Mutation in the *chm* gene (a stop codon position at 32 out of 666 amino acids) results in a 90% reduction in inner ear hair-cell number, causing deafness, disequilibrium and abnormality of the lateral-line organ and partial retinal degeneration by 5 days post-fertilization (Starr et al. 2004). Even though the loss of REP results in lethality of zebrafish larva, a transient rescue of the mutant is quite unexpected in light of the requirement for functional Rab-dependent vesicular transport machinery. This phenomenon has been attributed to the presence of the maternally derived *chm* transcript in the larva (Starr et al. 2004). Careful examination of the retinal phenotype of the *rep* mutant reveals consistency with CHM (photoreceptor degeneration, loss of visual function, and defects in RPE pigmentation and outer segment phagocytosis) (Krock et al. 2007). Further studies of the same *chm* KO zebrafish (Moosajee et al. 2009) revealed catastrophic degeneration of the retina after 4.5 days-post-fertilization with a severe multisystem disease. At the late stage of these symptoms accumulation of unprenylated Rabs in the cytosol was demonstrated. It is suggested that the absence of full-length REP is a lethal mutation in zebrafish and that once the maternal supply derived from the egg sac is exhausted, RGGT dysfunction results in general cellular malfunction and death. Additionally, a pool of geranylgeranylated Rabs of maternal origin might also persist for a time resulting in prolongation of the shortage of RGGT activity until the pool of prenylated Rabs had turned over (Moosajee et al. 2009).

Invertebrates

It has been suggested that in a model invertebrate *Caenorhabditis elegans*, the RGGT may function without REP activity, at least in some tissues (Tanaka et al. 2008). *rep-1* mutation is a single missense causing an amino acid substitution (E107K). This is probably a weak hypomorphic allele and not a null mutation.

This mutant shows a mild defect in synaptic transmission and in defecation behaviour. The disruption of *rep* by siRNA showed mild, Rab27-dependent and Rab3-independent defects in synaptic transmission at neuromuscular junctions. The defects of *rep-1* did not cause such strong effects on defecation as defects in the *rab27* gene or *rggta*. However, the exact level of REP inhibition in particular cells in the mutant strain or the effectiveness of the siRNA treatment with anti-*rep* siRNA was not specified. It is well known from the yeast (Bialek-Wyrzykowska et al. 2000), plant (Hala et al. 2005) and also mammalian (Detter et al. 2000) models that even a low level of enzyme activity sustains growth and basic functions of the organism. Mutant animals (*rep-1* and siRNA treated) produce many lethal embryos but still are able to reproduce in comparison to a complete sterility in *rggta* siRNA-treated animals. Probably the defect is correlated with abnormal germ-line development and gonad morphology.

In all organisms studied so far REP has a broad profile of expression but in *Caenohabditis elegans* the authors suggest that it is not present in every cell. In the *rep-1* mutant the patterns of localization of Rab5 and Rab7 have been disrupted partially and in *rep* RNAi this effect was stronger, comparable to *rggta* RNAi. The RNAi treatment has a smaller effect on localization of Rab10 and Rab11. In Y2H Rab5 and Rab7 showed interaction with REP and Rab10 and Rab11 did not.

The authors suggest that some Rabs may require REP for geranylgeranylation while some may be modified by the RGGT heterodimer alone. Moreover the requirement of REP for the same Rab may depend on its site of action. In contrast to other organisms, *C. elegans* RGGT would then possess a weak binding affinity to some Rabs. Alternatively REP interacts with all Rabs, but the binding affinity between REP and each Rab may be easily affected by their environment and correspond to the binding affinity of Rab proteins with REP.

Yeast

In yeast *mrs6/msi4*, a homologue of *REP1*, is an essential gene (Fujimura et al. 1994). The conditional mutant is impaired in protein transport to the vacuole on the ER to Golgi step. In mutant cells a soluble form of Ypt1p (Rab) accumulated, because level of Ypt1p geranylgeranylation was very low. *mrs6* overexpression caused a decrease in cell size on non-fermentable carbon sources (Ragnini et al. 1994) but depletion caused an increase in cell size in poor nutrients (Singh and Tyers 2009). An opposite effect should have been expected from a simple secretory system perturbation. Repression of *mrs6* while grown on glucose (fermentable carbon source) compromised cell growth and caused a G2/M delay. Microscopic observations of spores from a heterozygous *mrs6* strain (completely lacking one copy of the gene) show 2:2 segregation, the lethality was ascribed to the fact that spores either did not germinate or underwent not more than three cell divisions. A conditional mutant was lethal under non-permissive conditions, so the gene is important for both vegetative growth and germination (Ragnini et al. 1994). Overexpression of the mrs6 protein can suppress the thermosensitive phenotype of the $ypt^{N121I/A161V}$ mutant but not the absence of ypt1 protein (Ragnini et al. 1994). Mutational analysis of mrs6p led to the conclusions that mrs6p with deletions in the non-conserved C-terminal amino acid stretch or mutations in all but the first SCR were able to rescue the $mrs6^{-/-}$ conditional phenotype (Bauer et al. 1996). All the mutants sustaining growth were tested for geranylgeranylation activity and each showed a detectable (but sometimes lower than wt) activity. Mutated mrs6p and ypt1p showed to interact by the yeast two-hybrid assays and pull downs, albait with different strength. Interestingly, the C-terminal truncations of mrs6p lead to a protein with higher affinity to the ER and Golgi membranes (Miaczynska et al. 1997).

Interesting studies, disclosing more information on mrs6p function and the effects of single amino acid changes in this important protein, are based on mutant *mrs6-2* (Bialek-Wyrzykowska et al. 2000). This mutant, with reduced prenylation even at the permissive temperature, was constructed by random mutagenesis, causing a double mutation in a conserved region. Shift to a restrictive temperature causes no changes in growth of the mutant cells after 3 h, but reduces the amount of some Rab (sec4p) but not other (ypt1p) bound to membranes. For comparison, a complete lack of mrs6p prevents transport and causes the absence of Rabs on the membranes, as was mentioned earlier (Fujimura et al. 1994). Mutation *mrs6-2* also causes defects in polarization and budding (cell division). The phenotype is attributed to dysfunction in polar transport along the cytoskeleton rather than vesicle budding or fusion, because there is no vacuole fragmentation or vesicle clusters near the bud, but only the even distribution of the vesicles in the cytoplasm (Bialek-Wyrzykowska et al. 2000). Genes involved in cell wall maintenance rescue the phenotype. *mrs6-2* mutant does not show massive accumulation of ER, Golgi and PM as do $mrs^{-/-}$, $ypt^{-/-}$ and $sec4^{-/-}$, pointing to the fact that some Rabs remained functional, while others, underprenylated, could not perform their normal function.

It seems not clear whether CHM results from the independent or sequential layer-by-layer degeneration of retina, retinal pigment epithelium (RPE) and choroid. There are three hypotheses based on the clinical observations of CHM patients and on the animal models mentioned above. (i) The suggestion that RPE damage is a primary defect in CHM is based on a study on the zebrafish model which indicates that loss of REP1 from the RPE is sufficient for early photoreceptor degeneration (Krock et al. 2007); (ii) The concept of the independent photoreceptor and RPE degeneration has been drawn from the results of experiments with tissue specific *CHM* KO mice (Tolmachova et al. 2006); and (iii) Photoreceptor degeneration as the primary event has been based on the studies of retinas of female mice chimeric for a nonfunctional *REP1* (van den Hurk et al. 1997). This finding is consistent with the night blindness noted early in CHM male patients. So far the potential use of gene augmentation therapy (Tolmachova et al. 2012) and transplantation (Lund et al. 2001) have been proposed as therapeutic alternatives.

α subunit of RGGT (RGGTA)

Analysis of the genome sequences revealed presence of the *rggta* in all the eukaryotic species analyzed so far (Rasteiro and Pereira-Leal 2007). Duplication of the *rggta* has been noted exclusively for *Arabidopsis thaliana* but not for other plants (Hala et al. 2010).

The best studied animal model indicating the consequences of *rggta* mutation is *gunmetal* mouse (*gm* mutation results from a G→A substitution in a splice acceptor site) (Detter et al. 2000). RGGT activity and RGGTA protein levels were reduced 4-fold in *gm* platelets. In line with this was the hypoprenylation of the Rab27 protein. The residual RGGT activity is ascribed to the aberrant splicing that to some extent rescues RGGTA expression. The phenotype of the homozygous *gm* mouse manifests as prolonged bleeding caused by defects in platelets and megakaryocytes, macrothrombocytopenia and reduced platelet α- and δ-granule content (storage pool deficiency), the megakaryocyte count is higher but they have abnormal intracellular membranes, the animals also have partial cutaneous albinism (Detter et al. 2000). The *gm* phenotype resembles the rare human disorder gray platelet syndrome (GPS) and platelet α,δ-storage pool deficiency. Moreover, association of RGGTA with appropriate platelet function suggests applications of RGGT inhibitors as a novel therapeutic strategy for cure of thrombocytosis and clotting disorders, e.g., stroke (Detter et al. 2000).

In a follow-up study (Li et al. 2000) analysis of the 5′-untranslated structure of the human *RGGTA* gene in patients with deficiencies of platelet-dense granules (α,δ-storage pool deficiency) and GPS revealed similar exon/intron structural organization of the 5′-untranslated region of the human and *gm* mouse genes. However, exon alpha and intron alpha are not homologous between mouse and human. This analysis did not disclose any obvious disease-causing mutations of human *RGGTA*, however, several polymorphisms of *RGGTA* including a putative cryptic splicing mutation in intron 4 were identified. Based on phenotypic similarities *gunmetal* mutant is sometimes considered as a model of the human Hermansky-Pudlak syndrome (symptoms include albinism, bleeding tendency and lung disease); however, definite arguments supporting this concept are still missing (Hutagalung and Novick 2011).

Gunmetal mouse has also been used very recently to elucidate the effect of aberrant Rab prenylation on bone resorption (Taylor et al. 2011). *gm* osteoclasts exhibit a substantial reduction in resorptive activity *in vitro* while *gm* animals possessed slightly lower bone mass than controls, indicating also defects in osteoblasts. Interestingly, *gm* mice were partially protected from ovariectomy-induced bone loss, suggesting that levels of Rab prenylation in *gm* osteoclasts may be sufficient to maintain normal physiological levels of activity, but not pathological levels of bone resorption *in vivo*. In line with this bisphosphonates (inhibitors of farnesyl diphosphate synthase, an enzyme providing precursor for geranylgeranyl diphosphate synthesis) together with inhibitors of RGGT have been used to treat bone diseases characterized by extensive resorption, such as osteoporosis (Rogers et al. 2011, and references therein). Moreover, RGGT inhibitors have been shown to induce apoptosis in certain types of cancer (Lawson et al. 2008) indicating the involvement of geranylgeranylated Rab proteins in this process.

Studies in yeast revealed that loss of RGGTA activity is lethal in *S. cerevisiae* (Newman and Ferro-Novick 1987).

β subunit of RGGT (RGGTB)

The presence of the RGGTB encoding genes has been postulated in most of the analyzed eukaryotic genomes. In some cases two (*Tetraodon nigroviridis*, *Ciona intestinalis*, *Monosiga brevicollis*, *Naegleria gruberi*) or even three (*Danio rerio*) RGGTBs have been identified (Rasteiro and Pereira-Leal 2007). Duplication of the β subunit, although also found in many plants such as *A. thaliana*, *Vitis vinifera*, *Physcomitrella patens* and *Selaginella moellendorffii*,

seems to be independent in each of the lineages and not a plant-specific feature.

So far the effects of *rggtb* mutations have been characterized in *A. thaliana*. The sequence of AtRGGTB1 and B2 subunits are very similar to each other and to its mammalian homologue (Hala et al. 2010). According to the data from Genevestigator, the *rggtb1* transcript is abundant in all vegetative and generative tissues, *rggtb2* transcript is less abundant than *rggtb1* in vegetative, but expressed to a similar level to *rggtb1* in generative male tissue.

Disruption of *Arabidopsis rggtb1* results in pleiotropic growth and developmental effects prominent in the shoot, but less visible in the root (Hala et al. 2010). The plants were dwarfed, showed loss of apical dominance and had abnormally developed flowers, however, both female and male generative organs remained fertile. The first symptoms of ageing are delayed in the mutant plants. Similarly to other transport machinery plant mutants, the *rggtb1*$^{-/-}$ shows a gravitropic defect of the shoot and does not etiolate in the dark. The biochemical data point to the deregulation of both exo- and endocytosis as well as to accumulation of unprenylated Rab proteins in the cytosol of the mutant plants. Ablation of the *rggtb1* gene product results in the reduction of Rab geranylgeranylation activity to approx. 25% of the wt and the phenotypic changes affect the whole shoot and not only the specific organs or tissues as in mammalian mutants (*gunmetal* mice, CHM), suggesting a higher sensitivity of plants to defects of the secretion machinery. The authors speculate that deficient secretory pathway of the *rggtb1*$^{-/-}$ mutant can result in a defective cell wall composition accompanied by a stress response (as was proposed for yeast geranylgeranylation mutants (Newman and Ferro-Novick 1987, Bialek-Wyrzykowska et al. 2000) or a direct signalling feedback relay from the secretory pathway to the plant morphogenic program.

Loss of RGGTB activity (*bet2*, block in early transport) is lethal in yeast. The *bet2* mutant is thermosensitive for growth and accumulates ER membrane network even at a restrictive temperature making cells denser in transmission electron microscopy (Newman and Ferro-Novick 1987). This mutant has pleiotropic defects in many different steps of protein transport. Secretion of acid phosphatase to the periplasm and carboxypeptidase to the vacuole is inefficient and the accumulation of the immature ER form of invertase and alfa-mating factor precursors is evident.

Rab GTPases

Rabs, a diverse group of small GTPases, are master organizers of intracellular vesicular trafficking which ensure transport specificity and designate organelle identity. The role of disturbed vesicular trafficking and aberrant Rab function in inherited and acquired diseases has been summarized in an excellent review (Mitra et al. 2011) published recently. Below mentioned are the disorders resulting exclusively from the mutations in the *rab* genes resulting in defects of Rab geranylgeranylation.

Mutations in *Rab27A* have been found in Griscelli Syndrome Type 2 (GS2) patients characterized by immunological defects, immunodeficiency and pigmentary dilution of the skin and hair. Rab27A plays pivotal role in melanocytes as a member of a tripartite machinery (Rab27A-myosin Va-melanophilin) responsible for movement of melanosomes along the sublemmal actin network. Additionally, Rab27A also functions in granule release within cytotoxic T lymphocytes. Mutations in *RAB27A* account for most cases of GS to date (van Gele et al. 2009). Most often these are homozygous nonsense or frameshift mutations leading to a premature stop codon and resulting in a truncated protein devoid of C-terminal geranylgeranylation motif.

Similarly, truncation or missense mutations in *RAB23* have been identified as causative agents of Carpenter syndrome which is a pleiotropic disorder manifested by craniosynostosis, polysyndactyly, obesity and cardiac defects; such symptoms are also clinical characteristics of disorders associated with impaired Sonic Hedgehog signaling. Interestingly, Rab23 has been identified as a major negative regulator of the Sonic Hedgehog pathway (Mitra et al. 2011, and references therein).

The role of Rab in oncogenesis is broadly discussed since aberrant endocytosis, vesicle targeting and receptor recycling are involved in altering cell adhesion, migration, proliferation, polarity, asymmetrical division and overall survival. Indeed, aberrant expression of Rabs has been noted in various cancers. The best characterized example is Rab25 which is a determinant of tumor progression and aggressiveness of some cancers (prostate, ovarian and breast cancer); Rab25 does not play a role in tumor initiation but rather facilitates its progression (Chia and Tang 2009). Consequently Rabs might be considered as future biomarkers for various cancers while RGGT might serve as a target of anticancer therapy (Hutagalung and Novick 2011).

Conclusions

In summary, the last few years have brought an increasing interest in the function, mode of action and regulation of the RGGT complex. Advanced crystallographic studies have led to a mechanistic model of action of mammalian RGGT in complex

with REP and Rab substrate. Sequence analysis of the subunits of the complex proved a high level of conservation and despite obvious biochemical discrepancies, the modes of action of the RGGT from mammals and other organisms seem not to differ very much.

Much effort has been devoted to the construction of model organisms with knocked-out or down-regulated RGGT activity. The findings lead to the conclusion that the activity of this enzyme is indispensable for all eukaryotic organisms. The complete *rggt* or *rep* knock-outs in yeast, fish and mice are lethal. The mutant organisms with activity lowered either by mutation, siRNA treatment or silencing of one of two redundant genes revealed a general notion that even the low level of the RGGT activity is enough to sustain the basic functions in growth and development. The severity of the phenotype of the mutant is dependent on the particular level of function loss, with stronger manifestations in cells, tissues and processes strictly connected to vesicular transport.

The studies on organisms with residual RGGT activity together with yeast genetics revealed unexpected connections of RGGT subunits and processes of pre-mRNA splicing and nutrient sensing. The regulatory aspects of Rab geranylgeranylation and RGGT/REP additional cellular functions are promising directions for future investigations.

Manipulation of the activity level of RGGT by the use of specific inhibitors or gene therapy opens perspective for new therapeutic strategies against inborn syndromes such as CHM or GPS as well as bone and blood clotting diseases and cancer.

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