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REVIEW

Importance of organellar proteins, protein translocation and vesicle transport routes for pollen development and function

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Key message **Protein translocation.**

Abstract Cellular homeostasis strongly depends on proper distribution of proteins within cells and insertion of membrane proteins into the destined membranes. The latter is mediated by organellar protein translocation and the complex vesicle transport system. Considering the importance of protein transport machineries in general it is foreseen that these processes are essential for pollen function and development. However, the information available in this context is very scarce because of the current focus on deciphering the fundamental principles of protein transport at the molecular level. Here we review the significance of protein transport machineries for pollen development on the basis of pollen-specific organellar proteins as well as of genetic studies utilizing mutants of known organellar proteins. In many cases these mutants exhibit morphological alterations highlighting the requirement of efficient protein transport and translocation in pollen. Furthermore, expression patterns of genes coding for translocon subunits and vesicle transport factors in *Arabidopsis thaliana* are summarized. We conclude that with the exception of the translocation systems in plastids—the composition and significance of the individual transport systems are equally important in pollen as in other cell types. Apparently for plastids only a minimal translocon, composed of only few subunits, exists in the envelope membranes during maturation of pollen. However, only one of the various transport systems known from thylakoids seems to be required for the function of the "simple thylakoid system" existing in pollen plastids. In turn, the vesicle transport system is as complex as seen for other cell types as it is essential, e.g., for pollen tube formation.

Keywords Mitochondria · Plastids · Peroxisomes · Endoplasmic reticulum · Vesicle transport system · Organellar proteins

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Introduction

Cellular homeostasis depends on a multitude of processes including protein synthesis, folding and maintenance (Hartl et al. 2011). The proper distribution of proteins within cells and their insertion into membranes is one of the central processes for cellular function (Wang et al. 2004; Kessler and Schnell 2009; Schleiff and Becker 2010; Vögtle and Meisinger 2012) and is mediated by organellar protein translocation and vesicle transport systems (Bonifacino and Glick 2004; Wickner and Schekman 2005). The former facilitates the transport of proteins across the respective membranes of cellular subcompartments by specialized translocon components, e.g., translocase of outer/inner membrane of chloroplasts or mitochondria, or the complexes in the membranes of peroxisomes and endoplasmic reticulum (ER; Schleiff and Becker 2010; Paul et al. 2013). Vesicle transport refers to the transport of proteins from one compartment to the other via vesicles, e.g., COP-II vesicles transport proteins from ER to Golgi (Spang 2008; Duden 2009; Paul et al. 2014). The exact composition of the individual complexes depends on the cellular context, mostly reflecting the variation of organellar function in these structures. Highly specialized cell types exist for example in pollen, which might comprise altered protein transport complexes compared to other cell types.

As a result of asymmetric mitotic division, mature pollen is comprised of a large vegetative and a small generative cell forming a 'cell within a cell' structure (Fig. 1; Twell et al. 2006; Borg et al. 2009; Quilichini et al. 2015; Shi et al. 2015). Mature pollen is enclosed in a highly specialized wall comprised of inner (intine) layer mainly composed of cellulose and outer (exine) layer mainly composed of sporopollenin (Blackmore et al. 2007; Borg et al. 2009; Ariizumi and Toriyama 2011). Mature pollen has subcellular compartments typical for plant cells (Fig. 1). Therein, vacuoles act as storage sites and undergo extensive expansion and degradation during pollen development (Pacini et al. 2011), while an extensive endomembrane system is required for vesicle trafficking (Pertl et al. 2009). Mitochondria are essential for the metabolic capacity of pollen, and thus reduction or loss of function of biochemical pathways hosted in mitochondria often leads to cytoplasmic male sterility (Hoekstra 1979; Conley and Hanson 1995). Plastids are discussed to act as storage compartments (Nagata et al. 1999; Van Aelst et al. 2008); however, it is documented that the number of plastids is lower in pollen compared to most other cell types (Tang et al. 2009; Fujiwara and Yoshioka 2012). Thus, it is logical to assume that all types of organellar protein translocation machineries exist in pollen. The same holds true for the vesicle transport system, which plays a critical role in pollen germination, tube growth and thereby fertilization (Pertl et al. 2009). Pollen tube growth is directionally established by deposition of post-Golgi vesicles at the designated areas of pollen plasma membrane (Krichevsky et al. 2007). Further, new cell wall material is continuously added to the growing pollen tube (Steer and Steer 1989). The latter process requires the deposition of enzymes relying on vesicle-based enzyme transportation.

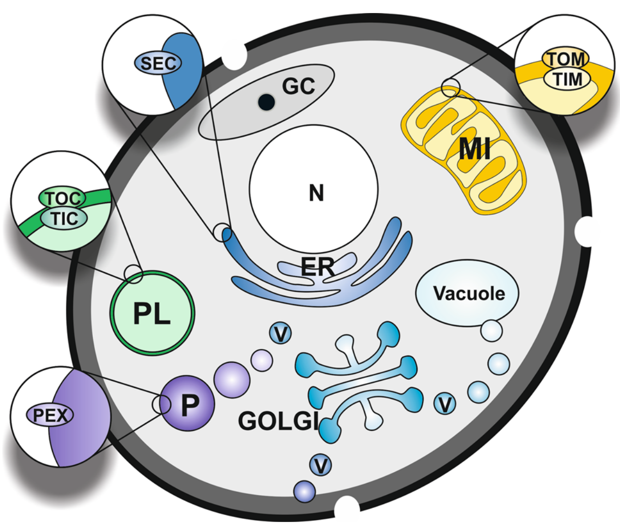

Fig. 1 Schematic structure of pollen. Shown is a scheme of the cellular components of pollen. Highlighted are the membranes in which protein translocation complexes are hosted. The complexes in the mitochondrial (MI) membranes are annotated as translocon of the outer/inner mitochondrial membrane (TOM/TIM), in the (PL) membranes of plastids as translocon of the outer/inner chloroplast envelope (TOC/TIC), in the membrane of the endoplasmic reticulum (ER) as SEC translocase, and in the membrane of peroxisomes (P) as peroxin (PEX). Shown are the nucleus (N), the Golgi system, the vesicles (V) and the generative cell (GC)

Keeping in mind the importance of protein homeostasis and the cellular structure of pollen and its reshaping during germination it is expected that protein homeostasis is prerequisite for pollen function and development. Experimental information on the impact of translocation components for pollen function is sparse as most are essential per se, which creates technical difficulties to derive their significance for pollen development. Moreover, there are not many studies with focus on protein transport in pollen. The general lack of substantial information on tissue-specific composition and regulation of transport components is the consequence of the current focus on understanding the fundamental principles of protein transport rather than tissue-specific variations. However, several indications point toward a central role of protein transport in pollen. For example, several plasma membrane proteins are essential for pollen development and their delivery depends on both classical ER-membrane translocation machineries and their subsequent distribution via vesicles (Yamamoto et al. 2008a; Ding et al. 2012; Dal Bosco et al. 2012). To this end, the importance of protein transport can be established on the basis of identified essential organellar proteins that require efficient protein transport and translocation alongside with a discussion of mutants of transport factors that lead to phenotypes in pollen structure, development and function. Furthermore, we utilize information obtained by publically available—*omics* studies in *A. thaliana* to define the core set of protein translocation-related proteins in pollen and discuss differences to other tissues (Honys and Twell 2004).

Importance of mitochondrial function for pollen

Protein translocation into pollen mitochondria is a fundamental process, which can be concluded from the importance of mitochondrial proteins and their functions for pollen development (Lee and Warmke 1979; Hoekstra 1979; Paul et al. 2015). For example, for the pollen-specific TIP5;1 and TIP3;1 aquaporins a mitochondrial and a vacuolar localization has been described (Soto et al. 2010; Wudick et al. 2014). They are involved in transport of water and urea within pollen and thereby in delivering nitrogen to the growing pollen tubes (Soto et al. 2010; Wudick et al. 2014). Another mitochondrial factor essential for pollen germination and tube growth is the GTPase Miro1 (Yamaoka and Leaver 2008). Miro is an essential regulator of mitochondrial morphogenesis and trafficking along microtubules (Reis et al. 2009) and thus required for proper mitochondrial streaming in pollen. Many mitochondrial carrier proteins have also been detected in pollen, namely the carnitine/acylcarnitine, the dicarboxylate–tricarboxylate, the phosphate and the ADP/ATP carrier (AAC; Paul et al. 2015), which signifies their important role in pollen homeostasis as well as the importance in pollen metabolism in general.

Besides general mitochondrial proteins, several pollen-specific mitochondrial proteins have been identified by either expression or functional analysis. For example the activity of the mitochondrial editing factor S8 was specifically assigned to pollen in *A. thaliana* (Verbitskiy et al. 2012), expression of atp2.3 coding for the catalytic ß-subunit of the mitochondrial ATPase/ATP synthase in *Nicotiana* was exclusively found in bicellular pollen (Lalanne et al. 1998; De Paepe et al. 1993), and the promotor of the sodA1 gene coding for a manganese superoxide dismutase in *Nicotiana plumbaginifolia* is only active in pollen, middle layer and stomium of anthers (van Camp et al. 1996). Therefore, the existence of nuclear-encoded pollen-specific mitochondrial proteins further documents the need of a translocation system in the surrounding membrane.

Another line of evidence for mitochondrial function in pollen comes from the analysis of chromosomal regions associated with cytoplasmic male sterility (CMS) that is defined by the inability of the plant to produce functional pollen grains. Many of the identified chromosomal regions encode for mitochondrial proteins (Hanson and Bentolila 2004; Horn et al. 2014; Touzet and Meyer 2014; Wang et al. 2015). Consequently, CMS lines with low F_0F_1–ATP synthase activity have been identified (Bergman et al. 2000; Li et al. 2013). In line, the MGP1 (male gametophyte defective 1) is a mutation of the F_Ad subunit of ATP synthase that alters ATP hydrolysis activity leading to mitochondrial destruction and subsequent pollen death (Li et al. 2010).

In addition, the mitochondrial genome hosts pollen-abortion-related genes or open reading frames (ORFs). As most of the mitochondrial ribosomal proteins are nuclear encoded (Woellhaf et al. 2014), the relevance of protein translocation is obvious. Here, ORF239 (known as sterility sequence), ORF297 (a putative polypeptide of 10.9 kDa), ORF720 (a putative polypeptide of 26.7 kDa) in common bean (Johns et al. 1992; He et al. 1996), ORF129 (12-kDa polypeptide loosely associated with membranes) in beets (Yamamoto et al. 2008b), ORFH79 (chimeric mitochondrial gene) and ORF352 (wild abortive-type CMS causing gene) in rice (Hu et al. 2012; Kazama and Toriyama 2014) were identified as essential genes for pollen development.

All of the mentioned examples document both (a) mitochondria are essential for pollen function as metabolic energy in form of ATP is required and (b) to perform this function the mitochondrial translocation system is essential as most mitochondrial proteins are either nuclear encoded or depend on the action of the mitochondrial ribosomes composed of nuclear-encoded ribosomal proteins.

The relation between peroxisomal, ER and plastidial function and pollen viability

As seen for mitochondria, peroxisomal proteins have been described to be central for pollen function. On the one hand, jasmonate synthesis appears to be central for pollen development. A double mutant of acyl-coenzyme A oxidase 1 and 5, which are involved in jasmonate biosynthesis, shows a reduced pollen viability and fertility (Schilmiller et al. 2007). The mutant of comatose, a peroxisomal ATP-binding cassette transporter required for biosynthesis of jasmonate, shows a reduced capacity of pollen germination as well (Footitt et al. 2007). However, jasmonate synthesis is not the only function of peroxisomes for pollen, as a mutant of a 3-ketoacyl-CoA thiolase and a double mutant of the peroxisomal long-chain acyl-Coenzyme A synthetases lacs6/lacs7, both with disturbed β-oxidation of storage lipids during germination, show a reduced capacity of pollen germination and in vitro tube growth (Footitt et al. 2007). These two examples document that peroxisomal function is essential for both pollen development and pollen tube growth.

The endoplasmic reticulum, a unit of endomembrane system, plays a critical role in vesicle transport as most proteins have to be inserted into the ER-membrane or lumen prior to vesicle transport. However, also ER-residual proteins appear to be essential for pollen development, like MIA (Male gametogenesis Impaired Anthers), and an ER-localized P-type ATPase cation pump as a mutation of this gene in *Arabidopsis* disturbs fertility and pollen morphology (Jakobsen et al. 2005). The same was found for the ADP/ATP antiporter (ER-ANT1) which is localized in the ER and which is crucial for regular supply of ATP. Again, by mutagenesis it was concluded that ER-ANT1 affects pollen grain development and function (Leroch and Neuhaus 2008). A central nucleotide sugar for co-translational

N-glycosylation of proteins imported into the ER is uridine 5′-diphosphate (UDP)-glucose, which serves as precursor for the synthesis of $Glc_3Man_9GlcNAc_2$ used for glycosylation. Two ER-localized transporters, UTr1 and UTr3, facilitate the translocation of UDP-glucose. In line with the central function of the ER for pollen development, mutagenesis confirmed that AtUTr1 and AtUTr3 are essential for pollen viability (Reyes et al. 2010).

However, pollen-specific ER proteins have been identified as well. The most prominent example is the pollen-specific auxin carrier Pin8 which is residual to the ER-membrane (Dal Bosco et al. 2012). Thus, insertion of proteins into ER-membranes is essential for pollen function.

The significance of plastids in pollen of flowering plants is under debate. It was described that plastids are transformed from a poorly differentiated organelle to a double-membrane structure containing simple thylakoids alongside pollen development (Kuang and Musgrave 1996; Tang et al. 2009; Jarvis and López-Juez 2013). However, plastid-localized glycolysis accounts for energy generation required for pollen tube elongation (Selinski and Scheibe 2014). Moreover, the function of plastid-localized energy-related enzymes (glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate mutase) is central for male gametophyte function (Muñoz-Bertomeu et al. 2009; Prabhakar et al. 2010; Zhao and Assmann 2011). In addition, at least for *Medicago truncatula* a biparental plastid inheritance has been observed (Matsushima et al. 2008). This suggests that at least a rudimentary plastid exists in pollen, which explains the detection of plastome-encoded mRNAs in mature pollen of *Solanum lycopersicum* as well (Paul et al. 2015). In line with this notion, some plastid proteins have been described to be higly expressed in pollen. For example, a specific function in pollen 'Fe-S cluster biosynthesis' was assigned to the plastid-localized SufE2 of *A. thaliana* (Murthy et al. 2007) and an impact on redox regulation of starch metabolism in pollen was assigned to the chloroplast targeted ß-amylase TR-BAMY in *A. thaliana* (Sparla et al. 2006).

Thus, protein translocation into plastids might be equally important as translocation into mitochondria. Indeed, even dual-targeted genes have been found to be essential for pollen development. The degradation of organellar DNA during pollen development is achieved by the exonuclease DPD1 (defective in pollen organelle DNA degradation 1; Wang et al. 2010a; Matsushima et al. 2011). The according mutant *dpd1* possesses elevated DNA levels in pollen plastids and mitochondria, which was shown by 4′,6-diamidino-2-phenylindole staining. This observation was discussed as an indication that the majority of the organellar DNA is maternally inherited (Matsushima et al. 2011; Schneider et al. 2015).

Organellar protein translocation machineries in pollen

Not many studies refer directly to the function of protein translocation machineries in pollen. Thus, it is noteworthy that in particular the function of a chloroplast translocation component in context of pollen was addressed. Tic40, a co-chaperone at the inner membrane of chloroplasts, is shown to be expressed in all stages of pollen development in *Brassica napus* with the exception of mature pollen (*BnaC.Tic40*; Dun et al. 2011). This result signifies the presence of plastidal translocation machinery in developing pollen. Moreover, the *B. napus* (*B. napus* 7365A) male sterile plant lines carrying a mutation in the Tic40 coding gene (Table 1) have defective tapetal secretory functions including retarded tapetal degradation, which result in abortive callose dissolution, absence of pollen exine and eventually generation of non-viable pollen (Dun et al. 2011). The essential function of Tic40 clearly links plastid function with pollen development. Thus, functional plastids appear to be essential for pollen development on both levels, i.e., at pollen tube growth stage for energy production (Selinski and Scheibe 2014) and on the indirect level by having a functional tapetum (Dun et al. 2011).

By analyzing the expression of assigned TOC components (Paul et al. 2013) in different pollen stages using global expression data for *A. thaliana* pollen (Honys and Twell 2004) the presence of transcripts of genes coding for the main translocation channels in outer and inner envelope, namely Toc75-III (Hinnah et al. 1997), Tic20 (Kouranov et al. 1998) and SecY2 (Skalitzky et al. 2011), became obvious in all pollen stages (Fig. 2a, left). Remarkably, the transcripts of the genes coding for the inter-membrane space protein Tic22-III and the inner membrane channel Tic20-IV are equally abundant in all pollen stages and as abundant as in leaves. In line with the experimentally confirmed importance of Tic40 for pollen development it is expressed in all pollen stages (Fig. 2a, left). Interestingly, transcript analysis shows that most of the genes coding for translocation components are not present in mature pollen (Fig. 2a, middle and right). Moreover, not all homologs are equally expressed suggesting a tissue or even cell specificity for example of the different Tic22 proteins.

As expected, almost all components of the thylakoid protein translocation systems are not expressed in pollen (Fig. 2a, right gray). In line, the two inner envelope translocon components that integrate the sensing of the redox state of chloroplasts into the regulation of the translocon (Oreb et al. 2008), Tic55 and Tic62, are not expressed in pollen either. These observations suggest that plastids of mature pollen have a rudimentary translocation

Table 1 List of components of protein translocation or vesicle transport systems of which mutants have been shown to affect pollen development

Organelle	Name of mutant	Effect on pollen	References
Plastids	*tic40* (BnMs3)	Pollen lethality	Dun et al. (2011)
Peroxisomes	dau (APEM9)	Aberration in pollen maturation and germination	Li et al. (2014)
	apm2 (Pex13)	Retarded pollen tube growth	Boisson-Dernier et al. (2008)
Vesicle transport	*sec24a/b* (Sec24)	Reduced pollen germination	Conger et al. (2011), Tanaka et al. (2013)
	tplate	Male sterility	Van Damme et al. (2006)
	ap1m1 and *ap1m2* (μ AP1)	Pollen lethality	Park et al. (2013)
	gnl1	Reduced pollen germination and pollen tube growth	Liao et al. (2010)
	rab2	Inhibition of pollen tube growth	Cheung et al. (2002)
	rabA4d	Bulged pollen tubes and reduced pollen tube growth rate	Szumlanski and Nielsen (2009)
	rabD2b/D2c	Pollen deformation and branched pollen tube tips	Peng et al. (2011)
	rab11b	Reduced pollen tube growth rate and fertility	de Graaf et al. (2005)
	rgt	Deformed pollen tubes	Gutkowska et al. (2015)
	rpa	Reduction in pollen tube growth	Song et al. (2006)
	syp41	Defect in pollen tube growth	Sanderfoot et al. (2001)
	sec22	Degenerated pollen grains	El-Kasmi et al. (2011)
	VAMP726	Defect in pollen tube growth	Guo and McCubbin (2012)
	pok/vps52	Very short pollen tube formation	Lobstein et al. (2004)
	vps45	Pollen lethality	Zouhar et al. (2009)
	vps15	Defect in pollen germination	Wang et al. (2012), Xu et al. (2011)
	atATG6 (VPS30)	Defect in pollen germination	Fujiki et al. (2007), Qin et al. (2007)
	sec5	Defective pollen germination and pollen tube growth	Hála et al. (2008)
	sec6	Defective pollen germination and pollen tube growth	Hála et al. (2008)
	sec8	Defective pollen germination and pollen tube growth	Cole et al. (2005), Hála et al. (2008)
	sec15a	Defective pollen germination and pollen tube growth	Hála et al. (2008)
	rop1	Inhibition of pollen tube elongation	Lin and Yang (1997)
	ren1	Depolarization of pollen tube growth	Hwang et al. (2008)

system in the envelope membranes only (Fig. 2b). However, two thylakoid translocation components (Fig. 2a, b; cpFTsY and ALB3; Schünemann 2007) are expressed in pollen, which supports the notion for the existence of simple thylakoid system in developing pollen (Kuang and Musgrave 1996).

Interestingly, an *Arabidopsis dau* mutant affecting a protein that encodes aberrant peroxisome morphology 9 (APEM9) causes defects in pollen maturation and germination (Li et al. 2014). Moreover, it is shown that *dau* pollen is impaired in peroxisomal protein import as APEM9 interacts with central protein import component—Pex13. Furthermore, it is also reported that Pex13 (APM2) is essential for the discharge of pollen tube and a mutation in PEX13 completely disrupts PTS1 (peroxisomal targeting signal 1)-dependent protein import into pollen peroxisomes (Boisson-Dernier et al. 2008). Both these reports strongly support the fact that functional peroxisomal protein import machineries exist in pollen. This is in line with the observed expression of almost all PEX components in all pollen stages (Supplementary table 1; Honys and Twell 2004).

Experimental evidence for the importance of translocon subunits of mitochondrial membranes for pollen development has not been provided. Interestingly, the mitochondrial processing peptidases involved in processing of nuclear-encoded precursor proteins after import were detected in mature pollen of rice and tomato (Dai et al. 2006; Paul et al. 2015). This indirectly signifies the necessity of the mitochondrial import apparatus. Moreover, expression of almost all mitochondrial components in all pollen stages is observed (Honys and Twell 2004). In line with the importance of the mitochondria for pollen development, most of the transcripts are about twofold higher expressed in pollen than in roots or leaves (Honys and Twell 2004). Thus, in contrast to chloroplasts, the translocon of mitochondria appears to exist in the composition as described for other cell types.

Similar to the situation for the mitochondrial translocon, the impact of the translocation machinery in the

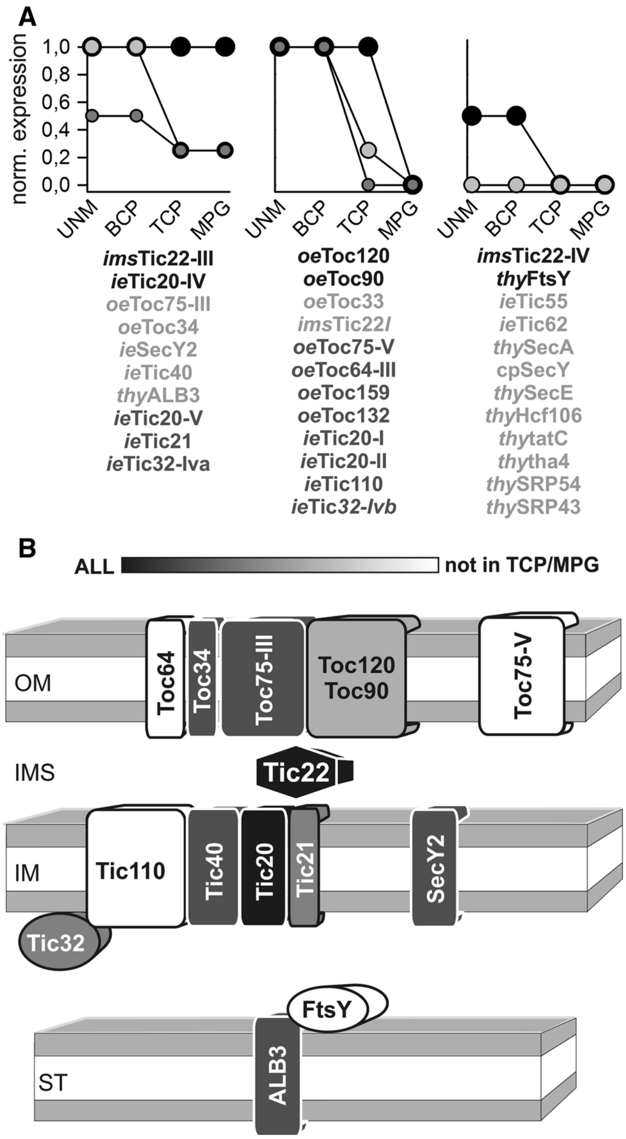

Fig. 2 Genes coding for plastid translocon components are differentially expressed. **a** The transcript abundance (Honys and Twell 2004) of the genes coding for the translocon components of the outer (OE) or inner envelope (IE), the intermembrane space (ims) or thylakoids (thy) in uninucleate microspores (UNM), bicellular pollen (BCP), immature tricellular pollen (TCP) or a homogeneous mature pollen grain (MPG) was normalized to that found in leaves/roots. The eight general profiles are shown. The proteins listed below are *color coded* according to the profile by which the according gene is expressed. **b** Shown are all components of the TOC and TIC system in the outer (OE) or inner envelope (IE) and the intermembrane space (IMS), as well as of the simple thylakoid system (ST) of which at least one isoform is expressed at least in uninucleate microspores and bicellular pollen. The *color code* indicates the degree of expression (*black* in all stages equally, *white* only in UNM/BCP)

endoplasmic reticulum has not been directly analyzed yet. However, with the exception of Sec63 (Paul et al. 2014), which is not expressed in mature pollen grains, all components are globally expressed (Honys and Twell 2004). The latter is in line with the importance of the ER translocon not only for the import into the lumen, but also for the subsequent transport by vesicles. In contrast, components of the ERAD pathway required for the export of proteins during unfolded protein response are significantly downregulated or even not expressed in immature tricellular pollen or mature pollen grains (Honys and Twell 2004). Particularly, the two components of the AAA ATPase machinery required for the transport of ubiquitinated proteins Cdc48 (encoded by At3g09840, At3g53230 and At5g03340) and Ufd1 (At2g21270, At4g38930 and At2g29070) are expressed at lower levels in the later stages, while no transcript could be identified for the anchoring components Dfm1 (At4g29330) and Ubx2 (At3g27310) in the later stages as well.

Role of proteins delivered by vesicle transport in pollen and pollen development

High levels of secretory activity have been reported to occur throughout pollen development (Tanaka et al. 2013). Vesicle transport systems mobilized by actin cytoskeleton play a prominent role in pollen tube growth (Cheung et al. 2002), because vesicles are packed with polysaccharides, glycoproteins, cell wall components and enzymes required for the tube growth (Roy et al. 1998; Krichevsky et al. 2007). Thus, the importance of vesicle transport for pollen function was concluded from manipulation of cytoskeletal function (Zhang and McCormick 2010; Peng et al. 2011; Conger et al. 2011). For example, downregulation of profilin decreased the amount of filamentous actin and reduced tip-directed vesicle transport in the pollen tube, which affected pollen tube growth (Liu et al. 2015). In turn, overexpression of α-tubulin leads to higher pollen germination and enhanced tube growth by stimulating vesicle transport (Yu et al. 2009).

A second line of evidence for the importance of vesicle transport system can be extrapolated from the impact of K^+- and H^+-transporting ATPases and other ion transporters on pollen germination and tube growth (Holdaway-Clarke and Hepler 2003; Certal et al. 2008; Pertl et al. 2009; Michard et al. 2009). For example, downregulation of the expression of the tonoplast-localized equilibrative nucleoside transporter 1 leads to defective pollen germination (Bernard et al. 2011). In the same way, a mutation in the catalytic subunit VHA-A of the vacuolar H(+)-ATPase leads to loss of pollen maturation (Dettmer and Schubert 2005) and the subunit VHA-E2 was even found to be pollen specific (Strompen et al. 2005).

Moreover, many enzymes distributed to various membranes by vesicle transport have been found to be essential for pollen function. For example, the mutant of the callose synthase 5, *cals5*, shows a severe drop in fertility, which is directly attributed to the degeneration of microspores (Verma 2001; Dong et al. 2005). In the same line, a mutant of the S-acyl transferase PAT family protein 10 (AtPAT10) localized in the Golgi stack, trans-Golgi network/early endosome and tonoplast results in reduced production and release of pollen, as well as in defective pollen tube growth (Qi et al. 2013).

Besides enzymes, membrane integral transporters, signal receptors or structural proteins are essential for pollen function as well. A double mutant of the plasma membrane-localized MAP3 Kε1 and MAP3 Kε2 leads to pollen lethality (Chaiwongsar and Otegui 2006) and overexpression of the pollen-specific plasma membrane-localized receptor-like kinase PRK1 from *S. lycopersicum* disturbs normal pollen tube formation (Gui et al. 2014). In turn, the C2 domain-containing plasma membrane protein (NaPCCP) interacts with the arabinogalactan proteins of the pistil extracellular matrix, a contact which is essential for fertilization (Lee et al. 2008a, 2009). The same holds true for the arabinogalactan proteins (AGPs) that are cell wall proteoglycans. For example, mutation of AGP6 and AGP11 leads to abnormal pollen development in *A. thaliana* (Coimbra and Costa 2009; Costa et al. 2013) and inactivation of the pollen-specific BM8 in *Brassica campestris* had a strong effect on pollen germination and pollen tube growth (Lin et al. 2014). Downregulation of the pollen-specific plasma membrane-localized hexose transporter HT1 of *Cucumis sativus* by antisense suppression leads to both inhibition of pollen germination and pollen tube formation (Cheng et al. 2015). In addition, the plasma membrane-localized monosaccharide (Stp6; Scholz-Starke et al. 2003) and ammonium (Amt1;4; Yuan et al. 2009) transporters of *A. thaliana* are exclusively expressed in pollen.

Lastly, the importance of vesicle transport for pollen development can be concluded from the impact of vacuoles, e.g., as calcium sink. It is well established that calcium acts as a central modulator for pollen tube growth. Calcium regulates the ion and vesicle transport as well as cytoskeleton reorganization in pollen (Pierson et al. 1996; Steinhorst and Kudla 2013), as well as it activates tonoplast-localized Ca^{2+}-sensor proteins. Overexpression of the calcineurin B-like CBL2 or CBL3 in *A. thaliana* was found to influence pollen germination and tube growth, while single (*cbl2* or *cbl3*) or double mutants (*cbl2/cbl3*) showed defects in pollen tube growth (Steinhorst et al. 2015). Moreover, mutation in CBL-interacting protein kinase 12 (CIPK12) also leads to impaired pollen tube growth (Steinhorst et al. 2015). Hence, vacuolar function is central for pollen development, and thus, vesicle transport is a critical process.

Vesicle transport in pollen and pollen development

The process of vesicle transport can be by and large divided into the transport from ER to Golgi by coat protein complex-II (COP-II) vesicles, from Golgi to ER by COP-I vesicles, from Golgi to plasma membrane by clathrin-coated vesicles (CCVs), from Golgi to endosome by retromer coat complex containing vesicles and from endosome to other cellular compartments by ESCRT (endosomal sorting complex required for transport) coat complex containing vesicles (Fig. 3). The importance of a functional vesicle transport for pollen development or pollen tube growth has been documented using mutants of several genes of the various pathways.

Individual mutants of atSec24A and atSec24B, which are components of COP-II vesicle, have been shown to be defective in pollen germination (Conger et al. 2011; Tanaka et al. 2013). In the same way, a mutant of Tplate that shows high similarity with COP-I coat proteins exhibits male sterility due to changes of the intine wall layer (van Damme et al. 2006). RNAi transgenic lines of Gnom-like 1 (GNL1), which is discussed to be involved in Golgi stabilization and COPI-vesicle recycling to the ER, showed a drastic reduction of pollen germination and pollen tube

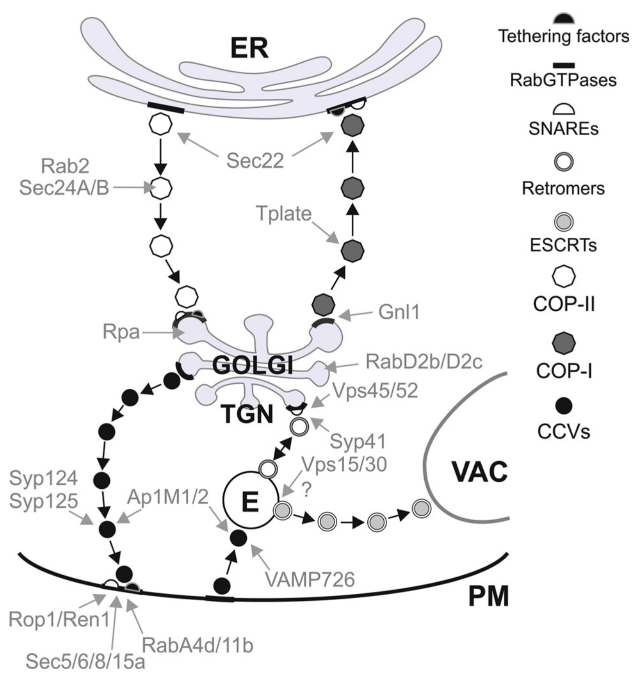

Fig. 3 Vesicle transport pathways and pollen-specific factors. The vesicle transport routes from endoplasmic reticulum (ER) via Golgi or trans-Golgi network (TGN) to plasma membrane (PM) or via endosome (E) to the tonoplast of the vacuole (VAC) is shown. The nomenclature of symbols is given on the *right*. The experimentally addressed and in the manuscript discussed factors are indicated in *gray letters*. *Arrowheads* point to the most likely cellular compartment the factor is associated with

growth (Liao et al. 2010). Coat proteins of clathrin-coated vesicles (CCVs) are essential for functional pollen as well. The single-knockout mutant of 1 μ subunit of the adapter protein complex AP-1, AP1M2, was shown to have arrested pollen growth, while the double-knockout mutant *ap1m1/ap1m2* of the μ subunits AP1M1 and AP1M2 were male gametophytic lethal (Park et al. 2013). Already these examples demonstrate that COP-II-, COP-I- and CCV-dependent vesicle flow is essential for pollen function.

Rab GTPases are known to regulate trafficking of vesicles between endomembrane compartments (Hutagalung and Novick 2011). For example, RabA4d is proposed to regulate vesicle targeting and its mutation resulted in bulged pollen tubes (Szumlanski and Nielsen 2009); while a dominant-negative mutant of Rab11b in *Nicotiana tabacum* caused a reduction of pollen tube growth and subsequently of pollen fertility (de Graaf et al. 2005). A similar phenotype was observed for Rab2 in *N. tabacum*, which blocks the secretory pathway and leads to inhibition of pollen tube growth (Cheung et al. 2002), while for the Rab2 of *A. thaliana* a predominant expression in pollen grains and seedlings was reported (Moore et al. 1997), but its function was not documented by mutagenesis. A double mutant of rabD2b and rabD2c, two Golgi-localized Rab GTPases of *A. thaliana*, shows morphological defects of pollen and of the pollen tube (Peng et al. 2011). Interestingly, a mutation in Rab geranylgeranyl transferase (RGT), which is known to assist Rab GTPases to anchor the membrane, results in deformed pollen tubes as well (Gutkowska et al. 2015), and a mutant of the Golgi-localized class II ARFGAP annotated as RPA was affected in pollen tube growth (Song et al. 2006).

SNAREs (soluble N-ethylmaleimide-sensitive attachment protein receptor) are involved in regulating fusion of vesicles to the destined compartment. Thereby they are critical for vesicle transport in pollen. For example the *SYP41* (syntaxin of plant 41) mutant has a defect in pollen tube growth (Sanderfoot et al. 2001), and a mutant of the vSNARE Sec22 involved in vesicle trafficking between ER and Golgi shows an abnormal growth during the bicellular stage leading to degenerated pollen grains (El-Kasmi et al. 2011). In turn, overexpression of VAMP726 in *Petunia inflata* inhibited pollen tube growth (Guo and McCubbin 2012). The pollen-specific Qa-SNAREs Syp124 and Syp125 from *A. thaliana* localize to apical vesicles at the plasma membrane (Silva et al. 2010; Ul-Rehman et al. 2011), and thus an important function for pollen development can be expected.

Other than their involvement in pollen development, secretory vesicles are known to regulate the pollen tube tip growth (Pertl et al. 2009). The exocyst complex is involved in targeting and tethering of Golgi-derived secretory vesicles to the plasma membrane (Synek et al. 2014). The exocyst is composed of eight subunits, and mutants of four (SEC5, SEC6, SEC8 and SEC15a) show defective pollen germination and pollen tube growth phenotypes (Hála et al. 2008; Synek et al. 2005). At the same side an indirect function of Rop1 is proposed. Rop1 is a pollen-specific plasma membrane Rho GTPases that is activated by the RhoGAP ROPenhancer1 (REN1; Hwang et al. 2008). ROP1 is thought to induce F-actin assembly and REN1-associated exocytotic vesicles accumulation in the pollen tube apex (Lee et al. 2008b). Thus, mutants of both ROP1 and REN1 lead to defects in pollen tube formation (Hwang et al. 2008; Lin and Yang 1997).

In line with the importance of vesicle transport to vacuoles and of vacuolar function, several mutants of this pathway have been described to affect pollen development or function. The vacuolar sorting protein 45 (Vps45) involved in sorting vacuolar receptors back to the *trans*-Golgi network is essential for pollen germination (Zouhar et al. 2009), and a T-DNA mutant line of Vps15 has been shown to be defective in pollen tube germination in vitro (Xu et al. 2011; Wang et al. 2012). Similarly, the mutant of poky pollen tube (POK) coding for a Vps52 homolog in *A. thaliana* shows a reduced pollen tube growth, while the protein is localized to the Golgi (Lobstein et al. 2004). Moreover, an *A. thaliana* mutant (*ATG6*) of VPS30, which is involved in autophagy and sorting of vacuolar hydrolases, has been reported to be defective in pollen germination (Fujiki et al. 2007; Qin et al. 2007; Harrison-Lowe and Olsen 2008). Finally, microinjection of small interfering RNAs affecting the level or vacuolar sorting receptors (VSRs) into lily pollen inhibits pollen tube growth (Wang et al. 2010b).

All of these examples document the relevance of the vesicle transport system in this specific cell type. Indeed, the analysis of the transcript abundance of components encoding for vesicular transport in pollen shows that only 3 out of 159 factors analyzed are not transcribed in pollen (Honys and Twell 2004). In turn 121 factors are expressed in all stages. This large number of expressed factors suggests that the entire vesicle transport system exists in pollen, which is a sign for its importance.

Conclusion

The existence of essential organellar proteins of pollen-specific organellar proteins and the expression of the translocon components strongly indicate that translocation complexes in the endoplasmic reticulum, peroxisomes and mitochondria (Fig. 1) are central for pollen function. In case of plastids, it should be suggested that the functional complexity of the translocon is significantly reduced during maturation of pollen, as only a minimal set of outer and inner envelope components are expressed that might form a minimal unit for translocation (Fig. 2). Nevertheless, this translocon remains essential for pollen development (Table 1; Dun et al. 2011). In line with the observed "simple thylakoid structure" (Kuang and Musgrave 1996; Tang et al. 2009; Jarvis and López-Juez 2013) expression of the membrane protein inserting Alb3 was observed in all pollen stages, while the components for other thylakoid import pathways are not expressed (Fig. 2b). With respect to the relevance of vesicle transport, evidence comes from four sides: (a) The massive restructuring of pollen cells during tube formation depends on massive vesicle transport. (b) The vacuole has central function in pollen development and receives proteins by vesicle transport. (c) Almost all factors identified to be involved in vesicle transport (Paul et al. 2014) are expressed, and in some cases even pollen-specific expressed factors exist (Honys and Twell 2004). And (d) mutants of several components involved in vesicle transport show severe defects in pollen development or germination (Table 1).

Interestingly, overexpression and inhibition of expression by mutagenesis have been performed for some components involved in vesicle transport. Remarkably, both alterations result in male function of pollen, which suggests that the balance of these factors is important for pollen function. Although experimental evidence for translocation systems does not exist, this notion can most likely be generalized for all systems. We propose that the sole enhancement of the abundance of individual subunits of the various translocons does not yield a benefit for pollen function as long as essential organellar proteins are not enhanced in expression as well. The only exception might be the ERAD system. The four components CDC48, Ufd1, Dfm1 and Ubx2 of this system are downregulated in the later stages of pollen development, and an enhancement might protect pollen from unfolded proteins. However, it is obvious that protein translocation and vesicle transport are essential for pollen development and alterations of their functions lead to severe defects.

Author contribution statement P.P., S.R. and E.S. planned, organized, wrote and reviewed the manuscript. All the authors approve the manuscript.

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