Plant Cell Monogr (3)
R. Malhó: The Pollen Tube
DOI 10.1007/7089_044/Published online: 17 February 2006
© Springer-Verlag Berlin Heidelberg 2006

Genomic and Molecular Analyses of Transporters in the Male Gametophyte

Heven Sze[1] (✉) · Sabine Frietsch[2] · Xiyan Li[1] · Kevin W. Bock[1] · Jeffrey F. Harper[2]

[1]Department Cell Biology & Molecular Genetics, HJ Patterson Hall, University of Maryland, College Park, MD 20742, USA
hsze@umd.edu

[2]Department of Biochemistry, Fleischmann Agriculture Bldg. #319, University of Nevada, Reno, NV 89557, USA

Abstract The major events of male reproductive development and function have been known for years, but the molecular and cellular bases of these processes are still poorly understood. Recent advances in cell biology coupled with molecular genetics and functional genomics are poised to offer tremendous opportunities to understand how membrane transport is integrated with male gametophyte development and physiology. Here we first propose the type of transporters necessary to affect the dynamics of Ca^{2+}, K^+, pH and others ions observed in polarized tip growth, and then show how pollen transcriptomics and molecular genetic tools are beginning to reveal the roles of specific transporters in microgametogenesis, pollen tube growth and male fertility.

1 Introduction

Little is known about the nutrients required for microspore proliferation and pollen maturation; however, it is well-established that pollen germination and tube growth depend on the continuous supply of ions and nutrients, like sucrose, Ca^{2+}, Mg^{2+}, K^+, $NO_3{}^{2-}$, $SO_4{}^{2-}$ and boric acid (Brewbaker & Kwack 1963). Since the 1970s, ion currents and fluxes were recognized as being critical in establishing and/or maintaining polarity of pollen tube growth (Weisenseel et al. 1975; Jaffe et al. 1975). Studies using ratiometric ion imaging, bioluminescent indicators and vibrating probes have revealed the dynamic nature of Ca^{2+}, H^+ and K^+ during pollen tube growth (Holdaway-Clarke and Hepler 2003; Hepler et al., this volume). Most of these studies were conducted using pollen from lily, tobacco, or petunia plants. The results, summarized in Fig. 1A, suggest that ion gradients, oscillations and fluxes are mediated by pumps, channels and carriers that are regulated in a spatial- and temporal-manner in the growing pollen tube. However the identities and properties of the transporters involved are largely unknown. A first step to understanding the nutrition, physiology and development of the male game-

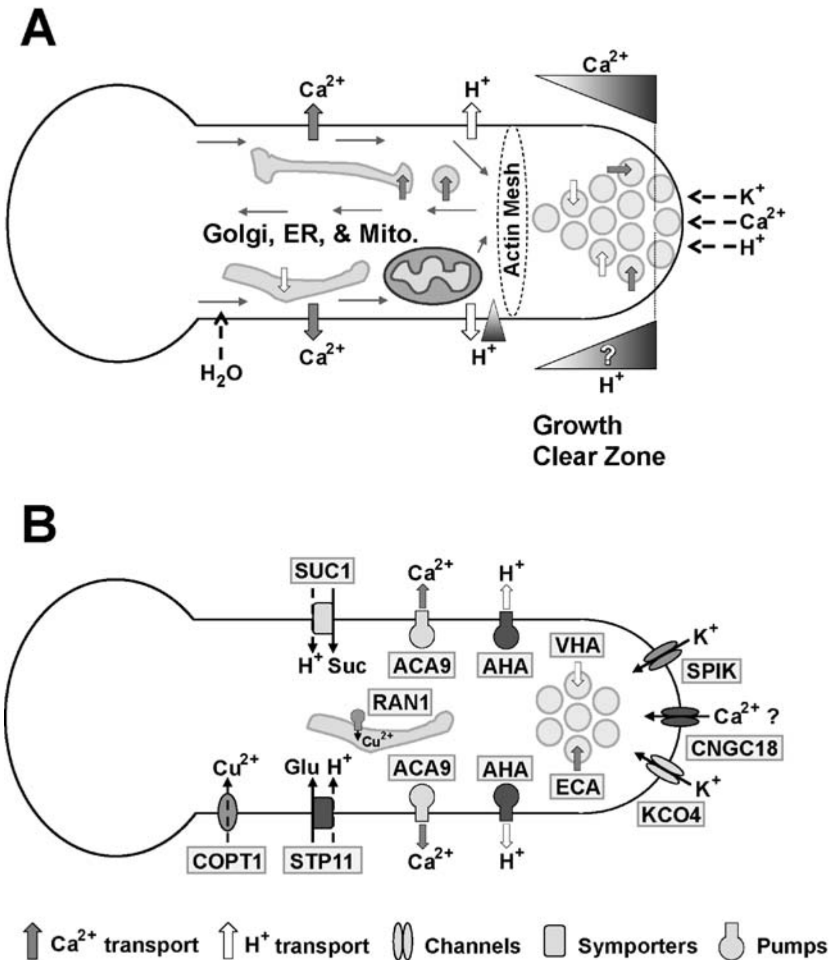

Fig. 1 Ion fluxes and specific transporters of pollen tubes. **A** Major ion fluxes and gradients detected in growing pollen tubes. A $[Ca^{2+}]_c$ gradient, high at the extreme tip, and a postulated pH_c gradient, acidic at the tip, of the pollen tube are denoted by *adjacent triangles*, respectively. Extracellular Ca^{2+} enters at the tip. Ca^{2+} is postulated to accumulate in internal stores and/or to be extruded across the plasma membrane. Release of internally stored Ca^{2+} could also increase $[Ca^{2+}]_c$. H^+ enters at the tip, and is pumped out at the shank. Extracellular K^+ enters at the tip. *Solid* and *dotted arrows* indicate active and passive transport, respectively. Active H^+ and Ca^{2+} transport is represented by *white-* and *grey-filled arrows*, respectively. *Line arrows* indicate direction of cytoplasmic streaming in the tube. Adapted from Holdaway-Clarke and Hepler (2003). **B** Selected pumps, cotransporters and channels identified in pollen tubes. Molecular studies have identified K^+ channels (SPIK and KCO4); putative Ca-permeable channel (CNGC18); Ca^{2+} pump (ACA9), and sugar symporters (SUC1 and STP11) at the plasma membrane. H^+ pumps AHA and VHA are presumed to be localized at the PM and endomembranes, respectively based on studies using sporophytic tissues. ECA Ca^{2+} pump and RAN1 Cu-ATPase are assumed to be on endomembranes. Cu transporter (COPT1) is likely localized to the PM

tophyte is to identify transporters expressed in pollen and to determine their specific functions.

2
Ion Current, Gradient and Oscillation During Pollen Germination and Tube Growth

2.1
Ca^{2+}

Ca^{2+} is the most prominent ion required for pollen germination and tube growth (Brewbaker & Kwack 1963). Depending on the plant species, extracellular Ca^{2+}, $[Ca^{2+}]_{ext}$, needed to support optimal tube growth *in vitro* varies from 0.1–5 mM (Steer & Steer 1989). At the apex, there is a tip-focused gradient of cytosolic free Ca^{2+}, $[Ca^{2+}]_c$ reaching 3–10 μM at the extreme tip and dropping sharply to basal levels of 150–300 nM within 20 μm from the apex (Fig. 1A, Hepler et al., this volume). The $[Ca^{2+}]_c$ gradient at the apex oscillates in phase with growth (Holdaway-Clarke et al. 1997; Messerli and Robinson 1997) and the oscillation, though large in magnitude (0.75–3 μM), remains at the extreme tip and is not propagated basipetally. Using yellow cameleon Ca^{2+} indicator, similar Ca^{2+} oscillations were observed at the tip of *Arabidopsis* pollen tube (Iwano et al. 2004). Changes of $[Ca^{2+}]_c$ within the apical dome also play a role in reorientation of the pollen tube (Malhó et al. 1995; Malhó and Trewavas 1996).

An intriguing question is how does pollen generate and maintain a $[Ca^{2+}]_c$ gradient that oscillates only at the extreme tip. With the electrical potential, negative inside (– 130 mV), and the pH gradients across the PM of plant cells (acid outside), Ca^{2+} is predicted to enter the cytosol passively via channels from the outside or from internal stores. Given the tip-focused Ca^{2+} gradient, it is likely that external Ca^{2+} enters via pathways concentrated at the pollen tip. Many experiments support this idea: (i) Autoradiography showed $^{45}Ca^{2+}$ is taken up at the tip (Jaffe et al. 1975); (ii) Mn^{2+} quenched indo-1 flourescence at the tip (Malhó et al. 1995); and (iii) vibrating electrode indicated influx of extracellular Ca^{2+} at the tip (e.g., Kuehtreiber & Jaffe 1990). $[Ca^{2+}]_c$ is most likely lowered by active Ca^{2+} pumps or H^+-coupled Ca^{2+} antiporters located on intracellular membranes and/or at the PM (Sze et al. 2000). Cytosolic Ca^{2+} oscillations are proposed to occur when Ca^{2+} release into the cytosol and its removal from the cytosol are temporally- and spatially-controlled by transporters localized at intracellular compartments and/or at the plasma membrane (Hepler et al., this volume).

The nature of the Ca^{2+} permeable influx and efflux pathways that create the tip-focused $[Ca^{2+}]_c$ gradient are poorly understood, though putative Ca^{2+} channel activities were recently reported. Suggested candidates for Ca^{2+}

permeable channels include stretch-activated ion channels as seen in fungal hyphae (Garrill et al. 1993), voltage-activated channels (Malhó et al. 1995) as observed in root hair cells (Very & Davies 2000), and cyclic nucleotide gated channels (CNGC) (Moutinho et al. 2001). Recently, Ca^{2+} conductance activated by symmetric positive or negative pressure was detected in lily pollen. The stretch-activated channel in the grain is restricted to a region where the tip emerges. Channel activity of the tube protoplast is lower in density and conductance than at the tip (Dutta & Robinson 2004). Spider venom, a blocker of stretch-activated ion channels, inhibited germination, tube growth and Ca^{2+} conductance. A hyperpolarization-activated inward Ca^{2+}-permeable channel was reported on the PM of *Arabidopsis* pollen (Wang et al. 2004) and of lily pollen protoplast (Shang et al. 2005). In lilies, the conductance was suppressed by trivalent cations, verapamil, nifedipine or diltiazem, and by calmodulin. The identities of these channels have yet to be determined at the molecular level. One possible candidate is CNGC18, a cation permeable channel, localized at the growing tip of *Arabidopsis* pollen (Frietsch S, Schroeder J, Harper JF, unpublished) (Sect. 4).

Assuming that pollen tubes are like other growing plant cells, it is likely that Ca^{2+} transporters on intracellular compartments participate in forming the tip-focused $[Ca^{2+}]_c$ gradient and Ca^{2+} oscillations. Secretory vesicles are abundant at the tip, thus they provide an enormous membrane area in close proximity to cytosolic Ca^{2+}. $[Ca^{2+}]_c$ increases at the extreme tip when extracellular Ca^{2+} enters the tube through activated PM-localized channels. Ca^{2+}-pumping ATPases (ACAs & ECAs) and H^+/Ca^{2+} antiporters (CAXs) are localized to the vacuole, ER, and perhaps in the Golgi and secretory vesicle/tubule abundant in growing cells, indicating that intracellular compartments serve as a rich source of stored Ca^{2+} (Sze et al. 2000). Chlorotetracycline fluorescence at the tip of growing pollen tube supports the idea for an apical gradient of Ca^{2+} associated with membranes (Reiss and Herth 1978; Malhó et al. 2000) but it has not been tested if $[Ca^{2+}]_m$ show the same type of dynamics observed for $[Ca^{2+}]_c$. Ca^{2+} release from the endomembrane vesicles through channels, such as those activated by hyperpolarization (e.g., Slow Vacuolar ion channel), and by ligands (IP_3, cADPR), could contribute to additional increase in $[Ca^{2+}]_c$. This rise in $[Ca^{2+}]_c$ would activate Ca^{2+} pumps and Ca^{2+}/H^+ antiporters in intracellular compartments and on the PM, thus lowering $[Ca^{2+}]_c$.

Ca^{2+} oscillations in pollen is thought to include temporally- and spatially-regulated "Ca^{2+} signatures" (Rudd and Franklin-Tong 2000; Sanders et al. 2002) that are involved in the specificity of the downstream responses, including turgor and cytoskeleton changes, and cell wall assembly. Despite recent electrophysiological evidence for calcium channel activity and isolation of the first Ca^{2+} transporter required for pollen tube growth and fertilization (Sect. 4), the molecular mechanisms controlling Ca^{2+} dynamics in polarized pollen tip growth remain a challenge.

2.2 pH and Oscillations

The role of pH in signaling is poorly understood in plants. *In vitro* pollen tube growth is optimal at pH 4.5–6, which is similar to root growth (Holdaway-Clarke et al. 2003). Recent studies using the pH indicator dye, BCECF-dextran, revealed the presence of a local pH gradient inside the pollen tube; an acidic domain estimated at pH 6.8 at the extreme tip in growing tubes, and an alkaline region of pH 7.5 at the base of the clear zone (Feijó et al. 1999). However the role of a pH_c gradient in tip growth is controversial. Several other studies showed an average pH_c of 7.1 with no standing pH gradient required for growth (Fricker et al. 1997; Parton et al. 1997; Messerli and Robinson 1998). Like Ca^{2+}, the internal pH (pH_i) oscillates at the apex (Feijó et al. 1999) and an acidic wave was observed to move basipetally (Messerli and Robinson 1998). How does oscillatory changes in pH occur? In plants, PM-localized pumps extrude H^+ to the outside, and V-ATPase and PPase pump H^+ into intracellular compartments (Sze et al. 1999). Vacuolar H^+-ATPases very likely affect pH in the cytosol, the compartments, and the outside, as these pumps are localized on Golgi, prevacuolar vesicles, and possibly secretory vesicles and endosomes. Unlike the case of Ca^{2+}, pH changes depend on a collaboration of co-transporters and channels in addition to H^+ pumps and passive H^+ fluxes. For instance, H^+ pumping into intracellular vesicles by a V-ATPase alone generates an electrical potential, positive inside, and a small change in ΔpH. However, anion influx (e.g., Cl^-) presumably via a voltage-activated anion channel will dissipate the charge difference and increase H^+ pumping to form an acidic domain at the lumenal side (Sze 1985). Conversely, activation of an H^+/K^+ exchanger at the vacuolar membrane where H^+ moves back to the cytoplasmic face, could raise the pH at the lumen face or locally acidify the cytosolic side. The local gradients would form albeit transiently due to the mobility of the H^+ ion and cytosolic buffering.

Thus, a pH oscillation would be formed by the temporal activation and inactivation of transporters that are localized on intracellular membrane and on the PM. The transporters include proton pumps, ion channels, H^+-coupled cotransporters or proton leak pathways. As K^+ is the major osmotic ion in cells, K^+ channels and H^+/K^+ exchangers are postulated to be prominent players. PM and vacuolar H^+-ATPases are highly expressed in mature pollen and in pollen tubes (Padmanaban et al. 2004; Dettmer et al. 2005; Sect. 3), though the identity and spatial location of ion channels, H^+-coupled cotransporters or proton leak pathways are poorly defined.

2.3 K^+

As the most abundant ion in plant cells (where $[K^+]_c$ is maintained at ~ 75 mM), K^+ serves various functions, including osmoregulation, cell expansion, cell movement, enzyme/protein activation, stress tolerance and modulation of the electrical potential. Pollen tube growth can be sustained with 0.1 or 1 mM K^+_{ext} indicating that K^+ is taken up via an energy-dependent H^+/K^+ symporter, and via K^+_{in} channels (Mouline et al. 2002). Various K^+ channel activities have been detected in protoplasts from pollen using the whole cell patch-clamp or single channel recording. These include (i) a hyperpolarization-activated cation channel (HACC), like an inward K^+ conductance that is insensitive to $[Ca^{2+}]_c$ but stimulated by acidic pH_{ext} (Fan et al. 2001; Mouline et al. 2002); (ii) depolarization-activated cation channel (DACC) that conducts K^+ outward (Fan et al. 2003); (iii) voltage-insensitive cation channel (VICC) that conducts K^+_{in} (Becker et al. 2004), (iv) voltage-insensitive and stretch-activated K^+, and (v) a spontaneous K^+ conductance (Griessner and Obermeyer 2003; Dutta and Robinson 2004). Genetic and functional studies have identified the first K^+ channels in pollen, SPIK/AKT6 as a Shaker K^+ inward rectifier (Mouline et al. 2002), and KCO4 as a voltage-insensitive K^+ channel (Becker et al. 2004) (Sect. 4). Nearly nothing is known about K^+ transporters on the vacuolar membrane of the vegetative cell, though vacuoles are very dynamic in growing pollen tubes (Hicks et al. 2004).

2.4 Other Ions-Chloride & Borate

Cl^- is considered a micronutrient that is needed for plant growth at an external concentration of $\sim 50\ \mu M$. Its major role is thought to regulate electrical potentials, and so influence turgor and cell volume (Ward et al. 1995). Interestingly, Cl^- is not required for *in vitro* pollen tube growth, suggesting its major role is in electrical signaling. Inorganic anions enter cells against an electrochemical gradient mediated by PM-localized H^+-anion symporters. When signals, like high $[Ca^{2+}]_c$, stimulate opening of an anion channel, anion efflux causes membrane depolarization which can lead to downstream physiological responses (e.g., Ward et al. 1995; Cho and Spalding 1996).

The relative contribution of anion flux, particularly Cl^- in pollen tube growth is currently controversial. Anion channel blockers, like DIDS (4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid), NPPB (5-nitro-2-(3-phenylpropylamide)benzoic acid) or niflumic acid, inhibited pollen germination and apparent Cl^- efflux (Zonia et al. 2002; Matveyeva et al. 2003). Cl^- efflux that oscillated in phase with growth was reported at the extreme apex (Zonia et al. 2002) but the selectivity of the microelectrode for Cl^- to other anions

(< 10 fold) or to MES (2.4 fold) was low, raising the possibility that H^+ influx contributed in large part to the signal (Messerli et al. 2004; Hepler et al., this volume). Inhibitor studies would suggest that Cl^- or other anion transport are involved in pollen germination and tube growth. Cl^- channel activity was, however, not detected by whole cell patch clamping under any condition tested (Dutta & Robinson 2004). Clearly, further studies are needed to determine the location of anion flux and the specific protein(s) mediating the transport.

Although external boric acid has long been recognized as an essential element for *in vitro* pollen tube growth, a biochemical role of boron in plant nutrition was recently established (Blevins and Lukaszewski 1998). One role is to stabilize cell wall structures. L-Fucose-deficient *Arabidopsis* mutants show only 50% cross-linked pectin and have a dwarfed phenotype that is reversed by exogenous borate and L-fucose (O'Neill et al. 2001). These results are consistent with the idea that borate ester connects and dimerizes rhamnogalacturonan II, a subclass of pectin. The finding that *Picea* pollen germinated in boron-deficient medium accumulated acidic pectin at the pollen tip (Wang et al. 2003) would support this idea. Combined with the requirement for extracellular fluxes of Ca^{2+} and pH in pollen tip growth, it has been proposed that binding of Ca^{2+} and borate to pectin may alter the mechanical properties of the apical cell wall (Holdaway-Clarke et al. 2003). Uncharged boric acid may enter the cell by diffusion, and borate is then supplied to walls via a PM-localized BOR1 efflux transporter (Takano et al. 2002).

3
Integrating Transport with Male Gametophyte Development and Function

One powerful approach to identify transporters that function in pollen development is through transcriptomics (Twell et al., this volume). The pollen transcriptome revealed for the first time most of the genes expressed in the mature pollen. Moreover, a unique dataset of four stages of male gametophyte development enables developmental analyses of gene expression (Honys and Twell 2004). Identification of specific transporter genes expressed in developing pollen is providing insights for strategic mutant analyses to integrate the roles of transporters in pollen development, pollen maturation, or post-pollinations events.

3.1
Identifying Transporter Genes Specific to Pollen in Arabidopsis

To identify transporter genes expressed in pollen, Bock et al. (2006) compiled a list of all known and predicted transporters from the *Arabidopsis thaliana* genome. Transport proteins are highly conserved from bacteria to eukaryotes;

Table 1 Partial list of transporter genes that are specifically (S) or preferentially (P) expressed in male gametophyte. The pollen transcriptome from 4 stages of development (Honys and Twell 2004) was compared with transcriptome data of 12 sporophytic tissues. MaxPo indicates the maximum expression level observed in any stage of male gametophyte; MaxSp refers to the maximum expression signal in any sporophytic tissue. Preferential expression was defined as genes showing a Fold Change or ratio pf MaxPo/MaxSp of > 3 (Bock et al. 2006)

AGI Name	TC #	FAMILY	Protein Description	PROTEIN	Pol	Max Po	Max Sp	Fold C	Clus
At2g25600	1.A.1	VIC	potassium channel	AtSPIK	S *	1852	0	Spec	1
At1g19780	1.A.1	VIC	put. cyclic nucleotide and calmodulin-reg. ion channel	AtCNGC8	S *	1153	0	Spec	2
At5g14870	1.A.1	VIC	put. cyclic nucl and calmodulin-reg. ion channel	AtCNGC18	P *	1099	95	11.5	2
At1g15990	1.A.1	VIC	put. cyclic nucl. and calmodulin-reg. ion channel	AtCNGC7	S *	426	0	Spec	2
At3g48010	1.A.1	VIC	put. cyclic nucl. and calmodulin-reg. ion channel	AtCNGC16	S *	420	0	Spec	2
At4g01470	1.A.8	MIP	putative tonoplast intrinsic protein 3 gamma	AtTIP1.3	P *	4787	27	176.7	18
At5g37810	1.A.8	MIP	NOD26-like intrinsic protein	AtNIP4.1	P *	1796	18	97.5	5
At3g47440	1.A.8	MIP	putative tonoplast intrinsic protein	AtTIP5.1	S *	1616	0	Spec	3
At5g23270	2.A.1.1	MFS	monosacc.-H^+ symporter	AtSTP11	P *	3814	140	27.3	1
At1g07340	2.A.1.1	MFS	monosacc.-H^+ symporter	AtSTP2	P	3403	60	57.1	29
At3g05150	2.A.1.1	MFS	glucose transport family, AtERD6 homol.	At3g05150	P *	2510	320	7.9	1

* indicates genes assigned by Pina et al. (2005) as pollen-specific or pollen-enriched in mature pollen. Cluster number (Clus) refers to distinct expression pattern of each gene assigned by Honys & Twell (2004)

Table 1 (continued)

AGI Name	TC #	FAMILY	Protein Description	PROTEIN	Pol	Max Po	Max Sp	Fold C	Clus
At1g50310	2.A.1.1	MFS	monosacc.-H^+ symporter, glucose-spec	AtSTP9	P *	1923	165	11.7	22
At3g03090	2.A.1.1	MFS	xylose transporter homolog	At3g03090	P *	1880	287	6.6	1
At5g28470	2.A.17	POT	proton-dependent oligopeptide transport	At5g28470	S *	1648	0	Spec	25
At4g35180	2.A.18	AAAP	putative lys/his transporter	AtLHT7	P *	5640	477	11.8	1
At1g71680	2.A.18	AAAP	Put. lys/his transporter	AtLHT8	S	4104	0	Spec	20
At5g25430	2.A.31	AE	putative boron transporter	At5g25430	P *	1497	46	32.2	1
At3g17630	2.A.37	CPA2	Put. cation-H^+ exchanger	AtCHX19	P	3133	107	29.2	22
At2g28180	2.A.37	CPA2	Put. cation-H^+ exchanger	AtCHX8	S *	3086	0	Spec	1
At2g13620	2.A.37	CPA2	Put. cation-H^+ exchanger	AtCHX15	S *	1585	0	Spec	2
At1g79400	2.A.37	CPA2	Put. cation-H^+ exchanger	AtCHX2	P *	1198	69	17.2	2
At5g01690	2.A.37	CPA2	Put. cation-H^+ exchanger	AtCHX27	P *	1084	268	4.1	2
At4g18790	2.A.55	Nramp	Put. ion metal transporter	AtNRAMP5	P *	1477	35	42.3	2
At2g07560	3.A.3	P-ATP	put. PM P3A-H^+-ATPase	AtAHA6	S *	8417	0	Spec	18
At1g80660	3.A.3	P-ATP	put. PM P3A-H^+-ATPase	AtAHA9	S *	6379	0	Spec	18
At3g42640	3.A.3	P-ATP	put. PM P3A-H^+-ATPase	AtAHA8	P *	6225	162	38.4	2
At3g21180	3.A.3	P-ATP	putative Ca^{2+} P2B-ATPase	AtACA9	P *	2781	349	8.0	3
At2g22950	3.A.3	P-ATP	putative Ca^{2+} P2B-ATPase	AtACA7	S *	2530	0	Spec	18

* indicates genes assigned by Pina et al. (2005) as pollen-specific or pollen-enriched in mature pollen. Cluster number (Clus) refers to distinct expression pattern of each gene assigned by Honys & Twell (2004)

Table 1 (continued)

AGI Name	TC #	FAMILY	Protein Description	PROTEIN	Pol	Max Po	Max Sp	Fold C	Clus
At1g54280	3.A.3	P-ATP	put. aminophospholipid translocase, P4-type	AtALA6	P *	1449	170	8.5	3
At3g13900	3.A.3	P-ATP	put. aminophospholipid translocase, P4-type	AtALA7	P *	1110	90	12.3	3
At1g26130	3.A.3	P-ATP	put. aminophospholipid translocase, P4-type	AtALA12	P *	861	154	5.6	3
At5g59040	9.A.12	Ctr2	copper transporter	AtCOPT3	S	3973	0	Spec	29

* indicates genes assigned by Pina et al. (2005) as pollen-specific or pollen-enriched in mature pollen. Cluster number (Clus) refers to distinct expression pattern of each gene assigned by Honys & Twell (2004)

thus, they can be classified into functional families according to the Transport Classification (Busch and Saier 2004). Using databases of genes encoding plant membrane proteins, including the Arabidopsis Membrane Protein Library (AMPL) (Ward 2001); PlantsT (Tschieu et al. 2003) and ARAMEMNON (Schwacke et al. 2003), a comprehensive list of all known or predicted transporters was assembled. Of 1106 classified transporter genes present on the ATH1 chip, ∼ 746 are expressed in pollen (Bock et al., accepted; Twell et al., this volume). By comparing the normalized gene expression level of pollen at four developmental stages (Honys & Twell 2004) to that of 12 sporophytic tissues, more than 120 genes that are specifically or preferentially expressed throughout pollen development were identified (Table 1). Genes that were present in pollen but absent in any sporophytic tissue were labeled as "pollen-specific". Genes that were expressed at least 3-fold higher level in any stage of pollen relative to the maximum expression in any sporophytic tissues were marked as "preferentially-expressed". In a study of mature pollen transcriptome, Pina et al. (2005) assigned 94 transporter genes as pollen-specific or pollen-enriched.

In spite of different methods used to analyze two pollen transcriptomes, 51 genes were identified by both groups as either specifically or preferentially expressed in mature pollen (see Table 1 for partial list). Highly expressed genes include PM H^+ pumps (AHA6, AHA8, AHA9), and Ca^{2+} pumps, (ACA9, and ACA7). Prominent channels include the SPIK K^+ channel, several CNGC channels and putative aquaporin TIP5.1. Putative cation/H^+ exchangers (CHX8, CHX15, CHX19), and one encoding a putative boron transporter (At5g25430) are preferentially expressed. Among metabolite transporters, several members of sugar transporters in the MFS superfamily (STP11), oligopeptide (At5g28470) and amino acid (LHT7, LHT8) transporters stand out. Most of these are expressed at the tricellular stage or in mature pollen grain. Analyses by Bock et al. (in press) showed that 5.2% and 7.1% of all genes expressed in microspores and mature pollen, respectively, are classified transporter genes. Furthermore, the proportion of transporter genes that are pollen – preferential (or specific) increased from 13.7% in microspore to 20.9% or 107 genes in tricellular and mature pollen (Honys and Twell 2004; Bock et al., in press; Twell et al., this volume). Thus the proportion of pollen preferential transporter genes being expressed increases as pollen matures, underscoring the significance of transport in the maturation and functions of the male gametophyte.

3.2 Early and Late Pollen Expressed Genes

While the transcriptome analyses indicates that pollen show expression for all the different classes of transporter genes that are also expressed in sporophytic tissues, analyses of individual gene families provides insights into

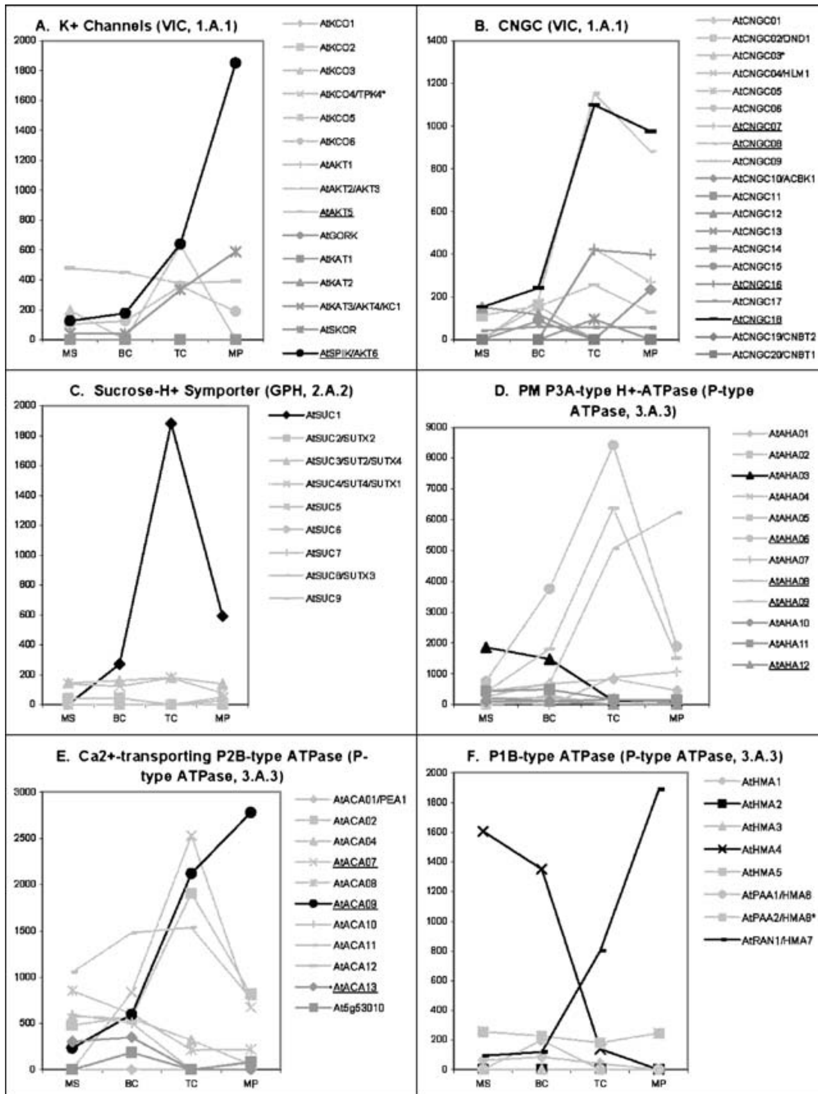

Fig. 2 Discrete subset of genes within selected gene families are expressed in male gametophyte in a developmentally-regulated manner. Relative expression of all genes within a gene family was monitored at the microspore (MS), bicellular (BC), tricellular (TC) and mature pollen (MP) stages. Each gene is identified by the given name when available or by the AGI number. *Underlined* gene name indicates those that are specifically or preferentially expressed in pollen. *Black line* highlights genes that have been genetically or functionally characterized. **A** VIC K^+ channel; **B** VIC Cyclic Nucleotide-Gated ion Channel (CNGC); **C** GPH Sucrose-proton symporter/sucrose transporter (SUC), TC# 2.A.2; **D** Plasma membrane P3A-type H^+-ATPase, TC# 3.A.3; **E** Calmodulin-regulated Ca^{2+}-transporting P2B-type ATPase, TC# 3.A.3; **F** Cu^{2+}-transporting and $Zn^{2+}/Co^{2+}/Cd^{2+}/Pb^{2+}$-transporting P1B-type ATPase, TC# 3.A.3

potentially significant isoform-specific patterns of developmental regulation. For example, the PM H^+ pump AHA3 (non-pollen specific) gene is the only AHA member expressed early in development at the microspore and bicellular stage. In contrast, AHA6, AHA8 and AHA9 are expressed late in development at the tricellular pollen stage at 5–7-fold higher levels than other AHAs (Fig. 2). Among autoinhibited Ca^{2+} pumps, several ACAs are expressed early in development, whereas ACA7, ACA2 and ACA9 are activated as pollen matures.

Expression patterns evaluated by other independent methods provide strong support for the normalized transcriptome results: (i) A portion of the pollen-specific or preferential genes has been verified by PCR amplification of reverse-transcribed pollen messages (Sze et al. 2004); (ii) The early and late pollen-expressed genes suggested by the microarray results have been confirmed by promoter::Gus staining of the anther or pollen at different floral stages for several CHX genes (Bock et al., in press); and (iii) differential expression of discrete gene members seen in transcriptomics were observed before by in situ hybridization or immunohistochemical staining. For instance, STP2 mRNA and protein were localized in the microspores but not found in tricellular or mature pollen (Truernit et al. 1999). In contrast, STP11 protein is exclusively in the pollen tube but not in the grains (Schneidereit et al. 2005). Transcriptomic analyses showed that STP11 message peaks at the tricellular stage; these results support the idea that many messages are stored until pollen germination.

The distinction of early and late pollen expressed genes is striking, indicating a sharp repression of certain genes at the bicellular stage, and an activation of other late transporter genes at the same time. It is then likely that early pollen-expressed genes are involved in microspore expansion and proliferation, whereas late-pollen expressed genes participate in pollen maturation, and in post-pollination events (Schiott et al. 2004; Twell et al., this volume).

4 Genetic and Functional Analyses of Transporters in Pollen Development and Tube Growth

The expectation is that most of the 746 pollen-expressed transporters in *Arabidopsis* will eventually be found to contribute to the development or fitness of pollen. The following are examples of gene knockout approaches that combined with other results have begun to reveal insights into pollen transporter functions. Although gene knockouts provide powerful tools, additional insights are expected from experimental strategies that include silencing, overexpression, and the use of dominant negative transgenes (Twell et al., this volume; Guermonprez et al., this volume).

4.1 Sugar Transporters

A large number of sugar transporters are specifically or preferentially expressed in the male gametophyte underscoring the demand for energy and carbon nutrient during microgametogenesis. STPs are monosaccharide/H^+ symporters at the plasma membrane and differential expression of STP2 and STP11 during microgametogenesis (Sect. 3), suggest that distinct STPs are used to take up monosaccharides at different stages of development (Truernit et al. 1999; Schneidereit et al. 2005). Other pollen-specific STPs (e.g., STP6, STP9) are co-expressed late in pollen development (Schneidereit et al. 2003; Scholz-Starke et al. 2003). This redundancy could account for the lack of phenotype in knockout mutants, such as in *stp6*.

Only one sugar transporter mutation has so far been shown to result in a pollen phenotype. A T-DNA gene disruption of SUC1 (*hap3*) was found to be completely male sterile (Johnson et al. 2004). *In vivo* cytological analysis indicated that mutant pollen tubes failed to enter the transmitting tract. SUC1 belongs to a family of sucrose/H^+ symporters localized at the PM (Stadler et al. 1999). Transcriptome data indicates it is the only SUC gene that is highly expressed relative to six other SUC family members in tricellular pollen (Fig. 2C). It is interesting that in spite of several monosaccharide/H^+ symporters in developing pollen, the genetic studies would suggest that a sufficient quantity of sucrose is critical to support pollen tube growth.

4.2 Proton Pumps

The expected function of multiple plasma membrane proton pumps (AHA, autoinhibited H^+-ATPase) in *Arabidopsis* is to energize the plasma membrane with 1) a proton gradient that drives nutrient co-transport systems, and 2) an electrical potential that drives ions through channels for both nutritional and signaling functions. The first null mutation reported for a plant AHA (*aha3*) was found to cause male sterility (Robertson et al. 2004). A cytological analysis indicated that the defect in *aha3* occurred early in pollen development but whether the defect in *aha3* is related to defects in signaling and/or nutrient uptake has not been determined.

The *aha3* loss-of-function phenotype provides an important new tool for investigating the structure and function of plant P-type proton pumps. Using *aha3* (–/+) plants, complementation was observed by the expression of a wild-type AHA3, but not a mutant AHA3 harboring a mutation of the penultimate residue in the C-terminal end (T948A) (Robertson et al. 2004). This mutation destroys a phosphorylation site critical to 14-3-3 binding. Since the binding of 14-3-3 can hyper-activate a plant P-type proton pump *in vitro*, it has been proposed as a key regulatory feature for controlling proton pump

activity *in planta* (Palmgren 2001). Strong evidence supporting this hypothesis is now provided by the failure to obtain complementation with the T948A mutant pump. These complementation experiments provide evidence that 14-3-3 is essential to pollen development, with at least one target being the plasma membrane proton pump.

The pollen sterile *aha3* null phenotype indicates that AHA3 provides an essential function that is not redundant with any of the other 11 AHA isoforms, or other proton pumping enzymes in plants (e.g., V-type proton pumps). Pollen transcriptome shows that *AHA3* appears to be the most highly expressed of the AHA genes in early pollen development (Fig. 2). At later stages of pollen development, different isoforms (AHA6, AHA8 and AHA9) are expressed at much higher levels. Whether one or more of these isoforms are involved in regulating the pH_c and pH_{ext} of growing pollen tubes has yet to be established.

Another proton pump, the vacuolar-type H^+-ATPase (VHA) acidifies various intracellular compartments, including the vacuole, ER, Golgi and undefined vesicles. The growing pollen tube is an exciting model to study the roles of the V-ATPase given its proposed functions in protein sorting, vesicle trafficking and membrane fusion (Sze et al. 1999, 2002; Padmanaban et al. 2004; Dettmer et al. 2005). In sporophytic tissues, the proton motive force energizes co-transport of ions and metabolites, and so is important for osmoregulation, cell expansion, and stress tolerance. However, other studies suggest that the functions of V-ATPase is much more diverse (Sze et al. 2002). In *Arabidopsis*, V-type ATPase complex of 12 subunits are encoded by 26 genes, most of which are highly expressed in pollen. Disruption of *VHA-A* (*vha-A*), a single copy gene for subunit A causes complete male and partial female gametophytic lethality (Dettmer et al. 2005). Evidence from RT-PCR analysis suggests small levels of *VHA-A* transcript in *vha-A* mutants. Nevertheless cytological analysis revealed abortion of *vha-A* pollen during development and Golgi stacks with abnormal morphologies after the first mitosis in mutant pollen grains, suggesting a crucial role of *VHA-A* in Golgi organization and protein trafficking (Dettmer et al. 2005).

The pollen lethal phenotype of *vha-A* suggests that proton pump activity of V-ATPase cannot be compensated by other H^+ pumps, such as the PPase AVP2 that is expressed during pollen development and that also targets to the Golgi (Dettmer et al. 2005).

4.3 K^+ Channels (VIC Family)

Of 15 genes encoding K^+ channels in the family of Voltage Gated-Ion Channels (VIC), only a few show relatively high expression in pollen according to transcriptome data (Fig. 2). SPIK, a Shaker channel, is highly expressed in male gametophyte late in development. Mutant pollen with gene disruption

in SPIK/AKT6 (At2g25600) showed decreased rate of tube growth in $[K^+]_{ext}$ ranging from 5 μM to 1 mM, and reduced hyperpolarization activated $K^+{}_{in}$ current (Mouline et al. 2002). The channel properties suggest SPIK is active at the PM and that it mediates bulk transport involved in K^+ nutrition. The *spik-1* mutation did show a general decrease in pollen fitness, suggesting that SPIK mediates K^+ uptake in growing pollen tube, and has a role in pollen tube development and pollen competitive ability. The residual K^+-dependent germination in the *spik-1* mutant is likely due to other K^+ permeable channels. However, gene disruption of another pollen-specific $K^+{}_{in}$ channel, TPK4 (KCO4), did not appear to alter pollen tube growth (Becker et al. 2004), though the ratio of the instantaneous current to the steady state current was reduced in the mutant pollen tube. TPK4 may be related to the spontaneously activating K^+ channel characterized in lily pollen (Dutta and Robinson 2004). Localized to the PM, TPK4/KCO4 is voltage-insensitive and is modulated by Ca^{2+} and pH, suggesting it has a role in K^+ homeostasis and voltage control (Becker et al. 2004).

The roles of four other K^+ channels of the VIC family expressed in pollen (Fig. 2) are not known though SKOR is most likely an outward K^+ rectifier as shown in xylem parenchyma cells (Very & Sentenac 2003). The roles of other putative K^+ transporters, including a K^+/H^+ symporter (KUP), K^+ $(Na^+)/H^+$ antiporters (CHX and NHX families), and other cation channels potentially permeable to K^+ (e.g., Glutamate receptors and CNGC) need to be investigated as a few members of these families are highly expressed in developing pollen (Table 1; Bock et al., in press).

4.4 Metal Transporters

Although the role of heavy metals in pollen development is not understood yet, their importance is highlighted by nutrition and genetic studies, and by the pollen transcriptome. For example, copper-deficient plants failed to produce grains possibly due to a defect in pollen formation (Graham 1997). It is thus interesting to find copper and other metal transporter genes (e.g., COPT3, COPT1) expressed at early and at late stages of pollen development (Bock et al., in press). Functional expression in yeast suggested COPT1 mediates Cu uptake at the PM, and this is confirmed by decreased Cu uptake and Cu content of transgenic *Arabidopsis* plants expressing anti-sense COPT1 (Sancenon et al. 2004). About 12% of the pollen grains from anti-sense plants showed slightly deformed morphology suggesting that Cu may be important for formation of the pollen wall.

The *Arabidopsis* genome encodes eight heavy metal-transporting P-type ATPases (HMA). The first loss-of-function mutant (*hma7/ran1*) was isolated in a genetic screen for ethylene response mutants (Woeste and Kieber 2000). Homozygous *hma7/ran1* plants show a copper deficiency and normally die

as young seedlings. If grown on agar media, mutants can survive till the reproductive phase but plants develop small flowers and are completely sterile. While this mutant clearly has a whole plant phenotype, there is also evidence for a pollen autonomous function of *hma7/ran1*. When pollen from plants heterozygous for *hma7/ran1* were crossed to wild type, there was a reduced transmission of the *hma7/ran1* mutant allele (i.e., segregation distortion). The *hma7/ran1* phenotype indicates that HMA7 provides essential function in plant development. Assuming RAN1 is localized to endomembrane compartments (e.g., Golgi or ER), the mutant phenotype suggests that Cu uptake into intracellular compartments is critical for yet undetermined aspects of pollen development or tube growth.

A double null mutation of isoforms HMA2 and HMA4, which are putative zinc translocating pumps, also result in severe growth defects, including anthers that do not produce any pollen (Hussain et al. 2004). These growth defects can be reversed by growing plants on high levels of zinc. HMA2 and HMA4 are expressed in vascular tissues, and HMA2 was localized to the PM. Both pumps are thought to have a role in regulating Zn^{2+} homeostasis in the whole plant.

HMA2 and HMA4 appear to have some level of functional redundancy or complementary activity since the sterility phenotype was only observed in the double knockout (Hussain et al. 2004). Expression profiling indicates that HMA4 and HMA7/RAN1 are the highest expressed isoforms at the early and late stages of developing pollen, respectively (Fig. 2F). Although expression for HMA2 was not detected in two independent microarray data sets, a positive result observed for a promoter-GUS reporter analysis suggests that HMA2 is expressed at an early stage of pollen development, but not in mature pollen (Hussain et al. 2004). It remains unclear if the male sterility of *hma2 hma4* double mutants is primarily caused by a general perturbation of Zn^{2+} homeostasis in the whole-plant or whether these pumps have an essential pollen-specific function as well.

4.5
Calcium Pumps

The *Arabidopsis* genome encodes 14 P-type calcium pumps (10 ACAs, autoinhibited Ca^{2+}-ATPase and 4 ECAs, ER-type Ca-ATPases) that are proposed to lower $[Ca^{2+}]_c$ by extruding Ca^{2+} into intracellular compartments or outside the cell (Sze et al. 2000). The first null mutation reported for a plant ACA (*aca9*) was found to cause partial male sterility (Schiott et al. 2004). The primary defect was a failure of *aca9* pollen to discharge sperm into the synergid. While mutant pollen tubes were able to reach ovules, they failed to complete fertilization in more than 50% of the interactions. Evidence indicates that the ACA9 pump is primarily located at the plasma membrane. A plasma membrane Ca^{2+} pump may either function in signal transduction by helping to

control the magnitude or duration of a Ca^{2+} signal (signaling function), or in cell wall biogenesis by pumping Ca^{2+} directly into the wall (nutritional function), or both. The fact that ACAs are activated by Ca^{2+}/calmodulin, indicates that they are directly connected to Ca^{2+} signaling.

The observation that the *aca9* null phenotype does not result in complete male sterile phenotype leaves open the question of whether other isoforms provide some low level of functional redundancy. In mature pollen, expression profiling studies show that *ACA9* is more highly expressed than any other ACA type calcium pump, including ACA2, ACA7 and ACA8 (Fig. 2). The expression of ACA8, a known plasma membrane Ca^{2+} pump (Bonza et al. 2000), under the control of the ACA9 promoter was shown to complement the *aca9* null mutation. While this indicates that *Arabidopsis* pollen do express pumps that have the potential to be functionally redundant, expression levels are not sufficient to completely compensate for the loss of the most abundant isoform, ACA9.

Interestingly, pollen-expressed ACA9 is also preferentially expressed in roots based on the transcriptome data of several sporophytic tissues (Bock K, Sze H, unpublished). As root hairs are known to elongate by tip growth, it is tempting to speculate that ACA9 performs a similar function in tip growth of both pollen tube and root hairs.

Pumps that load Ca^{2+} into endomembrane compartments also influence pollen tube growth. Although several ECA genes are expressed in pollen, homozygous mutants of ECA3 gene alone reduced pollen tube growth *in vitro* (Li X, Harper JF, Sze H, unpublished). Results suggest ECA3 affects lumenal $[Ca^{2+}]$ in compartments distinct from the ER where ECA1 and ACA2 are localized. It is possible that *eca3* mutants altered one or more of the following: (i) $[Ca^{2+}]_c$ dynamics needed for signaling; and (ii) lumenal $[Ca^{2+}]$ required to activate enzymes/proteins involved in protein sorting, modification or cell wall biosynthesis.

4.6 Cyclic Nucleotide-Gated Channels

CNGCs in plants, like animal homologs, are non-selective cation channels that are Ca^{2+} permeable (Talke et al. 2003). The first null mutation reported for a plant CNGC (*cngc2*) resulted in a dwarf plant with a lesion mimic/pathogen response phenotype, as well as poor fertility (Clough et al. 2000). While a specific role of CNGC2 in pollen has not been investigated, this gene is highly expressed at early stages of pollen development. In contrast to cngc2's weak fertility phenotype, a complete male sterile phenotype results from a null mutation of isoform CNGC18 (Frietsch S, Schroeder J, Harper JF, unpublished). Two independent T-DNA insertions in *CNGC18* were found to completely block pollen transmission of the mutant allele. A cytological analysis indicated that the primary defect was a failure of *cngc18* pollen

to undergo directional growth into the transmitting tract of the pistil. Evidence from a GFP tagging strategy suggests that CNGC18 has a tip-focused plasma membrane location. While the ion conductance properties of CNGC18 have not been directly tested, CNGCs are thought to be Ca^{2+} permeable, non-specific cation channels (Leng 1999; Talke et al. 2003) that are activated by cyclic nucleotides and inhibited by the binding of Ca^{2+}/calmodulin (Talke et al. 2003; Hua et al. 2003). The apparent tip-focused location of CNGC18 suggests that this channel may function in regulating tip-localized Ca^{2+} signals that coordinate the directional growth machinery (Fig. 1B).

The *cngc18* null phenotype indicates that CNGC18 provides an essential function that is not compensated by any of the other 6 or 7 pollen-expressed CNGC isoforms (Fig. 2B). In mature pollen, *CNGC18* and CNGC8 are the two most highly expressed *CNGC*s, with other isoforms showing less than half of their expression levels. Since some CNGCs are thought to form hetero-multimers, multiple isoforms expressed in plant pollen may form a highly diverse set of channels with different ion permeation or gating properties. Nevertheless, a function for these additional pollen isoforms has not yet been established.

5
Perspectives

Genetic studies are just beginning to provide valuable insights on the roles of specific pumps, carriers and channels in pollen nutrition, development and tube growth. Remarkably, almost 70% (746) of classified transporter genes are expressed in the male gametophyte of *Arabidopsis*. Most of these are expected to be expressed in the single vegetative cell that develops into the growing pollen tube, thus the developmentally-regulated expression of distinct transporters give clues for strategic functional analyses of each mutant. We anticipate that this system will provide a paradigm for transporter functions in general, and pollen development in specific. It is important to note that pollination and post-pollination events trigger pistil responses and guidance cues (Johnson et al., this volume) that conceivably modulate ion fluxes and gradients, and so orient pollen tube growth. Furthermore new methods are emerging to analyze *in vivo* and semi-in vivo pollen tube growth (Higashiyama and Inatsugi, this volume) and to image ion dynamics in targeted cells (Iwano et al. 2004). In the next ten years, it is reasonable to expect genetic, biochemical and cellular insights into the functions of most transporters in pollen, and their integration with pollen development in *Arabidopsis* and in other higher plants.

References

Becker D, Geiger D, Dunkel M, Roller A, Bertl A, Latz A, Carpaneto A, Dietrich P, Roelfsema MR, Voelker C, Schmidt D, Mueller-Roeber B, Czempinski K, Hedrich R (2004) AtTPK4, an Arabidopsis tandem-pore K^+ channel, poised to control the pollen membrane voltage in a pH- and Ca^{2+}-dependent manner. Proc Natl Acad Sci USA 101:15621–15626

Blevins DG, Lukaszewski KM (1998) Boron in plant structure and function. Annu Rev Plant Physiol Plant Mol Biol 49:481–500

Bock KW, Honys D, Ward JM, Padmanaban S, Nawrocki EP, Hirschi KD, Twell D, Sze H (2006) Integrating Membrane Transport with Male Gametophyte Development and Function through Transcriptomics. Plant Physiol. (in press)

Bonza MC, Morandini P, Luoni L, Geisler M, Palmgren MG, De Michelis MI (2000) AtACA8 encodes a plasma membrane-localized calcium-ATPase of Arabidopsis with a calmodulin-binding domain at the N terminus. Plant Physiol 123:1495–1506

Brewbaker JL, Kwack BH (1963) The essential role of calcium ion in pollen germination and pollen tube growth. Am J Bot 50:859–865

Busch W, Saier MH Jr (2004) The IUBMB-endorsed transporter classification system. Mol Biotechnol 27:253–262

Cho MH, Spalding EP (1996) An anion channel in Arabidopsis hypocotyls activated by blue light. Proc Natl Acad Sci USA 93:8134–8138

Clough SJ, Fengler KA, Yu IC, Lippok B, Smith RK Jr, Bent AF (2000) The Arabidopsis dnd1 defense, no death gene encodes a mutated cyclic nucleotide-gated ion channel. Proc Natl Acad Sci USA 97:9323–9328

Dettmer J, Schubert D, Calvo-Weimar O, Stierhof YD, Schmidt R, Schumacher K (2005) Essential role of the V-ATPase in male gametophyte development. Plant J 41:117–124

Dutta R, Robinson KR (2004) Identification and characterization of stretch-activated ion channels in pollen protoplasts. Plant Physiol 135:1398–1406

Fan LM, Wang YF, Wang H, Wu WH (2001) In vitro Arabidopsis pollen germination and characterization of the inward potassium currents in Arabidopsis pollen grain protoplasts. J Exp Bot 52:1603–1614

Fan LM, Wang YF, Wu WH (2003) Outward K^+ channels in Brassica chinensis pollen protoplasts are regulated by external and internal pH. Protoplasma 220:143–152

Feijó JA, Sainhas J, Hackett GR, Kunkel JG, Hepler PK (1999) Growing pollen tubes possess a constitutive alkaline band in the clear zone and a growth-dependent acidic tip. J Cell Biol 144:483–496

Fricker MD, White NS, Obermeyer G (1997) pH gradients are not associated with tip growth in pollen tubes of Lilium longoflorum. J Cell Sci 110:1729–1740

Garrill A, Jackson SL, Lew RR, Heath IB (1993) Ion channel activity and tip growth: tip-localized stretch-activated channels generate an essential Ca^{2+} gradient in the oomycete Saprolegnia ferax. Eur J Cell Biol 60:358–365

Graham RD (1975) Male sterility in wheat plants deficient in copper. Nature 254:514–515

Griessner M, Obermeyer G (2003) Characterization of whole-cell K^+ currents across the plasma membrane of pollen grain and tube protoplasts of Lilium longiflorum. J Membr Biol 193:99–108

Hicks GR, Rojo E, Hong S, Carter DG, Raikhel NV (2004) Geminating pollen has tubular vacuoles, displays highly dynamic vacuole biogenesis, and requires VACUOLESS1 for proper function. Plant Physiol 134:1227–1239

Holdaway-Clarke TI, Hepler PK (2003) Control of pollen tube growth: role of ion gradients and fluxes. New Phytol 159:539–563

Holdaway-Clarke TL, Feijó JA, Hackett GR, Kunkel JG, Hepler PK (1997) Pollen tube growth and the intracellular cytosolic calcium gradient oscillate in phase while extracellular calcium influx Is delayed. Plant Cell 9:1999–2010

Holdaway-Clarke TL, Weddle NM, Kim S, Robi A, Parris C, Kunkel JG, Hepler PK (2003) Effect of extracellular calcium, pH and borate on growth oscillations in Lilium formosanum pollen tubes. J Exp Bot 380:65–72

Honys D, Twell D (2004) Transcriptome analysis of haploid male gametophyte development in Arabidopsis. Genome Biol 5:R85

Hua BG, Mercier RW, Leng Q, Berkowitz GA (2003) Plants do it differently. A new basis for potassium/sodium selectivity in the pore of an ion channel. Plant Physiol 132:1353–1361

Hussain D, Haydon MJ, Wang Y, Wong E, Sherson SM, Young J, Camakaris J, Harper JF, Cobbett CS (2004) P-type ATPase heavy metal transporters with roles in essential zinc homeostasis in Arabidopsis. Plant Cell 16:1327–1339

Iwano M, Shiba H, Miwa T, Che FS, Takayama S, Nagai T, Miyawaki A, Isogai A (2004) Ca^{2+} dynamics in a pollen grain and papilla cell during pollination of Arabidopsis. Plant Physiol 136:3562–3571

Jaffe LA, Weisenseel MH, Jaffe LF (1975) Calcium accumulations within the growing tips of pollen tubes. J Cell Biol 67:488–492

Johnson MA, von Besser K, Zhou Q, Smith E, Aux G, Patton D, Levin JZ, Preuss D (2004) Arabidopsis hapless mutations define essential gametophytic functions. Genetics 168:971–982

Kuhtreiber WM, Jaffe LF (1990) Detection of extracellular calcium gradients with a calcium-specific vibrating electrode. J Cell Biol 110:1565–1573

Leng Q, Mercier RW, Hua BG, Fromm H, Berkowitz GA (2002) Electrophysiological analysis of cloned cyclic nucleotide-gated ion channels. Plant Physiol 128:400–410

Malhó R, Camacho L, Moutinho A (2000) Signalling pathways in pollen tube growth and reorientation. Ann Bot 85(suppl A):59–68

Malhó R, Read ND, Trewavas AJ, Pais MS (1995) Calcium channel activity during pollen tube growth and reorientation. Plant Cell 7:1173–1184

Malhó R, Trewavas AJ (1996) Localized apical increases of cytosolic free calcium control pollen tube orientation. Plant Cell 8:1935–1949

Matveyeva NP, Andreyuk DS, Yermakov IP (2003) Transport of Cl^- across the plasma membrane during pollen grain germination in tobacco. Biochemistry (Mosc) 68:1247–1251

Messerli M, Robinson KR (1997) Tip localized Ca^{2+} pulses are coincident with peak pulsatile growth rates in pollen tubes of Lilium longiflorum. J Cell Sci 110(Pt 11):1269–1278

Messerli MA, Robinson KR (1998) Cytoplasmic acidification and current influx follow growth pulses of Lilium longiflorum pollen tubes. Plant Journal 16:87–91

Messerli MA, Smith PJ, Lewis RC, Robinson KR (2004) Chloride fluxes in lily pollen tubes: a critical reevaluation. Plant J 40:799–812

Mouline K, Very AA, Gaymard F, Boucherez J, Pilot G, Devic M, Bouchez D, Thibaud JB, Sentenac H (2002) Pollen tube development and competitive ability are impaired by disruption of a Shaker K(+) channel in Arabidopsis. Genes Dev 16:339–350

Moutinho A, Hussey PJ, Trewavas AJ, Malhó R (2001) cAMP acts as a second messenger in pollen tube growth and reorientation. Proc Natl Acad Sci USA 98:10481–10486

O'Neill MA, Eberhard S, Albersheim P, Darvill AG (2001) Requirement of borate crosslinking of cell wall rhamnogalacturonan II for Arabidopsis growth. Science 294:846–849

Padmanaban S, Lin X, Perera I, Kawamura Y, Sze H (2004) Differential expression of vacuolar H^+-ATPase subunit c genes in tissues active in membrane trafficking and their roles in plant growth as revealed by RNAi. Plant Physiol 134:1514–1526

Palmgren MG (2001) Plant plasma membrane H^+-ATPases: Powerhouses for nutrient uptake. Annu Rev Plant Physiol Plant Mol Biol 52:817–845

Parton RM, Fischer S, Malhó R, Papasouliotis O, Jelitto T, Leonard T, Read ND (1997) Pronounced cytoplasmic pH gradients are not required for tip growth in plant and fungal cells. J Cell Sci 110:1187–1198

Pina C, Pinto F, Feijó JA, Becker JD (2005) Gene family analysis of the Arabidopsis pollen transcriptome reveals biological implications for cell growth, division control, and gene expression regulation. Plant Physiol 138:744–756

Reiss HD, Herth W (1978) Visualization of the Ca^{2+} gradient in growing pollen tubes of Lilium longiflorum with chlorotetraceycline fluorescence. Protoplasma 97:373–377

Robertson WR, Clark K, Young JC, Sussman MR (2004) An Arabidopsis thaliana plasma membrane proton pump is essential for pollen development. Genetics 168:1677–1687

Rudd JJ, Franklin-Tong VE (2000) Unravelling response-specificity in Ca^{2+} signaling pathways in plant cells. New Phytol 151:7–33

Sancenon V, Puig S, Mateu-Andres I, Dorcey E, Thiele DJ, Penarrubia L (2004) The Arabidopsis copper transporter COPT1 functions in root elongation and pollen development. J Biol Chem 279:15348–15355

Sanders D, Pelloux J, Brownlee C, Harper JF (2002) Calcium at the crossroads of signaling. Plant Cell 14 Suppl:S401–S417

Schiott M, Romanowsky SM, Baekgaard L, Jakobsen MK, Palmgren MG, Harper JF (2004) A plant plasma membrane Ca^{2+} pump is required for normal pollen tube growth and fertilization. Proc Natl Acad Sci USA 101:9502–9507

Schneidereit A, Scholz-Starke J, Buttner M (2003) Functional characterization and expression analyses of the glucose-specific AtSTP9 monosaccharide transporter in pollen of Arabidopsis. Plant Physiol 133:182–190

Schneidereit A, Scholz-Starke J, Sauer N, Buttner M (2005) AtSTP11, a pollen tube-specific monosaccharide transporter in Arabidopsis. Planta 221:48–55

Scholz-Starke J, Buttner M, Sauer N (2003) AtSTP6, a new pollen-specific H^+-monosaccharide symporter from Arabidopsis. Plant Physiol 131:70–77

Schwacke R, Schneider A, van der Graaff E, Fischer K, Catoni E, Desimone M, Frommer WB, Flugge UI, Kunze R (2003) ARAMEMNON, a novel database for Arabidopsis integral membrane proteins. Plant Physiol 131:16–26

Shang ZL, Ma LG, Zhang HL, He RR, Wang XC, Cui SJ, Sun DY (2005) Ca^{2+} influx into lily pollen grains through a hyperpolarization-activated Ca^{2+}-permeable channel which can be regulated by extracellular CaM. Plant Cell Physiol 46:598–608

Stadler R, Truernit E, Gahrtz M, Sauer N (1999) The AtSUC1 sucrose carrier may represent the osmotic driving force for anther dehiscence and pollen tube growth in Arabidopsis. Plant J 19:269–278

Steer MW, Steer JM (1989) Pollen tube tip growth. New Phytol 111:323–358

Sze H (1985) H^+-translocating ATPases: Advances using membrane vesicles. Annu Rev Plant Physiol 36:175–208

Sze H, Li X, Palmgren MG (1999) Energization of plant cell membranes by H^+-pumping ATPases. Regulation and biosynthesis. Plant Cell 11:677–690

Sze H, Liang F, Hwang I, Curran AC, Harper JF (2000) Diversity and regulation of plant Ca^{2+} pumps: insights from expression in yeast. Annu Rev Plant Physiol Plant Mol Biol 51:433–462

Sze H, Padmanaban S, Cellier F, Honys D, Cheng NH, Bock KW, Conejero G, Li X, Twell D, Ward JM, Hirschi KD (2004) Expression patterns of a novel AtCHX gene family highlight potential roles in osmotic adjustment and K^+ homeostasis in pollen development. Plant Physiol 136:2532–2547

Sze H, Schumacher K, Muller ML, Padmanaban S, Taiz L (2002) A simple nomenclature for a complex proton pump: VHA genes encode the vacuolar H(+)-ATPase. Trends Plant Sci 7:157–161

Takano J, Noguchi K, Yasumori M, Kobayashi M, Gajdos Z, Miwa K, Hayashi H, Yoneyama T, Fujiwara T (2002) Arabidopsis boron transporter for xylem loading. Nature 420:337–340

Talke IN, Blaudez D, Maathuis FJ, Sanders D (2003) CNGCs: prime targets of plant cyclic nucleotide signalling? Trends Plant Sci 8:286–293

Truernit E, Stadler R, Baier K, Sauer N (1999) A male gametophyte-specific monosaccharide transporter in Arabidopsis. Plant J 17:191–201

Tschieu JH, Fana F, Fink JL, Harper J, Nair TM, Niedner RH, Smith DW, Steube K, Tam TM, Veretnik S, Wang D, Gribskov M (2003) The PlantsP and PlantsT Functional Genomics Databases. Nucleic Acids Res 31:342–344

Very AA, Davies JM (2000) Hyperpolarization-activated calcium channels at the tip of Arabidopsis root hairs. Proc Natl Acad Sci USA 97:9801–9806

Very AA, Sentenac H (2003) Molecular mechanisms and regulation of K^+ transport in higher plants. Annu Rev Plant Biol 54:575–603

Wang Q, Lu L, Wu X, Li Y, Lin J (2003) Boron influences pollen germination and pollen tube growth in Picea meyeri. Tree Physiol 23:345–351

Wang YF, Fan LM, Zhang WZ, Zhang W, Wu WH (2004) Ca^{2+}-permeable channels in the plasma membrane of Arabidopsis pollen are regulated by actin microfilaments. Plant Physiol 136:3892–3904

Ward JM (2001) Identification of novel families of membrane proteins from the model plant Arabidopsis thaliana. Bioinformatics 17:560–563

Ward JM, Pei ZM, Schroeder JI (1995) Roles of ion channels in initiation of signal transduction in higher plants. Plant Cell 7:833–844

Weisenseel MH, Nuccitelli R, Jaffe LF (1975) Large electrical currents traverse growing pollen tubes. J Cell Biol 66:556–567

Woeste KE, Kieber JJ (2000) A strong loss-of-function mutation in RAN1 results in constitutive activation of the ethylene response pathway as well as a rosette-lethal phenotype. Plant Cell 12:443–455

Zonia L, Cordeiro S, Tupy J, Feijó JA (2002) Oscillatory chloride efflux at the pollen tube apex has a role in growth and cell volume regulation and is targeted by inositol 3,4,5,6-tetrakisphosphate. Plant Cell 14:2233–2249