

·Invited Review·

Diversification of the RAB Guanosine Triphosphatase Family in Dicots and Monocots

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Abstract

RAB guanosine triphosphatases (GTPases) are key regulators of vesicle trafficking and are essential to the growth and development of all eukaryotic cells. During evolution, the RAB family has expanded in different patterns to facilitate distinct cellular, developmental and physiological adaptations. Yeast has only 11 family members, whereas mammalian RABs have expanded to 18 RAB subfamilies. Plant RABs have diversified primarily by duplicating members within a single subfamily. Plant RABs are divided into eight subfamilies, corresponding to mammalian RAB1, RAB2, RAB5, RAB6, RAB7, RAB8, RAB11 and RAB18. Functional diversification of these is exemplified by the RAB11s, orthologs of which are partitioned into unique cell compartments in plants where they function to transport vesicles during localized tip growth. Similarly, the RAB2 family in grasses is likely involved in vesicle secretion associated with wall expansion, as determined by analysis of over-expression mutants. We propose that dicots and monocots have also diverged in their RAB profiles to accommodate unique cellular functions between the two groups. Here we present a bioinformatics analysis comparing the RAB sub-families of rice, maize and *Arabidopsis*. These results will guide future functional studies to test for the role of diversification of subfamilies unique to monocots compared to dicots.

Key words: dicot; GTP binding protein; monocot; phylogenetic analysis; RAB guanosine triphosphatase.

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Coordination of vesicle trafficking is essential to the growth and development of all eukaryotic cells. Members of the RAB family of guanosine triphosphatase (GTP) binding proteins are central to this process because they shuttle vesicles within specific cellular compartments and enable exocytosis, endocytosis, membrane cycling and other trafficking events (for review see

Molendijk et al. 2004; Grosshans et al. 2006). RABs belong to a larger superfamily of RAS proteins: all are highly conserved and share functional GTP binding domains across distantly related organisms (Pereira-Leal and Seabra 2001; Vernoud et al. 2003). Of all the RAS subgroups, RABs have expanded by duplication across and within organisms to become one of the largest families within the major group of RAS proteins. Because of this diversification and the central role of vesicle shuttling for cell growth, RABs have been under intensive study using functional and bioinformatics approaches (Pereira-Leal and Seabra 2001; Collins 2003; Vernoud et al. 2003; Buvelot Frei et al. 2006; Grosshans et al. 2006).

RABs insert into specific endomembranes by posttranslational modification at the carboxyl terminus. Most RABs have two cysteines, but some have conserved C terminal prenylation motif (CAAX) carboxyl terminus as described in Leung et al. (2007). Specificity of RAB function is also due to association of the activated RAB with diverse effector proteins that mediate vesicle transport, tethering, membrane fusion or other essential transport functions (Grosshans et al. 2006; Novick et al. 2006;

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Markgraf et al. 2007). RABs thus function within designated endomembrane compartments even for homologs across phylogenetically distant groups (Pereira-Leal and Seabra 2001; Stenmark and Olkkonen 2001; Zerial and McBride 2001; Vernoud et al. 2003).

RABs alternate between a cytosolic inactive and membrane-associated form. Research has recently focused on identifying and characterizing protein partners required for GTPase function of RABs (Grosshans et al. 2006; Markgraf et al. 2007). In all systems studied to date, RAB GTPases act as vesicle transporters by cycling between the GTP activated and guanosine diphosphate (GDP) inactivated state. Transitioning between GTP and GDP states involves numerous interacting proteins including GDIs (GDP dissociation inhibitors), which bind the inactive RAB, and which must be displaced by a membrane-bound GDF (GDI displacement factor) in order to become activated (Grosshans et al. 2006; Markgraf et al. 2007). A guanine nucleotide exchange factor (GEF) is required for GTP activation of the RAB, which in turn allows for RAB insertion into the target membrane and subsequent binding to numerous effector proteins. Eventually, vesicle binding, transport and/or fusion are initiated by the effector/RAB complex and the GTPase catalyzing function is mediated by a GAP (GTP hydrolysis activating protein). Members of all of these protein partners have been identified in yeast, mammalian systems, and are just being discovered in plants (Hala et al. 2005; reviewed in Yaneva and Niehaus 2005; Hanton et al. 2006; Heo et al. 2006).

Given that RABs share the conserved effector and GTP binding domains, important questions still remain about the degree of functional diversification of RABs across phylogenetic groups. Plant RABs are of particular interest because of the distinct cellular biology associated with ubiquitous cell expansion among higher plants and also because sequence conservation remains high between plants and other eukaryotic systems. Among the larger superfamily of RAS proteins, plants have been shown to contain four of the five subgroups including RABs, ARFs, RANs, RHOs (subfamily members of small GTP binding protein) and lacking the canonical RAS subgroup (reviewed in Yang 2002; Vernoud et al. 2003; Cole and Fowler 2006). Of these four groups, it appears that ROPs and RABs have diversified most extensively in plants.

ROPs (subfamily members of small GTP binding protein) have specialized to mediate tip growth, a unique plant-specific process, which requires localized secretion and transport to the elongating tip of root hairs and pollen tubes. Thus, molecular diversification of ROPs is consistent with acquisition of function during the evolution of unique plant cell functions. Several reviews discuss the molecular diversification of ROPs in relation to plant-specific functions (Yang 2002; Christensen et al. 2003; Cole and Fowler 2006).

RABs have also diversified on a molecular level in plants. Previously, 57 RABs were identified in *Arabidopsis* (Vernoud et al. 2003). Although some plant RABs share similar localiza-

tion patterns as non-plant RABs, it is likely that plant molecular diversification is associated with new cell growth functions that require vesicle trafficking. This hypothesis can be tested in plants once detailed functional studies are conducted with specific RABs involved in secretion and membrane cycling during cell expansion. Because of the molecular diversification of plant RABs, there is great need to investigate the underlying functional changes that accompanied molecular divergence. In this review, we discuss plant RAB molecular diversification and its relationship to predicted function, comparing the two major groups dicots and monocots that show unique cell biology.

The emergence of the full genomes of the monocot rice and dicot *Arabidopsis* allow for robust bioinformatics analysis of RABs (The Arabidopsis Genome Initiative 2000; International Rice Genome Sequencing Project 2005). *In silico* comparisons of protein structure are a starting point for designing well-guided functional studies, particularly when the plant sequences are compared with conserved and functionally characterized mammalian and yeast proteins. Here we review evidence for functional diversification of RABs in plant systems. First, we characterize the unique cell biology that necessitates diversified vesicle trafficking in plants. Next, we provide evidence for the acquisition of unique trafficking in both non-plant and plant systems. We identify rice and maize sequences and place them within the context of the known *Arabidopsis* family of RABs as presented by Vernoud et al. (2003). We present gene nomenclature for plant RABs based on the human numerical nomenclature (eg. RAB1, RAB2, etc. as in Botoko et al. 2000; de Graaf et al. 2005). Although some plant biologists have adopted an alphabetical classification (eg RABA, RABB, RABC etc. as in Vernoud et al. 2003), we consider that consistency of gene nomenclature with all other non-plant organisms is important due to the shared functions and high degree of sequence similarities.

The bioinformatic assessment presented here provides a molecular framework for future functional studies to test for the diversification of RABs in cell growth and expansion. This review points to the need for robust functional studies to clarify the unique roles of RABs in coordinating plant cell expansion.

Distinct Types of Cell Expansion in Plants Requires Diversified Function for RABs

The plant body grows by combined cell division and expansion. Cell expansion is notable in plants with some cells expanding up to 500-fold after cell division. Directional cell expansion is also essential to the normal functioning of most cell types, including small uniquely shaped cells such as stomatal complexes, as well as exceptionally long transport cells, such as vessels, tracheids, sieve cells and sclerenchyma fibers (Mauseth 1988). Furthermore, cell expansion provides the bulk of the biomass that makes the plant body. As an example of the pervasive

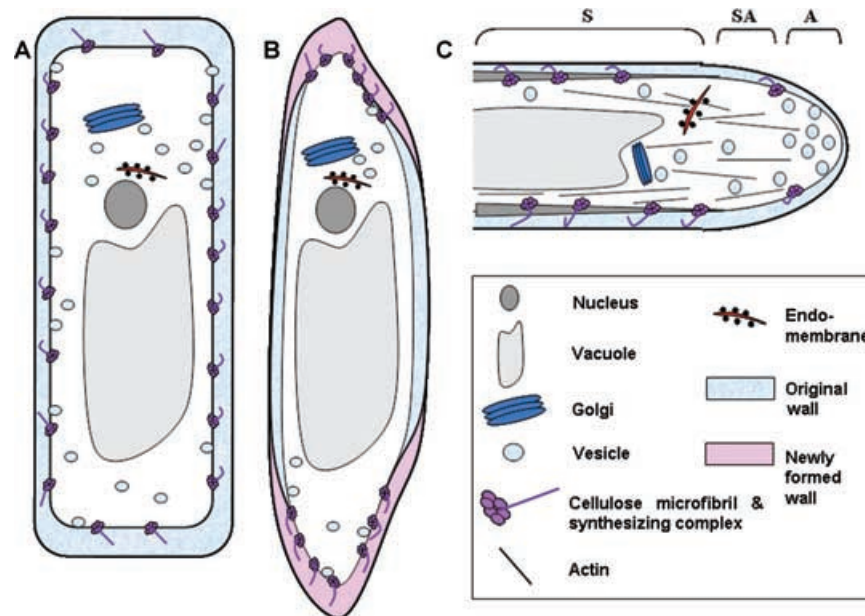


Figure 1. Three different patterns of cell wall expansion in higher plants.

The most common type of cell expansion is intercalative (**A**) where new cellulose microfibrils are synthesized in the plasma membrane and insert in amongst older cell walls, often in layers. Vesicles containing the wall matrix component are synthesized in the cytoplasm and traffic to the site of secretion at the plasma membrane. RABs are implicated in all steps of trafficking to allow for proper distribution of wall matrix components. The least common type of wall growth is intrusive (**B**) where new wall materials are synthesized and secreted locally at the ends of the cells. RABs are likely involved but no studies have yet confirmed their trafficking function. The third best-studied type is tip growth (**C**) as depicted here for root hairs (after Cole and Fowler 2006). RABs are involved in Golgi-derived vesicle packaging in the shank (S), transport through the subapical zone (SA), as well as contribute to membrane cycling at the apical growing tip (A).

importance of cell expansion, a corn plant would remain barely 30 cm high if cells did not expand to produce the tissues and cells capable of photosynthesis and normal function.

Plant cell expansion requires massive amounts of vesicle transport, secretion and membrane cycling because new wall material and membrane is synthesized and transported to the growing extracellular space. The plant cell wall is composed of two parts: cellulosic microfibrils, providing structural reinforcement, are oriented, embedded and held together by a complex extracellular matrix (summarized in Figure 1; for review see Cosgrove 2000). Cellulose is synthesized and organized into microfibrils from plasma membrane-bound cellulose synthase complexes. The matrix consists of a complex of glycoproteins, hemicelluloses, pectins, and expansins, all of which are synthesized within the endoplasmic reticulum (ER), packaged in Golgi into vesicles, which are then transported, targeted and secreted into the cell wall space (see Figure 1A for the most typical pattern). This latter process of wall matrix secretion requires extensive vesicle trafficking and hence, most likely requires the function of RAB GTPases.

Patterns of vesicular transport patterns depend on the type of wall extension as depicted in Figure 1 (for review see Mauseth

1988; Cosgrove 2000). The most common pattern of wall synthesis is intercalative (Figure 1A), where new microfibrils are added in amongst the old microfibrils, maintaining coordinated bidirectional growth. Vesicle transport typically must coordinate with cellulose synthesis and requires transport and secretion at the plasma membrane. All of these steps require RAB guidance for vesicle transport and targeting to the correct membrane location and at the right developmental time. To date, Zm-RAB2A1 is the only RAB definitively shown to moderate cell wall growth: hypermorphic mutations in ZmRAB2A1 cause maize leaf cells to overexpand (Sylvester et al., in review), suggesting the role of RAB2A in vesicle targeting to the cell wall space. Given that mammalian RAB2s are known to be involved in ER-Golgi trafficking, the effect on wall growth in ZmRAB2A mutants suggests diversified function.

In another pattern of plant cell expansion, microfibrils are synthesized in one location of the cell, rather than intercalated in amongst earlier formed microfibrils, thus causing the cell to grow at either end only (Figure 1B). This type of intrusive growth, although less common than the intercalative type, is found in solitary plant cells such as sclereids and sclerenchyma fibers. As depicted in Figure 1B, intrusive wall expansion must require

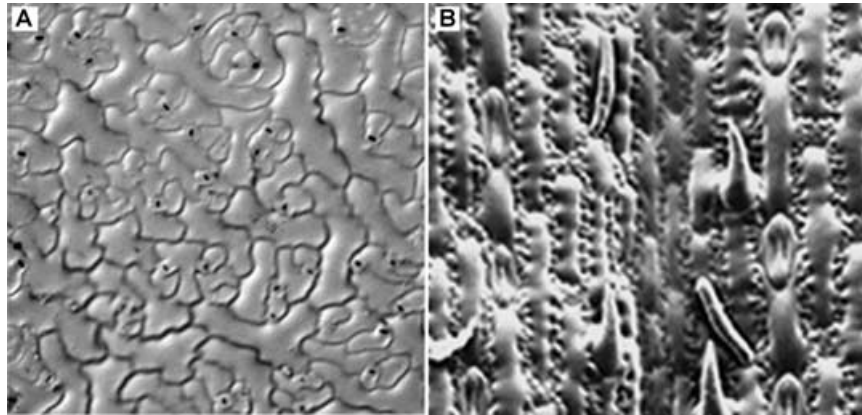


Figure 2. Epidermal cells in dicots and monocots.

Arabidopsis (A) has lobed and irregular epidermal cells compared with the rectangular and oriented epidermal cells of maize (B). Different strategies of cell expansion will require unique trafficking pathways, likely mediated by diversifications of RAB subfamilies.

vesicle transport of matrix components to the polarized end of the cell. RABs are likely to be involved in this process, but this has not yet been proven.

The most extensively studied type of wall growth occurs at localized tips of some cell types. Many rapidly elongating cells such as pollen tubes, root hairs, trichomes and other independently-elongating structures all grow by tip growth (Figure 1C). RABs contribute to tip growth through vesicle transport as well as membrane cycling, as described in more detail below. It is important to note that each of these three types of cell expansion all require vesicle trafficking, but they differ in terms of location of secretion to the wall space. Thus, we can assume that RABs must be involved in the transport and membrane cycling associated with each type of cell expansion.

Cell Architecture is Different in Dicots and Monocots, Suggesting Novel Regulation of Vesicle Trafficking in the Two Groups

In addition to differences in patterns of wall expansion, individual plant cells have distinct shapes. In turn, cell shapes are associated with unique functions, all dependent on coordinated vesicle trafficking. For example, stomatal complexes acquire distinct shapes to accommodate pore opening (for review see Li et al. 2006). These processes require proper targeting of membranes, wall material and proteins to specific sites in the cell.

Variations in plant cell shape in different species suggest that trafficking may be differentially regulated both spatially and temporally. A clear example of species-specific cell shape variation is seen in leaf epidermal cells of monocots and

dicots (Figure 2). The irregular jigsaw puzzle shape of the dicot epidermis contrasts with the highly bidirectional expansion of monocot cells. RABs are most likely involved in organizing these divergent cell patterns, but to date little is known about the specific roles of RABs in vesicle transport during cell expansion of the different cell types in dicots and monocots. We predict that functional studies will ultimately confirm unique diversification of RAB for plants.

Example of Molecular/Functional Diversification in Non-Plants: RAB7

There is good evidence that, despite conserved GTPase function, RABs are recruited for diverse compartmentalized activities in non-plant systems. Close homologs of RAB7 for example serve related, but clearly distinct functions, in distant groups such as yeast and mammalian systems. The yeast homolog to HsRAB7, Ypt7 is associated with vesicle trafficking around the yeast lysosome, with no distinction between late and early or endosomal events. In mammalian systems, different RABs localize and function in distinct endosomal to lysosomal compartments (Lazar et al. 1997; Pfeffer and Aivazian 2004). HsRab7 functions in distinct early or late endosomal compartments and also in trafficking from the trans-golgi network (TGN) to the lysosomal compartment (Meresse et al. 1995; Ihrke et al. 2004; Poteryaev et al. 2007).

Mutant studies suggest that RAB7 in worms may have been recruited for additional function by transporting vesicles from the TGN to the lysosomal compartment. In *rab7* deletion worms, the lysosomal marker LMP1 is mislocalized from the normal location in the lysosome to the early endosomal compartment (Ihrke et al.

2004). In human tissue culture cells, proper distribution of the protein and its vesicle transporting function is also dependent on the microtubule cytoskeleton (Meresse et al. 1995). Thus, RAB7 in animal systems functions in several compartments, dependent in part on species, but all associated with lysosomal trafficking.

In plants, RAB7 (classified as RABG by Vernoud et al. 2003) has been identified in *Arabidopsis*, tobacco and in rice (Haizel et al. 1995; Ueda and Nakano 2002; Nahm et al. 2003). Rice RAB7 tagged with green fluorescent protein (GFP) is localized to the *Arabidopsis* vacuole in heterologous transient assays, suggesting a potentially similar lysosomal function in plants as other eukaryotes (Nahm et al. 2003). These transient assays remain to be tested *in vivo* using direct tagging or deletion studies in plant systems.

Plant Homologs to Mammalian RABs have Diversified to Function During Plant Tip Growth

In plants, perhaps the best studied type of vesicular trafficking that involves RAB proteins is tip growth (for review see Yang 2002; Cole and Fowler 2006; Samaj et al. 2006). As described earlier, individual plant cells such as pollen tubes and root hairs exhibit localized growth where wall material is transported and secreted to the growing tip of the cell. The growing cell is therefore highly polarized by the localized network of interacting proteins mediating the process. This visibly distinct region of the cell (termed a LENS by Cole and Fowler, 2006) is characterized by active localized secretion mediated by the actin cytoskeleton, which helps direct vesicles toward the secretory tip. RABs are central to maintaining this localized growth through their vesicle transport function and also serve an important membrane recycling function.

AtRAB11 (classified as RABA by Vernoud et al. 2003) is implicated in tip growth in tobacco, in which mutant *rab11* caused abnormal tip growth (de Graaf et al. 2005). In this study, membrane addition at the growing tip is proposed to be the primary function for AtRAB11. Interestingly, RAB11 in animal systems is instrumental in membrane recycling during cleavage furrow formation and cytokinesis (Eathiraj et al. 2006; Yu et al. 2007). Despite sharing more than 50% identity at the amino acid level, these proteins have been recruited for two distinct functions in animals and plants, with the only shared characteristic that of membrane recycling. Localization studies in peas show that diverse RAB11s localize to different membranes not directly involved in membrane recycling, suggesting further diversification (Inaba et al. 2002).

Another RAB involved in tip localization at least in root hairs is another duplicate of RAB11, identified as AtRAB11E2 (referred to as RABA4B by Vernoud et al. 2003), which shares high

amino acid similarity to HsRAB11 and HsRAB25, both reported to function in the TGN specifically associated with late vesicle processing (Vernoud et al. 2003; Preuss et al. 2004, 2006). Preuss et al. (2004) showed that AtRAB11E2 localizes to the membrane at the root hair tip and phosphoinositide signaling is involved (Preuss et al. 2006). Although vesicular location is not apparent in this case, the study shows that AtRAB11E2 may be involved in signaling as well as tip membrane cycling.

RABs in Non-Tip Growing Plant Cells: Diversification of the RAB1, RAB2, RAB11 Subfamilies

RABs may also be involved in hormonal signaling. Overexpression of NtRAB11 and antisense *rab11* both cause similar cellular and morphological defects (Sano et al. 1994). Similarly, an antisense *rab11* in tomato causes distinct growth defects such as loss of apical dominance normally associated with altered auxin signaling (Lu et al. 2001). Effects on the brassinosteroid hormonal pathway have been shown in pea, where RAB11 (referred to as PRA2 by Kang et al. 2001) was shown to have a direct signaling role through binding to a cytochrome instrumental in light-regulated signaling (Kang et al. 2001). These results combined with the potential signaling role for AtRAB11E2, as described above, suggest multiple plant-specific functions: signaling combined with or instead of vesicular trafficking.

Both ZmRAB1A and ZmRAB2A appear to function together in grasses to mediate cell growth. Mutations in one of the proteins cause partial expansion defects as evident by the appearance of overly expanded wart-like cells in mutant leaves (Sylvester et al., in review). Both together complement yeast null mutants of the homolog *ypt1*, suggesting these plant RABs serve similar functions. Also, both show similar expansion-specific expression patterns in the growing maize leaf. Sugarcane homologs to ZmRAB1A and ZmRAB2A show similar complementation and expression patterns (Zhang et al. 2006), suggesting RAB1 and RAB2 function during cell expansion is conserved in the grasses.

Shared localization has been one way to begin confirming potential shared function. Interestingly, similar to the yeast homology YPT1, AtRAB1B was shown to localize in a similar ER to the Golgi compartment, but with characteristics unique to the morphology and function of plant Golgi (Batoko et al. 2000). Similarly, GFP-tagged NtRAB2 localizes to Golgi bodies during pollen growth, regulating secretion during active expansion of the tube tip (Cheung et al. 2002). A dominant negative mutant of AtRAB8 (referred to as AtRABE by Zheng et al. 2005) prevents secretion by inhibiting Golgi function, but this action is downstream of AtRAB1 (referred to as AtRABD by Zeng et al. 2005), suggesting sequential interaction of these RABs in plants (Zheng et al. 2005).

Table 1. RAB guanosine triphosphatases (GTPases) in human, *Arabidopsis*, rice and maize

Human		<i>Arabidopsis</i>		Rice		Maize	
Gene name	Accession no.	Gene name	AGI no.	Gene name	IRGSP number	Gene name	Accession no.
HsRab1A	NP_004152	AtRab1A1	At3g11730	OsRab1A1 ^a	Os05g010520	ZmRab1A1	X63277 AY109984
HsRab1B	NP_112243	AtRab1B1	At5g47200	OsRab1A2 ^b	AK060839	ZmRab1A2	DQ246177
HsRab2A	NP_002856	AtRab1B2	At4g17530	OsRab1B1 ^b	AK062838	ZmRab1B1	AY104866
HsRab2B	NP_116235	AtRab1C1	At1g02130	OsRab1B2	Os01g0558600	ZmRab1B2	DQ246111
HsRab3A	NP_002857	AtRab2A1	At4g17170	OsRab1C1	Os01g0179700	ZmRab1B3	DQ244598
HsRab3B	NP_002858	AtRab2A2	At4g35860	OsRab1C2	Os02g0658100	ZmRab1C1	X63278
HsRab3C	NP_612462	AtRab2A3	At4g17160	OsRab1C3	Os02g0653800	ZmRab1C2	AY103767
HsRab3D	NP_004274	AtRab5A1	At3g54840	OsRab2A1	Os04g0470100	ZmRab1C3	AY105385 T019232
HsRab4A	NP_004569	AtRab5B1	At5g45130	OsRab2A2	Os02g0586400	ZmRab2A1	AY103978 AY103978
HsRab4B	NP_057238	AtRab5B2	At4g19640	OsRab2B1	Os10g0208800	ZmRab2A2	U22433
HsRab5A	NP_004153	AtRab6A1	At5g64990	OsRab2B2	Os10g14150	ZmRab2A3	X77795
HsRab5B	NP_002859	AtRab6A2	At4g39890	OsRab5A1	Os03g0151900	ZmRab5A1	DQ245832
HsRab5C	NP_958842	AtRab6A3	At2g44610	OsRab5A2	Os10g0441800	ZmRab5B1	AY107131
HsRab6A	NP_942599	AtRab6A4	At2g22290	OsRab5B1	Os03g0666500	ZmRab5B2	AY104686 M95071
HsRab6B	NP_057661	AtRab6A5	At5g10260	OsRab5B2	Os12g0631100	ZmRab6A1	AY103845
HsRab6C	NP_115520	AtRab7A1	At1g22740	OsRab5B3 ^b	AY029301 AAK38149	ZmRab7A1	AF467541
HsRab7A	NP_004628	AtRab7A2	At4g09720	OsRab5C1	Os06g0687100	ZmRab7B1	AY108223
HsRab7B	NP_796377	AtRab7A3	At2g21880	OsRab5D1	Os05g0341600	ZmRab7B2	DQ245017
HsRab7L1	NP_003920	AtRab7B1	At1g52280	OsRab6A1	Os07g0496000	ZmRab8A1	AY103763 BT016282
HsRab8A	NP_005361	AtRab7B2	At3g16100	OsRab6B1	Os03g0914	ZmRab8A2	DQ246120
HsRab8B	NP_057614	AtRab7B3	At3g18820	OsRab6B2 ^a	Os03g0191400	ZmRab8A3	BT016418
HsRab9A	NP_004242	AtRab7B4	At1g49300	OsRab7A1	Os05g0536900	ZmRab8B1	AY108224
HsRab9B	NP_057454	AtRab7C1	At5g39620	OsRab7A2	Os01g0714900	ZmRab8C1	DQ245083
HsRab10	NP_057215	AtRab8A1	At3g53610	OsRab7B1	Os01g0227300	ZmRab11A1	AY107805
HsRab11A	NP_004654	AtRab8A2	At5g59840	OsRab7B2	Os05g44050	ZmRab11A2	DQ244975
HsRab11B	NP_004209	AtRab8A3	At3g46060	OsRab7B3	Os05g0516600	ZmRab11B1	AY105457
HsRab12	NP_001020471	AtRab8B1	At5g03520	OsRab8A1 ^b	AY576526 AAS88430	ZmRab11B2	BT023992 DQ245314
HsRab13	NP_002861	AtRab8B2	At3g09900	OsRab8A2	Os05g0461300	ZmRab11C1	AY110109
HsRab14	NP_057406	AtRab11A1	At1g09630	OsRab8A3	Os07g0239400	ZmRab11C2	DQ244859
HsRab15	NP_941959	AtRab11B1	At5g59150	OsRab8A4 ^a	Os07g13530	ZmRab11C3	DQ245392
HsRab17	NP_071894	AtRab11B2	At3g46830	OsRab8A5	Os03g0819900	ZmRab11D1	AY103999
HsRab18	NP_067075	AtRab11B3	At5g45750	OsRab8B1	Os07g0195100	ZmRab11D2	DQ245244
HsRab19	NP_001008749	AtRab11C1	At4g18430	OsRab11A1	Os03g0843100	ZmRab11E1	AY104562
HsRab20	NP_060287	AtRab11C2	At1g28550	OsRab11A2	Os05g0105100	ZmRab11E2	AY107436
HsRab21	NP_055814	AtRab11C3	At2g33870	OsRab11B1	Os05g0280200	ZmRab11E3	AY104765
HsRab22	NP_065724	AtRab11C4	At5g60860	OsRab11B2	Os03g0823700	ZmRab11G1	AY106908
HsRab23	NP_057361	AtRab11C5	At3g15060	OsRab11B3	Os01g0848700	ZmRab11M1	D31906
HsRab24	NP_570137	AtRab11D1	At1g16920	OsRab11C1	Os01g0667600	ZmRab11M2	D31905
HsRab25	NP_065120	AtRab11D2	At1g06400	OsRab11C2	Os09g0327100	ZmRab18A1	AY104355
HsRab26	NP_055168	AtRab11D3	At4g18800	OsRab11C3	Os05g0564400	ZmRab18A2	DQ245235
HsRab27A	NP_899059	AtRab11D4	At5g45750	OsRab11D1	Os06g0551400	ZmRab18B1	AY108713

Table 1. Continued

Human		<i>Arabidopsis</i>		Rice		Maize	
Gene name	Accession no.	Gene name	AGI no.	Gene name	IRGSP number	Gene name	Accession no.
HsRab27B	NP_004154	AtRab11E1	At5g65270	OsRab11D2	Os01g0750000		
HsRab28	NP_001017979	AtRab11E2	At4g39990	OsRab11D3	Os05g0516800		
HsRab30	NP_055303	AtRab11E3	At2g22390	OsRab11E1	Os10g0377400		
HsRab31	NP_006859	AtRab11E4	At3g12160	OsRab11E2	Os09g0281700		
HsRab32	NP_006825	AtRab11E5	At5g47960	OsRab11F1	Os06g0714600		
HsRab33A	NP_004785	AtRab11F1	At1g01200	OsRab11F2	Os09g0527600		
HsRab33B	NP_112586	AtRab11G1	At5g47520	OsRab11G1	Os07g0634200		
HsRab35	NP_006852	AtRab11G2	At3g07410	OsRab11G2	Os08g0525000		
HsRab36	NP_004905	AtRab11G3	At2g43130	OsRab18A1 ^b	AK111647		
HsRab37	NP_001006639	AtRab11G4	At2g31680	OsRab18B1	Os10g0456600		
HsRab38	NP_071732	AtRab11G5 ^a	At1g05810	OsRab18B2	Os03g0146000		
HsRab39A	NP_114140	AtRab11H1	At1g73640				
HsRab39B	NP_741995	AtRab11H2	At1g18200				
HsRab40A	NP_543155	AtRab18A1	At1g43890				
HsRab40B	NP_006813	AtRab18B1	At5g03530				
HsRab40C	NP_066991	AtRab18B2	At3g09910				
HsRab41	NP_001027898						
HsRab42	NP_689517						
HsRab43	AAH62319						
HsRab44	CAD92808						

^aMissing or incorrect 5' or 3' ends or inner part were found by carefully comparing genomic sequences and related mRNA sequences. ^bGenes for which an International Rice Genome Sequencing Project number is not available.

molecular phylogeny, adding monocots to the RAB family classifications.

Molecular Identification and Classification of RAB GTPases in *Arabidopsis*, Rice and Maize

Prior studies identified all of the RABs in the *Arabidopsis* genome and classified them within the context of human homologs (Vernoud et al. 2003). Here we identify homologs from the completed rice genome and partially completed maize genome. Lists of RAB GTPases in the human and *Arabidopsis* genome were first accumulated according to previous reports (Bock et al. 2001; Vernoud et al. 2003). BLASTP (used to query protein databases) programs were run against human and *Arabidopsis* genome databases to search for additional RAB sequences. Genes from the same locus were combined, based on known map information and depicted in Figure 3. All plant RABs were classified here using the same numerical nomenclature of human (Bock et al. 2001), as discussed above.

We identified 61 Rab genes with map information in the human genome (Table 1), with several minor classification differences with the published study of Bock et al. 2001. Genes or isoforms without map information, such as Rab4C and Rab9C,

were not included in our study. We also identified new duplicates and changes after manual annotation. For example, we identified two Rab39 and two Rab7 instead of the one Rab39 and one Rab7 reported in Bock et al. 2001. Another Rab HsRab7L1 (map location 1q32) was reclassified to the Rab32/Rab38 sub family. We believe that more Rab loci will be found in the human genome after more extensive analysis. We identified 57 Rabs in *Arabidopsis*, consistent with prior studies (Vernoud et al. 2003).

A phylogenetic tree of Rab gene families in human and *Arabidopsis* was generated and these sequences were used as templates to group RABs in rice and maize. To identify OsRABs, the BLASTP program (Altschul et al. 1997) was run against the rice genome databases from the International Rice Genome Sequencing Project. Contaminants from varieties other than Nipponbare and those derived from alternative splicing were removed. To correct annotations, the predicted coding DNA sequences by the genome project were compared with expressing sequence tag (EST) databases and available mRNA sequences. Incorrect annotations were modified by hand.

As shown in Figures 3 and 5 and in Table 1, 52 RABs were identified in rice. To identify Rab gene sequences of maize, rice cDNA sequences were used as queries to Blast the maize nucleotide databases (<http://www.maizegdb.org/>, <http://www.plantgdb.org/> and <http://www.maizesequence.org/index.html>). Resulting sequences were analyzed with the

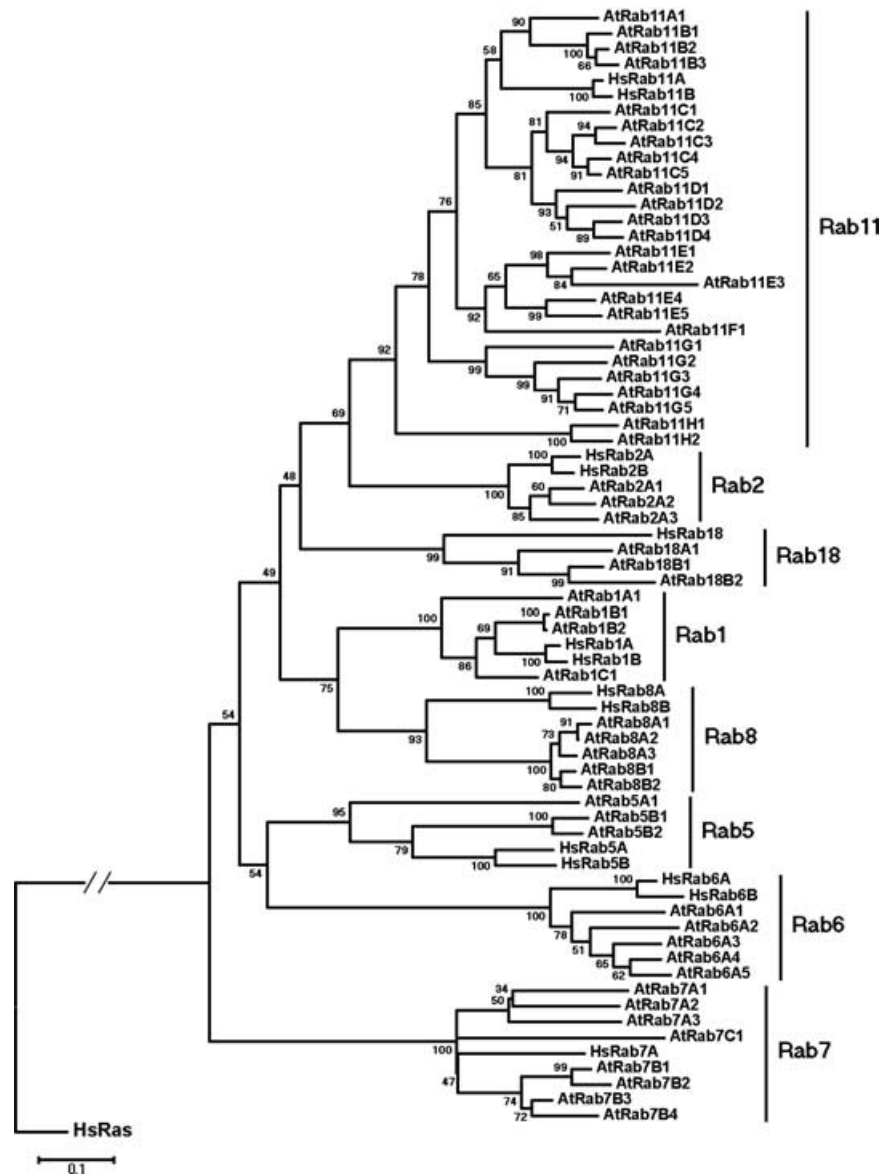


Figure 4. Neighbor-joining trees of Rab family in *Arabidopsis* using representative Rab members in humans as references.

The trees are rooted with a human RAS protein. Bootstrap values were calculated for 1000 replicates and are shown on branches. Branch lengths reflect the evolutionary distances indicated by the scales. Bar, 0.1 residue difference per residue.

MacVector program, and translated into protein sequences (MacVector, Inc. Cambridge UK). Accessions coding for the same protein were combined. Currently, 41 ZmRABs were identified from the partial build of the genome and 40 of these were used for phylogenetic analysis due to high similarity between two (RAB18A1 and RAB18A2). Given the incomplete nature of the maize genome, these 41 RABs are a preliminary assessment and will require additional annotation as more sequences emerge with the projected completion of the maize genome in 2008.

To classify RABs from *Arabidopsis*, rice and maize, protein sequences were aligned together with the 61 human RABs using Clustal X (Jeanmougin et al. 1998) and edited manually to ensure the correct alignments of the conserved functional domains. Phylogenetic trees were built using the neighbor-joining method incorporated in Mega3.1 (Kumar et al. 2004) with 1000 bootstrap replicates. After phylogenetic analysis, we found that, unlike the human RAB family, which can be divided into as many as 18 subfamilies, plant RABs can be grouped into mainly eight subfamilies, corresponding to the RAB1, RAB2,

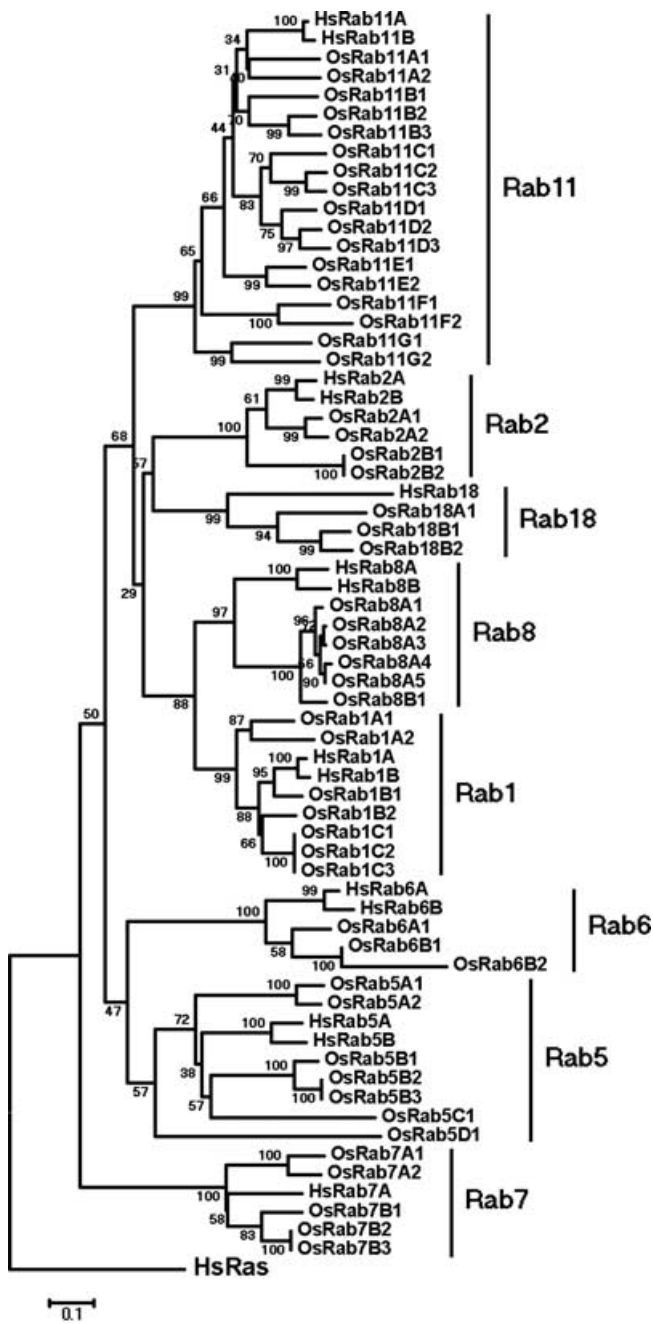


Figure 5. Neighbor-joining trees of Rab family in rice using representative Rab members in humans as references.

The trees are rooted with a human RAS protein. Bootstrap values were calculated for 1000 replicates and are shown on branches. Branch lengths reflect the evolutionary distances indicated by the scales. Bar, 0.1 residue difference per residue.

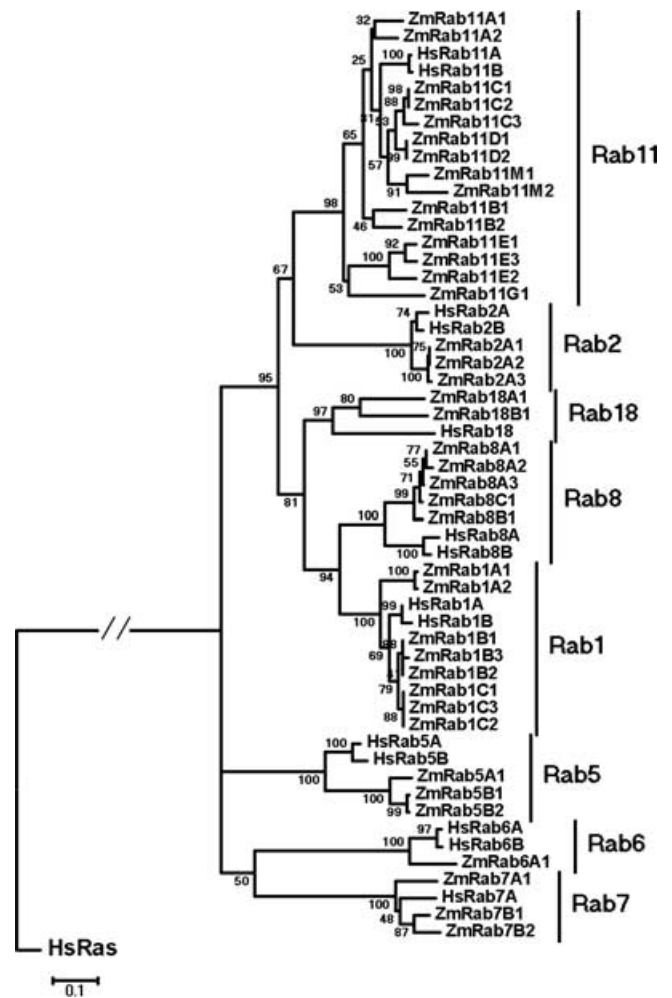


Figure 6. Neighbor-joining trees of Rab family in maize using representative Rab members in humans as references.

The trees are rooted with a human RAS protein. Bootstrap values were calculated for 1000 replicates and are shown on branches. Branch lengths reflect the evolutionary distances indicated by the scales. Bar, 0.1 residue difference per residue.

RAB5, RAB6, RAB7, RAB8, RAB11 and RAB18 subfamilies of human RABs, suggesting different profiles between mammals and plants. At the same time, plants have more homologous members in each of the eight subfamilies.

Comparative Analysis of RAB Families in Rice, Maize and *Arabidopsis*

The topologies of the phylogenetic trees of RABs in three genomes are similar (Figures 4–6), with eight subfamilies of similar member proportions. In spite of the sequence similarities,

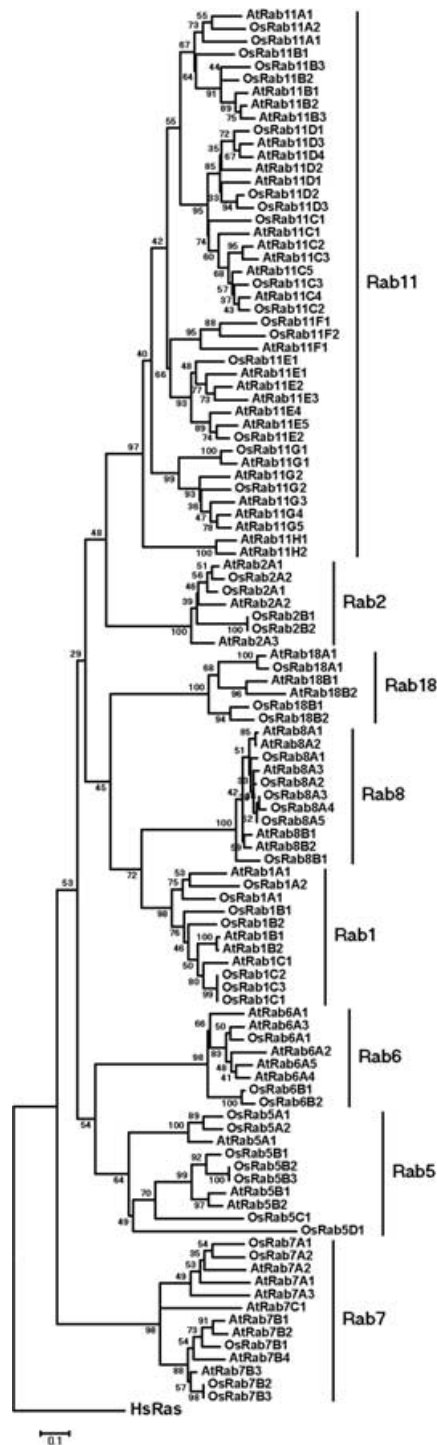


Figure 7. Comparative analysis of Rab families in *Arabidopsis* and rice.

The tree was generated using the Neighbor-joining method and rooted with a human Ras protein. Bootstrap values were calculated for 1000 replicates and are shown on branches. Bar, 0.1 residue difference per residue.

gene organizations of each subfamily in different plant genomes are conserved. All *Rab1* and *Rab8* genes in the three genomes have eight exons, while *Rab2*, *Rab6* and *Rab18* members have six exons, *Rab5* (except *OsRab5D1*) and *Rab7* members have seven exons, indicating common ancestral origin of the genes in the same subfamily.

RAB11 is the largest subfamily in plants. Humans have only three RAB11 members; rice has at least 17 members and *Arabidopsis* has 24. These RAB11 members can be further divided into six to eight subfamilies depending on genomes (Figures 4–7), in which the RAB11A, RAB11B, RAB11C, RAB11D, RAB11E, RAB11G subgroups are shared by all three of the plant genomes, while the RAB11H subgroup is *Arabidopsis*-specific and the RAB11M subgroup is maize-specific. RAB11 members also have the most highly diverged gene organization with exon numbers ranging from one to four, indicating their complicated origin. The subgroups of RAB11 might be considered as subfamilies, and some subgroups may serve the cells at locations different from the mammalian RAB11. As discussed earlier, plant RAB11s may have significantly diversified to accomplish the unique vesicular transport functions during tip growth.

Another significant difference between *Arabidopsis* and rice is the *OsRAB5D1*, which is located between RAB5 and RAB6 subfamilies in the tree (Figure 7). *OsRAB5D1* is located on chromosome 5, has the same gene organization (six exons) with RAB6, but its sequence is more like RAB5, especially the carboxyl terminus cysteine residues (CCS). RAB5s usually have CCS terminus, whereas all other RAB6s have CSC as carboxyl ends. The *OsRAB5C1* does not have an *Arabidopsis* counterpart either, which has no prenylation site at the C terminus. Thus, this gene is one example of an intermediate state, and would be an excellent target for evaluating functional divergence between monocot and dicot RABs.

Summary and Prospects

Plants have greatly diversified RABs within a smaller number of subfamilies compared with mammalian systems. Unique cellular requirements most likely explain this molecular diversification. Interestingly, basic functions, such as membrane recycling, as in RAB 11 homologs, are shared, despite divergent compartments and cellular processes involved in the recycling. This suggests that effector protein diversification could be driving cell-specific functions. Analysis of mutual evolution between plant effectors and RAB subfamilies will help to clarify how this functional recruitment has occurred in plants. Further, RAB diversification within plants could be due to functionally specific changes during development of unique cell types between monocots and dicots. Bioinformatic groupings described here provide essential information that will help guide future functional studies.

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