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GTP-binding proteins in plants

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Abstract. GTP-binding proteins are found in all organisms. They are important switches that cycle between an active and an inactive state, ensuring vectorial flow of information on the expense of guanosine triphosphate (GTP). In this review, we discuss current progress in the molecular characterization and functional analysis of plant genes encoding heterotrimeric and small GTPases. An up-to-date list including all cloned plant GTPase genes is given and a systematic classification is proposed.

Key words. GTPase; G protein superfamily; heterotrimeric G proteins; WD40 repeat protein; G protein-coupled receptors; small G proteins; signal transduction; vesicle transport; secretion; *Arabidopsis thaliana*; *Nicotiana tabacum*; *Solanum esculentum*.

More than 20 years ago, François Jacob proposed that evolution makes use of preexisting materials rather than designing new genes and structures from scratch [1]. Two decades of molecular genetic research have shown how valid and influential these ideas were. The genome projects and their offspring are now disclosing that an increasing number of genes from many different organisms share similarities at the basic structural and functional level, thereby representing the 'tinkering' by which evolution was able to recruit the raw materials to reassemble new functions and build novel regulatory networks. In fact, characterization of specific genes has demonstrated that many intermediary elements of developmental pathways remained highly conserved, particularly proteins that act in signal transduction pathways. A remarkable example illustrating Jacob's visionary hypothesis have been the GTPases, molecular switches and timers that function via conformational changes resulting from the binding and hydrolysis of GTP by intrinsic activities [2, 3]. They are inactive as GDP-bound species because of reduced affinity for downstream effectors. GTPases are activated by exchange of guanosine diphosphate (GDP) for GTP, a process mediated by various regulatory factors such as heptahelical receptors (G protein-coupled receptors, GPCRs) or guanine nucleotide exchange factors (GEFs). GTPases have been found to be highly conserved from yeast to mammals. In view of their important regulatory function, it is not surprising that they play an important role in many plant processes, too. Currently, several laboratories are concentrating on elucidating the role of different GTPases in various plant-signalling pathways. Rapid molecular progress has resulted and has been summarized in several previous reviews [4–10]. In the light of advances made over the last 3 years, we concentrate on organizing current information on plant GTPases in a unifying structure according to well-described functionally related groups of eukaryotic GTPases. As many new genes encoding GTPases are disclosed by the rapidly progressing *Arabidopsis thaliana* genome sequencing project and various EST sequencing projects from maize, pine, rice and other plants, we need to replace the old tradition of naming genes fortuitously by a systematic nomenclature that easily allows comparison with the many GTPases from other eukaryotes. Therefore, we propose a systematic classification (see table 1) according to the mammalian nomenclature [11, 12]. We will briefly summarize general information describing the well-characterized groups of GTPases and then add more specific information on plant GTPases reported over the last few years.

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Table 1. Genes coding for subunits of heterotrimeric G proteins.

Systematic group	Gene name used by the authors	Genbank accession no.	Expression profile	Reference(s)
AtGα1	GPA1	M32887	ubiquitously expressed, but not in mature seeds strongest expression in shoot and root meristems	24, 58–60
NtGα1	NtGα	Y08154		-
NpGα1	NpGα	Z72389		-
PsGα1	PsGPA	U97043		
LlGα1	LlGpro	X99485		
GmGα1	SGA1	L27418	highest expression in elongating regions	26
GmGα2	GmSGA2	X95582		23
LjGα1	LjGPA1	X77250		27
StGα1	StGPA1	X87836		-
LeGα1	TGA1	M74419		25
AfGα1	AfGα1	AF010476	low expression, not affected by GA	32
OsGα1	RGA1	L28001, D38232	highest expression in young leaves, light-regulated	56
AGβ1	AGβ1	U12232	found in all tissues	34
NtGβ3	NtGβ	X98161		
NtGβ1	NtGβ1	Z84820		-
NtGβ2	NtGβ2	Z84821		-
NpGβ1	NpGβ1	Y09513		-
StGβ1	StGβ1	X87837		-
AfGβ1	AfGβ1	AF033357	n.d.	32
AfGβ2	AfGβ2			
OsGβ1	OsGβ1	X89737		33
ZmGβ1	ZGβ1	U12233	root, leaf, little higher expression in tassels	34
Putative G protein-coupled receptors				
AtGCR1	GCR1	U95143	low expression in roots, stems, leaves	66, 67
BnGCR1	BnGCR	U95144	n.d.	67
WD-40 repeat proteins				
AtArcA	AtArcA	U77381	auxin-induced expression in auxin-starved cells	69
BnArcA	BGB1	Z33643	ubiquitously expressed	71
NtArcA	arcA	D17526	auxin-induced expression	68
MsArcA	Msgbl	Y08678	expressed in young embryos and leaves	72
OsArcA	RWD	D38231		73
CrArcA	Cblp	X53574		70
Arf/Sar-related genes				
AtSar1	AtSar1	M95795	expressed in all tissues, but lower in mature leaves; mRNA upregulated by cold treatment	204, 211, 213
AtSar2	ASar1	U56929, AF001308		
NtSar1	NtSar1	D87821	in BY2 stronger expression during growth, expressed in all tissues but higher in roots	215
NtSar2	Ntgb2	U46928	n.d.	119
NtSar3	Ntgb3	U46929	n.d.	119
NtSar4	NtSar1	X97967		
NpSar1	NpSar1	Y08423		
NpSar2	NpSar1	Y08424		
BcSar1	Bsar1a	U55035	found in all tissues	206
BcSar2	Bsar1b	U55036	found in all tissues	206
	LeSar2	L12051	expressed in all tissues, highest in fruits	205
AfSar1	AfSar1	AF084005		
MdSar1	MdSar1	AF048825		
AtSEC12	AtSEC12,Stl2	M95796	expressed ubiquitously (GEF for Sar)	204, 213

Table 1. (Continued).

Systematic group	Gene name used by the authors	Genbank accession no.	Expression profile	Reference(s)
AtArf1	AtArf	M95166	highest expression in roots	198, 211
AtArl2	AtArf3	X77385		216
AtArl1	Atgb1	U46924	n.d.	119
BcArl1	BcArf	U38470		
CaArf1	CaArf	AF005238		
DcArf1	DcArf1	D45420	somatic embryogenesis	201
	PsArf	not cloned		214
SbArf	SbArf	AB003377		
StArf1	StArf	X74461	high in young leaves	200, 207
VuArf1	VuArf	AF022389		
ZmArf1	ZmArf	X80042	n.d.	203
OsArf2	OsArf	D17760	highly expressed in young seedlings and seeds	199
OsArf1	OsArf1	AF012896	early elicitor induced	
CrArf1	CrArf1	U27120	light-regulated	208
Rab1-related genes				
AtRab1a	EST	T42619		
AtRab1b	Ara5	D01027,U89959	ubiquitously expressed	116
AtRab1c	ESSA-contig	Z97343	n.d.	117
NtRab1a	Ntrgp	X72212		
LeRab1a	LeRab1A	U38464	expressed in root, increase in mature fruits	113
LeRab1b	LeRab1B	U38465	mRNA levels highest in young leaves	113
LeRab1c	LeRab1C	U38466	mRNA levels highest in young leaves	113
PhRab1a	PhRab1	U35026	mRNA levels highest in opened flowers	121
PsRab1a	Pra8	D12547	expressed in roots and in leaves	114
PsRab1b	Pra9A	D12548	high in roots, less in leaves	114
PsRab1c	Pra9B	D12549	high in roots, less in leaves	114
PsRab1d	Pra9C	D12550	high in roots, less in leaves	114
VfRab1a	VfaYPT1	Z29590	expressed weakly in leaves, strongly in cotelydons	127
LjRab1a	LjRab1A	X97853	highly expressed in developing root nodules	115
LjRab1b	LjRab1B	Z73931	highly expressed in mature root nodules	115
LjRab1c	LjRab1C	Z73932	constitutively expressed	115
LjRab1d	LjRab1D	Z73933	constitutively expressed	115
LjRab1e	LjRab1E	Z73934	constitutively expressed	115
GmRab1a	sypt	L14929	roots (repressed during etiolation)	125, 126
GmRab1b	sra2	U58854	expressed in roots and shoots, not light-regulated	PGR97-181
ZmRab1a	ZmYPT1m	X63277	high in developing pollen	122
ZmRab1b	ZmYPT2m	X63278	highest in flower tissue	122
OsRab1a	Ric1	S66160	expressed in seedlings, higher in calli	129
VcRab1	YptV1	M93438	constitutively expressed	120, 132
CrRab1	YptC1	U13168		131, 132
Rab18-related genes				
AtRab18a	AtRabα	D89824	strongly expressed in stems and roots	133
AtRab18b	AtRab18	U75603		
LjRab18a	LjRab1X	Z73935		115
LjRab18b	LjRab1Y	Z73936		115
VcRab18	YptV3	L08129		141
Rab2/Rab4-related genes				
AtRab2a	AtRab2a	Y09314	high expression in cotelydons, fruits and pollen	123
AtRab2b	AtRab2b	Y09315	n.d.	118
AtRab2c	Atgb	U46925	n.d.	119
GmRab2	srab2	U32185	predominantly found in plumule region	126

Table 1. (Continued).

Systematic group	Gene name used by the authors	Genbank accession no.	Expression profile	Reference(s)
LjRab2a	LjRab2A	Z73937	increased expression in mature nodules	115
ZmRab2a	ZmYPT3		mainly found in male flower tissue	118
VcRab2	YptV4	L08130	expressed constitutively, also found in flagella	141, 281
CrRab2	YptC4	U13167		130, 131
Rab5-related genes				
AtRab5a	Rha1	X59152		160
AtRab5b	Rha1-isolog	H77002		EST
NtRab5a	NtRab5	X63875	expressed in flower tissue	161
NtRab5b	NtRgp2	X71609		
NpRab5a	Rhn1	X64941		166
NpRab5b	NpYPT3	X63874		161
VfRab5a	Vfa-ypt5	Z37503	expressed in roots and cotyledons	127
LjRab5a	LjRab5A	Z73938		115
LjRab5b	LjRab5B	Z73939		115
ZmRab5a	EST	M95071		
Rab7-related genes				
AtRab7a	AtRab7	Y09821		162
AtRab7b	EST	Z30937		
AtRab7c	EST	R64738		
AtRab7d	EST	T43379		
NtRab7a	Nt-rab7a	L29274		138
NtRab7b	Nt-rab7b	L29275	highest expression in stems and fruits	138
NtRab7c	Nt-rab7c	L29276		
PsRab7	PsRab7	X65650	expressed in developing pea pods	167
GmRab7	Srab7	L14930		125
VfRab7	Vrab7	L14928	mainly expressed in nodules	125
LjRab7a	LjRab7a	Z73940	root, high expression in leaves	115
LjRab7b	LjRab7b	Z73941	root, high expression in leaves	
LjRab7c	LjRab7c	Z73942	high expression in root nodules	
LjRab7d	LjRab7d	Z73943	housekeeping gene	
PaRab7	PaRab7	U82219	only expressed in ripening fruit	168
CrRab7	YptC5	U13170		130, 131
VcRab7	YptV5	L08131	constitutively expressed, also found in flagella	130, 281
McRab7	McRab7	U87142		
Rab6-related genes				
AtRab6	AtRab6	L26984	highest expression in liquid root culture	139
NtRab6	NtRab6	L29273	highest expression in stamen,in petals and in unripe fruits	138
Rab8/ Rab10-related genes				
AtRab8a	AtAra3	D01025		116
AtRab8b	AtRab8	U82434	n.d.	
LeRab8a	LeYPT2	X69980	expressed in apical shoot meristem	
PsRab8a	psse354gp	Z49899	isolated from etiolated pea leaves	140
PsRab8b	psgtp6	Z49900		
PsRab8c	psgtp11	Z49901		
PsRab8d	psgtp13	Z49902		
DcRab8a	Dc-Rab8	AJ001367	isolated from suspension cells	PGR97-185

Table 1. (Continued).

Systematic group	Gene name used by the authors	Genbank accession no.	Expression profile	Reference(s)
LjRab8a	LjRab8A	Z73944	constitutively expressed	115
LjRab8b	LjRab8B	Z37945	constitutively expressed	
LjRab8c	LjRab8C	Z37946	constitutively expressed	
LjRab8d	LjRab8D	Z37947	constitutively expressed	
LjRab8e	LjRab8E	Z37948	constitutively expressed	
VcRab8	YptV2	L08128	expressed only during embyogenesis	132, 141, 151
Rab11-related genes				
AtRab11d	Ara	M25471		
AtRab11e	Ara2	D01024		116
AtRab11f	Ara4	D01026	ubiquitously expressed, root lower level	116, 144, 145
AtRab11g	Atgb3	U46926	n.d.	119
AtRab11a	AtRab11A	Y08904	n.d.	142
AtRab11b	AtRab11b	L18883	highly expressed in roots	143
AtRab11c	AtRab11c	U74669	n.d.	
BnRab11a	BnBra	L12395	ubiquitously expressed	152
FsRab11a	FsGTP1	X98540	ABA-induced expression in embryos	149
NtRab11a	NtRab11a	L29271	n.d.	138
NtRab11b	NtRab11b	L29269	n.d.	138
NtRab11c	NtRab11c	L29268	n.d.	138
NtRab11d	NtRab11d	L29270	highest expression in stem and unripe fruits	138
NtRab11e	NtRab11e	L29272	highest expression in stem and unripe fruits	138
NpRab11a	NpYPT3	X63874	highest expression in petals, stigma and stamen	161
McRab11	Rab11e	U87143	partial	
PsRab11a	PsPra1	D12540	high expression in leaves and roots	114
PsRab11b	PsPra2	D12541	light-regulated expression (see text)	124, 148
PsRab11c	PsPra3	D12542	light-regulated expression (see text)	124, 148
PsRab11d	PsPra4	D12543	high expression in roots, less in leaves	114
PsRab11e	PsPra5	D12544	low levels in roots and leaves	114
PsRab11f	PsPra6	D12545	moderate levels in leaves and roots	114
PsRab11g	PsPra7	D12546	high expression in roots, less in leaves	114
GmRab11a	sra1	U58853	expressed in roots and shoots, not light-regulated	PGR97-181
VfRab11a	VfYpt3a	Z29591	scarcely detectable by Northern	127
VfRab11b	VfYpt3b	Z29592	high expression in all organs	127
VfRab11c	VfYpt3x	Z29593	scarcely detectable by Northern	127
LjRab11g	LjRab11G	Z73955	higher expression in aerial parts	115
LjRab11a-j	LjRab11A-J	Z73949-58	constitutively expressed	115
OsRab11a	Osrgp1	X59276	constitutively expressed, but not found in young plants	150, 153, 154
OsRab11b	Osrgp2	D13152	stem, highest expression in young seedlings	155
OsRab11c	Osric2	D13758	seedling, but expressed mainly in callus	129
VcRab11	YptV6	U13169		131, 132
Interacting proteins				
AtRabGDI1	AtGDI	D83531	ubiquitously expressed, little higher expression in liquid root culture	175, 176
AtRabGDI2	AtGDI2	AB005560, AJ001397	mRNA levels lower than AtRabGDI1, much higher expression in suspension culture and root	177, 178
NtRabGDI1		AF012823	expression induced by aluminium stress	PGR97-133
VcRabGDI	GDIV1	U62866	ubiquitously expressed (entire life-cycle)	179
t-SNARE	KNOLLE	U39452	highly expressed in flowers and siliques	171, 172
t-SNARE	AtPEP12	L41651	low expression in leaves, siliques and cell suspension, strong expression in flowers, highest mRNA levels in stems and roots	169, 170
t-SNARE	AtVAM3	U88045		174
v-SNARE	AthSar1	M90418		

Table 1. (Continued).

Systematic group	Gene name used by the authors	Genbank accession no.	Expression profile	Reference(s)
v-SNARE?	AtSYBR1a	AC004809		
v-SNARE?	AtSYBR2a	AC002334		
v-SNARE?	AtSYBR2b	AC002334		
v-SNARE?	AtSYBR2c	AC004681		
v-SNARE?	AtSYBR4	Z97339		
v-SNARE?	Synaptobrevin	AB007651		
Rac-related genes				
A	ARac1,Rop3At	U41295	ubiquitously expressed, also in pollen	239, 242
A	ARac2	U43026	restricted to stems and roots	239
A	ARac3,Rop6At	U43501	ubiquitously expressed, but not in pollen	239, 242
A	ARac4,Rop2At	U45236	ubiquitously expressed, but not in pollen	239, 242
A	ARac5,Rop4At	U52350	ubiquitously expressed, but not in pollen	239, 242
A	ARac6	AF079487		239
B	ARac7	AF079484		239
B	ARac8	AF079486		239
B	ARac10	AF079485		239
A	ARac11	AF085480		239
A	Rop1At	U49971	expressed only in pollen tubes and mature pollen	242
A	Rop5At	AF031429	expressed in pollen and vegetative organs	242
A	Rac-like Br	AF042330		
A	Rop1Ps	L19093	ubiquitously expressed, highest expression in pollen	240
A	Rho1Bv	Z49191	n.d.	
A	GhRac9	S79309	expressed in roots and fibers (stage-specific)	241
A	GhRac13	S79308	expressed in fibers (stage-specific)	241
A	LjRac1	Z73961	ubiquitous	115
A	LjRac2	Z73962	expressed in developing root nodules	115
A	EST (Bn)	L37455		
B	EST (rice)	D41794		
B	EST (rice)	D48393		
B	EST (rice)	D23963		
B	EST (rice)	D41104		
B	EST (citrus)	C21854		
C	Rac-like	U88402		243
	AtRHO-GDI	T43578		
	AtRHO-GAP	H77115		
Ran-related genes				
	AtRan1	X97379	all tissues, highest expression in gynoecium	272
	AtRan2	X97380	all tissues, highest expression in stems	272
	AtRan3	X97381	all tissues, highest expression in stems	272
	AtRan-BP1a	X97377	all tissues, highest expression in gynoecium	272
	AtRan-BP1b	X97378	n.d.	272
	NtRan-A1	L16767	all tissues, highest expression in root and stem	275
	NtRan-A2	L16786	n.d.	275
	NtRan-B1	L16787	all tissues, highest expression in root and stem	275
	NtRan-B2	L16788	n.d.	275
	LeRan1	L28713	all tissues, low expression in mature leaf	273
	LeRan2A	L28714		273
	LeRan2B	L28715		273

Abbreviations of species: Af/*Avena fatua* (wild oat), At/*Arabidopsis thaliana*, Bc/*Brassica campestris*, Bn/*Brassica napus*, Br/*Brassica rapa*, Bv/*Beta vulgaris*, Ca/*Catharanthus roseus*, Cr/*Chlamydomonas reinhardtii*, Dc/*Daucus carota*, Fs/*Fagus sylvaticus*, Gh/*Gossypium hirsutum* (cotton), Gm/*Glycine max*, Le/*Lycopersicon esculentum*, Lj/*Lotus japonicus*, Mc/*Mesembryanthemum crystallinum*, Md/*Malus domestica*, Np/*Nicotiana plumbaginifolia*, Nt/*Nicotiana tabacum*, Pa/*Prunus armeniaca* (apricot), Ph/*Petunia hybrida*, Os/*Oryza sativa*, Sb/*Salix bakko*, St/*Solanum tuberosum*, Vc/*Volvox carteri*, Vf/*Vicia faba*, Vu/*Vigna unguiculata*, Zm/*Zea mays*. PGR = plant gene register.

Heterotrimeric G proteins

General

Heterotrimeric G proteins are composed of three different subunits (Gα, Gβ, Gγ). They comprise a large gene family mediating a vast array of signalling processes in all eukaryotes [13, 14], serving as a bridge between heptahelical GPCRs and effectors such as phospholipases, adenylate cyclases, phosphodiesterases, ion channels and protein kinases. Binding of an extracellular ligand to a GPCR alters the conformation of the receptor molecule, which promotes its association with an intracellular heterotrimeric G protein. GPCRs catalyze the exchange of GDP for GTP on Gα subunits, leading to the dissociation of Gα subunits and Gβγ dimer. Treatment with the drug Mas7, an amphiphilic cationic tetradecapeptide from wasp venom, results in activation of heterotrimeric G proteins by a mechanism that mimics receptor activation. Either free Gα or free Gβγ subunits, or in some cases both, regulate downstream effectors. Inactivation occurs by the intrinsic GTPase activity of the Gα subunit which, in its GDP-bound form, again reassociates with the Gβγ dimer. Treatments which block intrinsic GTPase activity, such as adenosine dinucleotide (ADP) ribosylation by cholera or pertussis toxin, or addition of nonhydrolyzable GTP-analogs like GTPγS lead to persistent activation of heterotrimeric G proteins [15].

Gβ-subunit proteins contain a structurally conserved motif: WD40 repeats of approximately 40 amino acids and several additional conserved amino acids, including a Trp-Asp dipeptide. These structural motifs are part of seven β-sheets, each containing four antiparallel strands radiating outwards from a central core with approximately sevenfold symmetry forming a circularized propeller-like structure [16]. This structure has also been found in a number of Gβ-related WD40 proteins, a family of proteins consisting of six subfamilies involved in (i) signal transduction, (ii) RNA processing, (iii) gene regulation, (iv) vesicular traffic, (v) regulation of cytoskeleton assembly and cell cycle and (vi) yet unknown functions [17].

Heterotrimeric G proteins in plants

Plant genes. For a long time the existence of heterotrimeric G proteins in plants was only indicated by indirect evidence such as ADP ribosylation or kinetic analysis of GTPγS binding to microsomes or purified plasma membranes [18–22]. Recently, several genes encoding α-subunits of heterotrimeric G proteins have been cloned from *Arabidopsis*, tomato, soybean, *Lotus japonicus* and rice [23–29]. They encode 44-kD proteins of about 380 amino acids with similarity to all known Gα subunits. The predicted proteins show highest homology to one another (70–87%), but levels of homology were also high to nonplant Gα subunits, for example approximately 26–36% identical and about 50–75% similar to rat G_{i1-3} and bovine transducin [30, 31]. Plant Gα subunits corresponding to other well-characterized animal Gα subclasses have not yet been found except for recently isolated novel partial complementary DNA (cDNA) clones from barley [32]. Known plant Gα-like proteins contain all the motifs essential for GTP binding, binding to βγ-subunits, an N-terminal site for myristoylation to anchor Gα proteins to membranes and a specific arginine residue for cholera toxin-mediated ADP ribosylation [23–25, 28, 29, 32].

Important constituents of heterotrimeric G proteins are β and γ subunits. While no plant Gγ's have yet been reported, several genes encoding Gβ subunits have been isolated from *Arabidopsis* and maize, encoding proteins with approximately 40% identity to yeast and animal Gβ subunits [33, 34]. Like the other Gβ subunits, they contain seven moderately conserved WD40 motifs.

Expression and functional information. Numerous molecular and functional studies support a role for heterotrimeric G proteins in various plant-signalling processes. Especially, stomatal guard cells respond to a variety of environmental and internal factors to regulate their stomatal aperture. These cells are differentiated from other plant cells by the ability to receive, integrate and transduce signals like light, CO_2 and abscisic acid into changes in cell shape. Thus, many data on heterotrimeric G protein-mediated signalling resulted from studies using this unique system. The GTP analogs GTPγS and GDPβS, cholera and pertussis toxins and the small peptide Mas7 have all been shown to modulate in a complex manner the activity of inward-rectifying K^+ channels of guard cells [35–40]. Heterotrimeric G proteins also seem to play a role in the regulation of outward-rectifying K^+ channels in mesophyll cells [35, 37], regulation of Ca^{2+} channels by fungal elicitors [41, 42], defense against pathogens [43–45], responses to blue and red light [46–49], regulation of biosynthetic pathways [50], Nod factor signalling [51] and hormone signalling [52–55]. To study the expression and biochemical properties of Gα subunits, antibodies were raised against recombinant Gα subunits and against synthetic oligopeptides corresponding to the C terminus of AtGα1 from *Arabidopsis* (formerly GPA1) [56–59]. AtGα1 was shown to be associated with both the plasma membrane and the endoplasmatic reticulum (ER) [58]. Immunolocalization studies revealed that AtGα1 was expressed during all stages of development and in all organs examined except in mature seeds [59]. Particularly high AtGα1 levels were observed in shoot and root meristems, during flower development and in dividing microspores. These immunological data were confirmed in transgenic plants expressing a *GUS* gene

under control of the *AtGα1* promoter [60]. Elevated expression levels were found in vegetative organs, root tips and root elongation zones. It was concluded that AtGα1 might be involved in several different signalling pathways affecting cell growth, differentiation and nutrient transport. Other studies indicated a role for heterotrimeric G proteins in Ca^{2+} channel regulation [41, 42]. In order to demonstrate this directly, the effects of wild-type LeGα1 (formerly TGA1) and GTP-locked mutant LeGα1-Q223L recombinant proteins on Ca^{2+} currents were analyzed by patch clamp analysis in single channel recordings. Both LeGα1 and LeGα1-Q223L increased Ca^{2+} channel activities, although the effect of LeGα1-Q223L on the mean open probability of these channels was significantly higher [61]. Indirect evidence was provided for G protein-mediated activation of inward-rectifying K^+ channels in xylem parenchyma cells using various GTP analogs [62].

One Gα and two Gβ subunits have been isolated from oat aleurone, and their implication in the induction of α-amylase was suggested by Mas7-induced α-amylase gene expression [32]. Therefore, it was concluded that gibberellin induction of α-amylase expression may be mediated via a GPCR. Consistent with this conclusion was the finding that GTPβS completely prevented gibberellin induction of an α-Amy::GUS reporter construct [32]. Recently, heterotrimeric G proteins were identified in green algae. Specific high-affinity ^{35}S-GTPγS binding and GTPase activity was detected in eyespot fractions. The GTPase activities were suppressed by addition of different anti-Gα antibodies to the extracts, suggesting the presence of Gα subunits. To differentiate from small GTPases, the identity of putative Gα subunits was confirmed by two-dimensional polyacrylamidel gel electrophoresis (2D-PAGE) followed by immunodetection and by ADP ribosylation using cholera toxin. Interestingly, GTPase activity and ADP ribosylation were specifically suppressed by green light treatment of eyespots. Hence, a regulatory role of Gα subunits in rhodopsin-based light signalling, for example phototropism, was suggested [63].

Heptahelical receptors in plants

The existence of heptahelical receptors in plants was suggested by work using the G protein agonist mastoparan Mas7 [64, 65]. In spite of numerous polymerase chain reaction (PCR) cloning attempts, the first sequences for GPCRs were finally found by EST database searching [20, 66]. On this basis, full-length cDNA and genomic clones for *AtGCR1* from *Arabidopsis* were reported [67]. The predicted AtGCR1 protein contains seven transmembrane-spanning segments and a long loop between the second and third membrane segments typical for the GPCR superfamily. AtGCR1 shares several conserved peptide motifs with other GPCRs in the transmembrane segments and extracellular loops [67]. Highest similarity was found with several members of the *Dictyostelium* family of cyclic adenosine monophosphate (cAMP) receptors with 20–23% identity over a region of 210 to 285 amino acids encompassing the seven transmembrane segments. Phylogenetic tree analysis revealed a closer relationship with the cAMP receptors from *Dictyostelium* than with calcitonin, serotonin and olfactory receptors from animals. Reverse transcriptase-PCR (RT-PCR) analysis showed very low expression in leaves, roots and stems at different stages of development. Transgenic *Arabidopsis* expressing antisense *AtGCR1* cDNA displayed a *Dainty* phenotype as characterized by reduced cotyledon and leaf expansion and a single flowering stem. An antisense line displayed reduced sensitivity to the cytokinin benzyladenine. The inhibition of root growth and hypocotyl elongation by cytokinin treatment was lower in the antisense line than in control plants, but no differences were found in ethylene-mediated root growth inhibition [67]. In fact, cytokinin levels were altered in the antisense line. This led to the conclusion that AtGCR1 may be involved in cytokinin signalling.

WD40 repeat proteins in plants

The *ARC* genes from tobacco, *Arabidopsis* and *Brassica*, as well as a related gene from *Chlamydomonas*, encode members of the Gβ-like class of WD40 proteins [68–70]. They are thought to belong to the RACK group of proteins acting as receptors for activated protein kinase C [71]. Another member of the RACK subfamily is *Msgb1* from *Medicago sativa*, a gene that is expressed in young embryos, leaves and in dividing cells of nodule primordia. It is induced by cytokinins, but not by auxin [72] in contrast to other auxin-inducible *ARC* genes from *Arabidopsis* and tobacco [68, 69]. A more distantly related gene has been cloned from rice [73]. Another protein containing seven WD40 repeats is the carrot DcWD1 protein. This protein shares homology with proteins controlling cell cycle progression in yeast and animals. However, the expression of DcWD1 in both proliferating and differentiating cells led to the conclusion that DcWD1 may be involved in functions not directly releated to cell division [74].

Small GTPases

Small GTPases are involved in diverse functions

The superfamily of monomeric small GTPases can be divided into distinct families: the Ras family, the Rab family, the Arf/Sar family, the Rho family and the Ran family (fig. 1). They have been shown to regulate an assortment of cellular processes ranging from nuclear

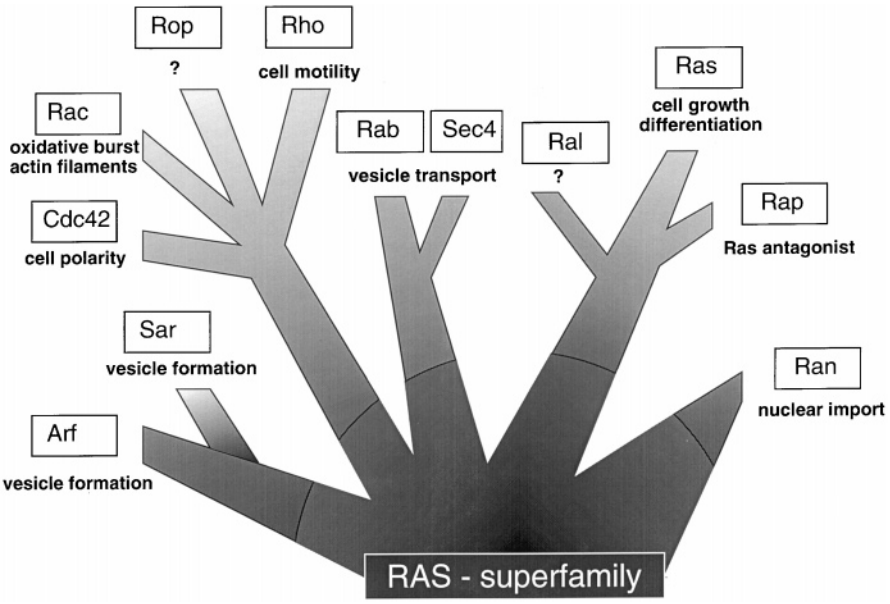

Figure 1. Phylogenetic tree of the Ras superfamily. The different subfamilies and their relationship is depicted. The general function of proteins belonging to each subfamily is indicated.

import to endocytic and exocytic membrane traffic, maintenance of organelle integrity, assembly of vesicle coat proteins, expansion of the ER, vesiculation of the Golgi, regulation of the cytoskeleton, activation of lipases and control of transcription [12, 75].

These GTPases cycle between GTP- and GDP-bound states through the action of GTPase-activating proteins (GAPs) and GEFs [76–78]. The GTP-bound form is active and interacts with its effectors, while the GDP-bound form is inactive. Ras, Rab and Rho GTPases are posttranslationally modified by C-terminal isoprenylation and Arf by N-terminal myristoylation. These modifications allow membrane localization. While Ras is always membrane-bound, Arf, Rab and Rho GTPases cycle between cytosol and membranes. Cytosolic Rho and Rab proteins occur in the GDP-bound form complexed to a specific GDP dissociation inhibitor (RabGDI or RhoGDI). Arf, Rho and Rab GTPases translocate to the membrane by a not fully understood mechanism. There, they undergo GDP/GTP exchange facilitated by GEFs and probably go through several rounds of GTP hydrolysis and GDP/GTP exchange. GTPases in the GDP form can be stripped from the membrane by GDI proteins to again form a cytosolic complex. Ran GTPases are different and will be discussed in detail in the Ran section.

Due to the wide variety of processes in which different members of these families are involved, we will give a brief summary of the main activities in which each group is involved and then discuss current information on plant GTPases and their functional relevance.

The Rab family members function in intracellular protein traffic

The Rab family constitutes a large family of GTPases consisting of more than 20 different members that regulate vesicular traffic between specific compartments of the endocytic and exocytic pathways of eukaryotic cells [79]. Extensive studies in many organisms revealed that the components of the machinery used in constitutive vesicle trafficking are highly related from yeast to humans and likely plants as well [80–83]. Current information suggests that Rab proteins regulate the specificity and directionality of vesicular transport from a source to a target compartment [84]. The vesicles are covered with integral membrane proteins, called v-SNAREs, which are only compatible with a subset of t-SNARE molecules on target membranes. The specificity of membrane fusion is therefore determined by the interaction of v-SNAREs and t-SNAREs, which is controlled by Rab proteins [85–87]. Figure 2 illustrates schematically the site of action of several GTPases at endo- and exocytic compartments and their known plant SNARE proteins. Originally, it was proposed that each transport step in the endo- and exocytic pathways involves at least one distinct Rab protein. More recently, it became clear that Rab GTPases fulfill different

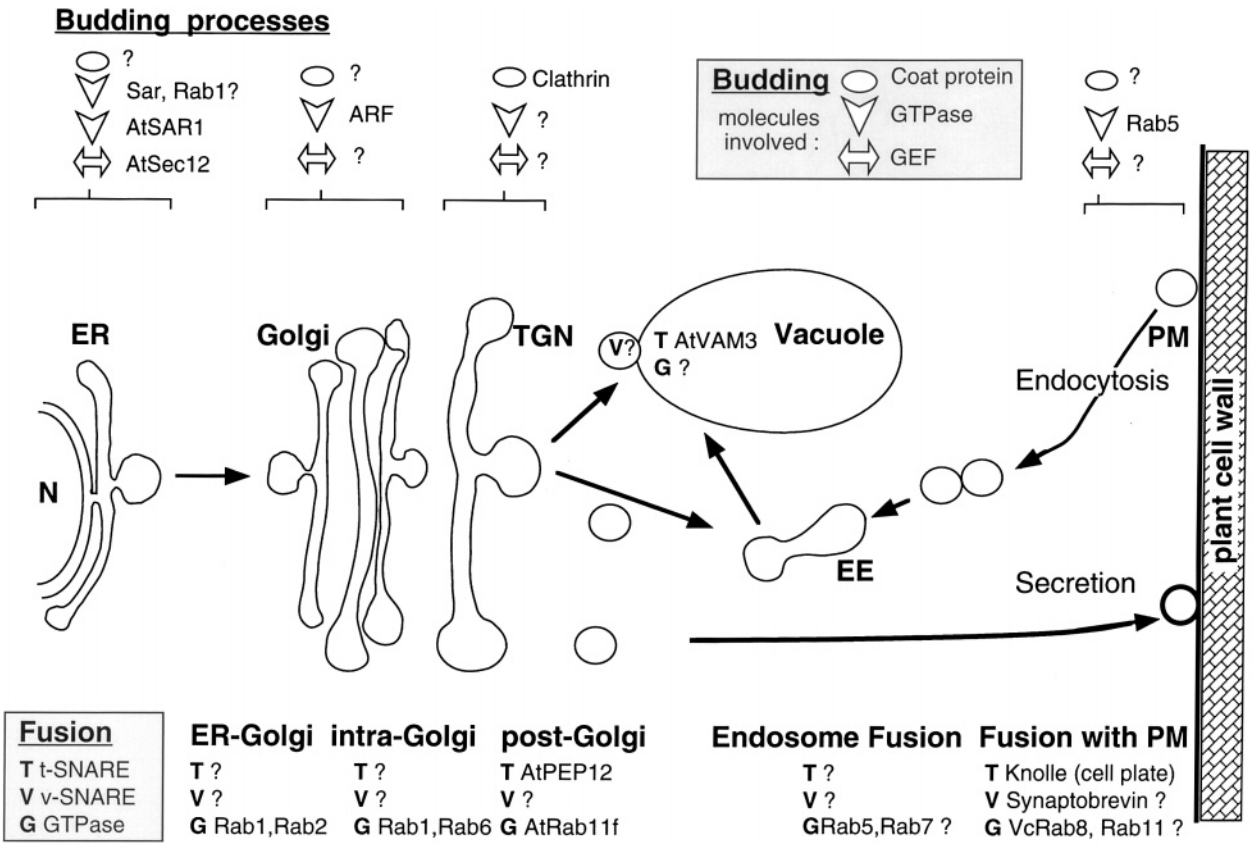

Figure 2. Diagram showing the different cellular compartments and the vesicular traffic between them. The different compartments [ER, Golgi, trans-Golgi network (TGN), vacuole, early endosomes (EE) and the plasma membrane (PM)] are depicted and the vesicular traffic between them (arrows). In the upper row, the coatomers, GTPases and guanine exchange factors (GEF) involved in budding processes at a particular compartment are indicated. In the lower row, the t-SNAREs and v-SNAREs as well as the GTPases for the corresponding fusion events are listed. The plant homologs are indicated by their subfamily name (for example Rab1). For proteins which have been specifically localized, the full protein name is given (for example AtRab11f). Compiled from references [144, 151, 169–172, 174, 281].

functions which can overlap with those of other Rab GTPases. For example, Rab1 proteins are located in the ER-to-Golgi intermediate compartment as well as in cis-Golgi cisternae and are apparently required for both ER-to-Golgi and intra-Golgi transport [79]. In yeast, spatial overlap of transport steps in which the Rab1 homolog YPT1 and the YPT3 protein are involved, was confirmed by a functional link between both GTPases [88]. Rab2 proteins were shown to be residents of pre-Golgi intermediates and of a cluster of small vesicles and tubules [89]. Although the precise functions of these proteins are not yet known, they seem to be required in the early secretory pathway for protein transport from the ER to the Golgi and act in the segregation of anterograde and retrograde protein transport after protein export from the ER [90–92]. Rab3 proteins are expressed exclusively in cells that have a high activity of regulated exocytosis [93, 94]. Rab4 and Rab5 are present in early endosomes [95, 96] and are involved in regulation of vesicular transport between the plasma membrane and early endosomes [97, 98]. Rab6 is an ubiquitous protein associated with the membranes of the Golgi apparatus and the trans-Golgi network functioning in intra-Golgi transport [99–101]. Rab7 and Rab9 are associated with late endosomes and are required for transport to or from late endosomes [102, 103]. A large number of Rab GTPases (for example Rab4, 5, 17, 18, 20) seem to be associated with early compartments of the endocytic pathway, suggesting a highly complex organization and function of the peripheral sorting endosome and the recycling endosome compartment [95, 96, 102–105]. Based on their intracellular localization and on mutant analysis, several other Rab proteins (for example Rab1, 2, 3, 6, 8 and 11) have been associated with different exocytic steps [99].

Like other members of the Ras superfamily, the Rab proteins contain four highly conserved sequence motifs required for guanine nucleotide binding [106]. In addition, they contain several nonconserved regions that confer unique functions for the regulation of distinct transport events (the effector domains around amino acids 52–58 as well as highly divergent N and C termini [107]). Of particular importance is the posttranslational processing at the C terminus resulting in addition of geranylgeranyl lipids, a modification essential for membrane association and function [108]. Moreover, the hypervariable region composed of the C-terminal 30 amino acids is thought to determine the targeting of Rab proteins to specific endo- and exocytic compartments [109].

The plant Rab family

Rab1 and Rab2 controlling the ER–Golgi traffic

General. Much information is available on the action of Rab1/YPT1 and Rab2 from mammalian and yeast cells. The small GTPase Rab1 has been assigned to an ER–Golgi intermediate compartment in mammals. The docking process of ER-derived COPII-vesicles with the cis-Golgi has been shown to require Rab1 or YPT1 GTPase [110, 111]. Consequently, ER membranes and vesicles with an average diameter of 50 nm accumulated in yeast cells in which the *YPT1* gene was disrupted [112].

Plant genes. A large number of Rab1-related genes have been cloned from various plants including green algae (see table 1). They encode proteins of about 22 kD with 70–80% identity to human Rab1. There are at least three genes present in *Arabidopsis*, three in tomato [113], four in pea [114] and five in *Lotus* [115]. *AtRab1b* (formerly *Ara5*) is located at the very top of chromosome 1 near the RI marker *acc2*, and *AtRab1c* is located on chromosome 4 [116, 117]. Three *Arabidopsis Rab2* genes (*AtRab2a*,*b*,*c*) were identified sharing 79%, 68% and 76% homology with human *Rab2* genes, respectively. Two of these genes, named *AtRab2a* and *AtRab2b*, are located on chromosome 4 between the markers AG and alb358 [117, 118]. *AtRab2c* (formerly *ATGB2*) was cloned from an expression library and identified by its property to bind guanine nucleotides [119].

Expression and functional information. Plant Rab1 homologs were found to be ubiquitously expressed in most tissues consistent with their role in ER–Golgi traffic (see table 1). Interestingly, VcRab1 (formerly YptV1) protein levels did not change throughout the whole life cycle of *V. carteri* [120]. Elevated expression levels were found in rapidly growing cells or in cells requiring active membrane biogenesis, such as germinating pollen or flower tisssue where *PhRab1*, *ZmRab1a*, *ZmRab1b*, *ZmRab1c* and *AtRab2a* genes were highly expressed [118, 121, 122]. Using a promoter-*GUS* fusion, the *AtRab2a* gene was shown to be transcribed in maturing pollen which accumulate ER and Golgi membranes to sustain pollen tube growth after germination [123]. In addition, strong GUS staining was found in elongating hypocotyls as well as in other actively growing tissues. *Rab2* genes of pea and soybean were differentially regulated by light. In pea the *PsRab1b* messenger RNA (mRNA) (formerly *Pra9A*) was slightly repressed by light whereas *PsRab1c* mRNA was shown not to be influenced by any light treatment [124]. In soybean, *GmRab1* (formerly *sypt*) mRNA was present at lower levels in roots of dark-grown seedlings than in roots of light-grown ones [125, 126].

Rab1 expression also correlated with highly specialized secretory processes such as the development of root nodules as shown for LjRab1a [115] and GmRab1 [125]. GmRab1 expression is required during *Rhizobia* endocytosis and peribacterioid membrane biogenesis of root nodules in legume [125]. Elevated levels of GmRab1 coincided with nodule formation. Reduced GmRab1 levels in antisense plants resulted in pleiotropic effects including reduced nodule size and reduced nitrogen fixation although endocytosis of *Rhizobia* was normal. Furthermore, small unfused vesicles accumulated in the cells of antisense plants probably due to an inhibition of general vesicular transport from the ER [125]. Together with the strong homology to mammalian Rab proteins, the available data obtained from plants suggest a specific involvement of Rab1 and Rab2 in ER–Golgi traffic. Additional functions may be present in green algae, where localization studies were carried out. Antibodies were raised against recombinant VcRab1 from *Volvox carteri* and used in immunogold electron microscopic studies. Gold grains were found to decorate the contractile vacuole of *V. carteri* and *Chlamydomonas reinhardtii* somatic cells. In *V. carteri* embryos, immunohistochemical studies also showed staining of the perinuclear regions where many Golgi complexes were found, surrounded by an extensive ER structure [281]. Similar localization studies in higher plants did not show these structures, which may be due to different morphological appearances and functions of the ER in higher plants [127].

Rab GTPases 6,8 and 11 are involved in later steps in the secretory pathway traffic

General. Several small Rab GTPases are involved in transporting secretory vesicles from the trans-Golgi network to the plasma membrane. In mammals, they comprise Rab3, Rab6, Rab8, Rab11 and Rab15 proteins.

Rab3 proteins are expressed especially in neurons and are implicated in regulation of secretion, mainly in late exocytic events [81]. In MDCK cells, Rab8 is localized to the Golgi region and to the basolateral membrane and regulates the transport of vesicles between both compartments [134]. Recent evidence indicates that HsRab11 is associated with the pericentriolar recycling endosome and regulates traffic through this compartment [135]. Rab6 associates with membranes of the Golgi apparatus and the trans-Golgi network [136] and may have a role in retrograde Golgi transport: transfection of HeLa cells with the GTP-bound form of Rab6 induced changes in Golgi morphology and the redistribution of Golgi-resident proteins into the ER [101], and addition of the Rab6-interacting domain of the motor protein Rabkinesin-6 specifically inhibited this effect [137].

Plant genes. Tobacco *Rab6* homologs have been isolated by plaque hybridization with oligonucleotides corresponding to conserved amino acid motifs [138], while *Arabidopsis AtRab6* has been isolated by complementation of the Rab6-deficient *Schizosaccharomyces pombe rhn1* mutant [139]. Plant *Rab8*/*Rab10*-related genes are represented by two members from *Arabidopsis*, five from *Lotus*, four from pea, one from tomato and one from *V. carteri* [115, 116, 140, 141]. The Rab11 class consists of a large group of genes that were found in all plant species (see table 1). They encode proteins of around 24–25 kD with 50–70% identity to human HsRab11. Seven members of this gene family were found in *Arabidopsis*, five in tobacco and seven in pea [114, 116, 142, 143, 138]. The high diversity of this subclass may suggest that these proteins play important regulatory roles during secretion and in maintenance of cell polarity in plants.

Expression and functional information. *Arabidopsis* AtRab11f (formerly Ara4) was shown to be localized to Golgi-derived vesicles in wild-type and in transgenic *Arabidopsis* plants expressing the *AtRab11f* gene under control of a heat-inducible promoter [144]. In wild-type plants, AtRab11f was ubiquitously expressed in roots albeit at low levels [145]. A specific function could not yet be assigned to the protein, as overexpression of AtRab11f under control of the CaMV 35S promoter in transgenic tobacco plants resulted in instable morphological abnormalities [146]. Likewise, overexpression in transgenic tobacco of OsRab11a (formerly rgp1), a Rab11 homolog from rice, resulted in pleiotropic changes such as reduced apical dominance, tillering and altered morphology of leaves [147]. A detailed immunological expression analysis was performed for pea Rab11 homologs: PsRab11b und PsRab11c were found to be mainly expressed in the growth zone of etiolated pea stems. PsRab11b (formerly Pra2), but not PsRab11c (formerly Pra3) expression was restricted to rapidly expanding cells which contain a characteristic highly active Golgi apparatus [148]. In parallel with the inhibition of hypocotyl elongation after light exposure, the mRNA levels of *PsRab11b* and *PsRab11c* were repressed by light. Evidence for phytochrome-regulated expression was provided by red-light pulse treatments that drastically and transiently lowered mRNA levels of *PsRab11b* and *PsRab11c*, but not of *PsRab1c*. Red light-induced repression was overcome by subsequent far-red exposure [124]. Upon light exposure, PsRab11c protein levels decreased selectively in stems, but not in roots [148]. A similar expression profile during elongation was found for *FsRab11a* from *Fagus sylvaticus*. These experiments showed that abscisic acid enhances *FsRab11a* expression in elongating cells of the embryonic axis [149]. Interestingly, in transgenic tobacco plants overexpressing OsRab11a, cytokinin levels were shown to be elevated [150]. Further investigations are needed to clarify the significance of this observation and to demonstrate the role of hormones in Rab11-mediated secretion.

No functional data are yet available for Rab8 homologs from higher plants. Using indirect immunofluorescence, VcRab8 (formerly YptV2) was found to be highly concentrated just below the cell surface in a granular pattern [151]. The VcRab8 protein was only detectable during embryogenesis and during the later process of inversion, by which the *Volvox* embryo turns inside out. VcRab8 expression ceased during growth and cytodifferentiation. Thus, in contrast to other Rab-related proteins from *Volvox*, only VcRab8 expression coincided with dividing gonidia, which dramatically increase their membrane surface during this phase [151].

Rab5 and Rab7 GTPases involved in endocytosis

General. Rab5 promotes membrane fusion between early compartments of the endocytic pathway. It was generally assumed that Rab5-GTP is membrane-bound, whereas Rab5-GDP is cytosolic and complexed with RabGDI [83]. RabGDI is required for efficient targeting of Rab5 GTPase to the endosome membrane where Rab5 promotes homotypic endosome fusions. In contrast to the simple switch model, it was shown that Rab5 undergoes several rounds of hydrolysis and GDP/GTP exchange on the endosome membrane, acting as a timer for early endosome fusion [156]. Several effectors and regulators of Rab5 have been identified in mammals, for example Rabaptin-5, a soluble protein essential for membrane fusion, which forms a complex with a Rab5-specific GEF, Rabex-5 [157].

In contrast to Rab5, Rab7 is not required for early internalization events, but crucial in downstream events which lead to the degradation of the vesicle content

[102]. It was shown that Rab7 is associated with the late endosomal compartments [95]. The cytotoxin VacA from *Helicobacter pylori* was shown to alter Rab7 activity and a role for Rab7 in homotypic fusion of late endosomes and formation of large vacuoles in HeLa cells was demonstrated [158]. Similarly, the disruption of the yeast YPT7 gene led to fragmented vacuoles and impaired transport of proteins to this compartment [159].

Plant genes. The *Arabidopsis AtRab5a* (formerly *Rha1*) 21.7-kD gene product has 60% identity with mammalian Rab5 [160]. A second gene is represented by an EST (see table 1). Two *Rab5* homologs were also found in tobacco and in *L. japonicus*, respectively [115, 161]. Several *Rab7* homologs were cloned from several plant species encoding proteins of 23 kD with up to 70% identity to Rab7 (see table). One *Arabidopsis* homolog was found on chromosome 1 at the PFL locus; the others are not yet mapped [162]. Three homologs were isolated from *N. tabacum* [138], and four from *L. japonicus* [115].

Expression and functional relevance. For *AtRab5a*, Northern analysis revealed high mRNA levels in root and callus tissue and weak expression in leaves, inflorescences, stems and seed pods [160]. Wheat *Rab5* mRNA levels decreased during the early stages of caryopsis development [163].

In transgenic plants containing an *AtRab5a* promoter-*GUS* construct, GUS staining was limited to guard cells of young leaves and was prominent in root tips [164]. When guard cell maturation was blocked in transgenic plants, GUS staining was still observed in leaves. This indicates that AtRab5a protein is already expressed in the guard cell mother cell. It was suggested that this GTPase is involved in cell plate formation, as Rab5 can mediate homotypic fusion [97, 164]. Such fusion events of numerous vesicles with a coherent electron-dense content have been observed during guard cell maturation [165]. Whether plant Rab5 proteins play a role in endocytosis has been discussed for VfRab5 and remains to be clarified [128]. The strong expression of *NpRab5* in roots might be linked to a high endocytotic activity in root hairs [166].

Tobacco *Rab7* homologs were highly expressed in stems and in immature fruits [138]. Borg et al. [115] showed differential expression of four distinct *Rab7* genes in *L. japonicus* (see table 1). *LjRab7c*, *VaRab7* and *GmRab7* genes were expressed in root nodules [125]. Interestingly, *NtRab7b*, *PaRab7* and *PsRab7* mRNAs were found at high levels in developing fruits [138, 167]. In apricots, *PaRab7* expression seems to be restricted to ripening tissue [168].

The precise control of degradation events seems to play a crucial role in the symbiosis of *leguminosae* and *Rhizobia*. In transgenic plants in which the high expression of GmRab7 protein in nodules was repressed by overexpressing of an antisense *VaRab7* mRNA, the nodule development was impaired. The repression of GmRab7 protein level resulted in a phenotype similar to changes observed in yeast cells in which the *YPT7* gene was disrupted: many small unfused vesicles and some large multivesicular bodies accumulated around the nuclear region [125, 159]. It seems that in transgenic plants the natural maturation of the membranes that envelope intruding symbiotic *Rhizobia* was affected during nodule development. It was suggested that in wild-type soybean plants, Rab7 proteins prevent the degradation of endosymbiotic bacteria in the vacuole [125]. However, it cannot be excluded that these and other Rab7 homologs might also have other functions as their mRNAs are present in different organs, for example root meristems and leaves (see table 1).

Proteins interacting with Rab GTPases

Rab GTPases ensure the compatibility of the docking molecules on the vesicle membrane (v-SNARE, for example synaptobrevin) and on the target membrane (t-SNARE for example syntaxin). Some components of this machinery have been cloned in plants (see fig. 2). For instance, three syntaxin-related molecules were identified in *Arabidopsis*. The t-SNARE AtPEP12 complemented a yeast *pep12* mutant. It was shown to be located on a post-Golgi compartment likely involved in vacuolar transport [169, 170]. However, no phenotype was reported for transgenics overexpressing AtPEP12. The KNOLLE protein encodes a cytokinesis-specific t-SNARE protein. KNOLLE protein was expressed specifically in mitotically dividing cells and localized to the plane of cell division during cytokinesis. Analysis by electron microscopy revealed that vesicle fusion was impaired in cells of *knolle* mutant *Arabidopsis* plants. Vesicle traffic itself was not affected as the dynamin-like protein ADL1 accumulated at the plane of cell plate formation [171, 172]. ADL1 belongs to the dynamin-class of GTP-binding proteins and was also shown to be involved in the biogenesis of thylakoid membranes [173]. The third t-SNARE is encoded by the gene *AtVAM3*, which is able to complement defective vacuolar assembly of the Δ*vam3* yeast mutant [174]. AtVAM3 protein was localized on vacuolar membranes and might function in vacuolar assembly in *Arabidopsis*.

Another important constituent of the Rab GTPase machinery is the GDP dissociation inhibitor RabGDI. Two AtRabGDI proteins have been identified from *Arabidopsis* using complementation of the *sec19* yeast mutant [175–178]. *AtRabGDI1* mRNA was present at similar levels in different tissues whereas *AtRabGDI2* mRNA levels were much higher in suspension culture and in roots than in other tissues [175–177]. A broad

expression pattern was also shown for VcRabGDI protein, which is the only RabGDI protein in *Volvox* [179].

The Arf and Sar-families: regulators of membrane traffic and organelle structure

General. The ADP ribosylation factor (Arf) family consists of different groups of structurally related GTPases of about 21 kD and includes the Arf-like GTPases (Arl) [180]. Arf GTPases were initially discovered as cofactors required for ADP ribosylation of the stimulatory Gα subunit of heterotrimeric GTPases by cholera toxin. ADP ribosylation blocks the GTPase activity of Gα subunits, resulting in persistent stimulation of adenylate cyclase and an increased level of cAMP [181]. More recently, they were also shown to be important components in several vesicular trafficking pathways and to be the primary activators of phospholipase D (PLD) [182–187]. PLD has been implicated in vesicle formation as well as in various signal transduction pathways [188, 189]. Membrane-associated Arf-GTP interacts with coat proteins, thereby promoting vesicle budding [190, 191]. Brefeldin A, a fungal toxin that reversibly blocks protein secretion and causes disintegration of the Golgi apparatus, is known to block the GDP/GTP exchange reaction on Arf, illustrating the important role of Arf in intracellular transport [192, 193]. The targets of brefeldin A are homologs of SEC7, a guanine nucleotide-exchange factor for Arf [194]. The *sec7* yeast mutant was isolated in a screen for secretion deficiencies. Another secretion-deficient mutant, *sec12ts*, is defective in vesicle formation at the ER [195]. Searching for multicopy suppressor genes of this mutation, Sar1 from *Saccharomyces cerevisisae* was isolated and shown to be distantly related to the Arf family. SEC12 was subsequently shown to be a guanine nucleotide-exchange factor for Sar [196, 197].

Plant Arf and Sar genes. Many plant *Arf* genes and cDNAs have been identified [119, 198–203], as well as a number of *Sar* genes [204–206]. In *Arabidopsis*, an *Arf* gene (*AtArf1*) was found with homology to human *Arf1*. It encodes a protein of 20.6 kD [198]. In addition, an *Arabidopsis* Arf-like protein (formerly ATGB1) has been described, which represents a novel group of Arf-like GTPases [119]. A third *Arabidopsis* Arf-like protein and one from *Brassica* (BcARL1) were related to human Arl1. A potato *StArf1* gene encoding a 197-amino acid protein of 22.6 kD with about 78% identity to the *Arabidopsis* Arf1 protein and 72–79% identity to fungal and human proteins has also been reported [207].

In *Arabidopsis* cell suspension cultures, *AtArf1* mRNA levels were similar in different stages [198]. In potato plants, *StArf1* mRNA levels were high in roots and tubers and lower in sink and source leaves, whereas StArf1 protein levels showed a reverse expression pattern [207]. In synchronized cultures of *Chlamydomonas*, a homolog of animal Arf1 had a biphasic expression pattern and was transiently expressed at the start of the light period [208]. The EMB30 protein, which was found to be mutated in the *Arabidopsis* embryo mutant *gnom* [209] contains an SEC7 domain and is therefore a candidate for a guanine nucleotide-exchange factor for Arf proteins [210].

Arabidopsis homologs of *Sar1* and its guanine nucleotide exchange factor (*SEC12*) have been cloned by complementation of the *sec12ts* yeast mutant [204]. Western and Northern analysis as well as in situ hybridization revealed equal expression levels in all cell types except a lower expression of AtSar1 in older leaves [211].

Expression and functional relevance. The function of Arf protein in plants has been studied using antisense transgenic potato lines with reduced levels of *StArf1* mRNA and protein [207, 212]. Using an ADP ribosylation assay, differences were observed between antisense and control plants in the relative amounts of labelling of two putative Gα subunits; proteins of either 40 or 42 kD were preferentially labelled with nicotinamide adenine dinucleotide (^{32}P-NAD) in antisense or control plants, respectively. Antisense plants with reduced *StArf1* mRNA and protein levels also accumulated a 27-kD 14-3-3 protein, and cAMP levels in these plants showed a 2–3 fold decrease [212]. Consistent with the high *StArf1* mRNA levels in wild-type potato tubers, these organs were strongly affected in transgenic antisense plants, resulting in knobby tubers and reduced stolon number. It was suggested that StArf1 might influence starch metabolism. AtSar1 and AtSEC12 were found to be associated with the ER, consistent with their role in secretory COPII-coated vesicle formation at the ER. Cold shock and tunicamycin, which block secretory protein transport, caused increased levels of *AtSar1* mRNA in *Arabidopsis* seedlings and suspension cultures, whereas *AtArf1* mRNA levels were not affected [211]. However, a 12-h cold treatment of suspension cells seemed to lead to a slight decrease in AtSar1 protein levels [213]. Cold caused a decrease, especially in the relative amount of membrane-bound AtSar1, suggesting a correlation between secretory activity and the amount of ER-associated AtSar1 protein. This correlation was strengthened by the fact that transgenic *Arabidopsis* plants, overexpressing either *AtSar1* or its guanine nucleotide-exchange factor *AtSec12*, did not contain more membrane-bound AtSar1 protein than control plants [214].

The RHO family: mediators in various signal transduction processes

General. In this paragraph we will use 'RHO' for the GTPase family consisting of Rho, Rac and CDC42

subfamilies, and 'Rho' for the Rho subfamily. RHO proteins share 50–55% homology with each other and have been linked to the regulation of a wide range of processes, including the organization of the actin cytoskeleton [217–220]. Thereby they control various aspects of cell morphology and motility, depending on the particular RHO protein involved. Members of the Rho subfamily can be activated by extracellular ligands, leading to the formation of stress fibers and associated focal adhesions in mammalian cells [221]. Rac subfamily GTPases are activated by different agonists, resulting in membrane ruffling and oxidative bursting depending on the cell type [222–224]. Activation of CDC42 induces filopodia and microspikes in mammalian cells [225, 226]. Because most of these responses are dependent on actin rearrangements, RHO GTPases are thought to be important regulators linking cell surface receptors to the organization of the actin cytoskeleton [220, 227–230]. Besides the effects on the actin cytoskeleton, RHO GTPases play a role in membrane traffic, transcriptional activation and cell growth control [217, 231]. The effects on the actin cytoskeleton and the long-term effects on transcriptional activation are mediated by RHO's ability to activate targets like the protein kinases PAK and ROK, which in turn activate MAPK pathways [232–234]. A major advance in understanding how Rho may regulate some of its activities has been the report that Rho may activate PLD [235–237]. This lipase generates phosphatidic acid, an intracellular messenger which can be metabolized to various lipids with different signalling functions in various pathways [238]. PLD is primarily activated by Arf and, like Arf, is involved in most cellular transport pathways. The role of Rho GTPases in intracellular transport is thought to be dependent on the ability to activate PLD by direct molecular interaction [189].

Plant genes. To date, no real plant homologs of the yeast and mammalian subfamily Rho and CDC42 GTPases have been found [239]. While the full repertoire of plant RHO genes will only be known after the completion of the *Arabidopsis* genome-sequencing project, the currently available sequence information already may suggest that, like Ras GTPases, members of the Rho and CDC42 subfamilies might be absent in plants. However, two groups of plant-specific RHO-like GTPases have been described. One is represented by a multigene family, members of which have been found in various monocot and dicot plants [239–242]. Sequence comparison indicates that these GTPases do not belong to the Rac subfamily as represented by mammalian Rac1 and *Dictyostelium* Rac1A [239, 242], but instead represent a novel plant-specific subgroup of RHO GTPases. This subgroup has been named Rop (RHO of plant) [240]. Multiple Rop genes have been detected in pea (about 6), cotton (around 8–10) using Southern analysis, and 13 are known from *Arabidopsis* [239–242]. The Rop genes can be divided in two subgroups based on amino acid differences in the effector and RHO insert regions [239]. Differential gene expression of several Rop genes has been demonstrated in cotton and *Arabidopsis* (see table 1). It is not clear whether the different Rop proteins are functionally equivalent or have diverged to allow interaction with different effectors.

Another group of plant-specific RHO-like GTPases is represented by a protein which is about 50% identical to the Rops. The molecule has been named'Rac-like', has a higher molecular weight than the Rops and a C-terminal farnesylation peptide motif [243]. RHO proteins are typically geranylgeranylated; in some rare cases they are also farnesylated [244]. The functional relevance of these differences in isoprenylation is currently unknown. Sequence comparison of the plant RHO GTPases with other RHO subfamilies found in *Dictyostelium* indicates that they resemble RacE. Both plant Rho groups and *Dictyostelium* RacE [245] share a unique two-amino acid deletion in the RHO insert region, including a conserved leucine residue. Especially the plant Rac-like sequence is clearly related to the RacE subfamily. Because the plant RHO proteins have no clear counterparts in mammals or yeast, we will maintain the original names in this section and in the table.

GDP dissociation inhibitor and, RHO GTPase-activating proteins are represented as ESTs in the *Arabidopsis* database (see table 1), but a plant RHO-GEF has not yet been identified. The RHO-GAP EST is not full-length and encodes the C-terminal domain of RHO-GAP. In mammalian cells and yeast, a large number of RHO-interacting proteins and effectors have been identified [217]. Information on RHO effector proteins from plants is still lacking.

Expression and functional relevance. The role of Rop GTPases has been studied in pollen tube growth. Tip growth in pollen tubes is dependent on the integrity of the actin cytoskeleton and on the presence of a Ca^{2+} gradient with the highest concentration of Ca^{2+} at the tube apex. Ca^{2+} enters the pollen tube at the extreme apex during growth [246]. Cytoplasmic streaming (the movement of cell organelles and the generative cell) is dependent on the pollen tube actomyosin system. Golgi-derived vesicles are transported to the pollen tube apex, where they dock and fuse with the cell membrane. This docking and fusion of vesicles at the tip is dependent on elevated Ca^{2+} levels at the tip [247].

The Rop1 GTPase from *Arabidopsis* is specifically expressed in mature pollen and pollen tubes as revealed by a *Rop1At* promoter:*GUS* fusion and by RT-PCR [242].

The pea homolog of Rop1 is similarly expressed in pollen and pollen tubes and is concentrated in the cortical region of the tube apex and also in the periphery of the generative cell. This localization suggests that Rop might be involved in controlling actin-dependent tip growth and possibly actin-dependent movement of the generative cell. Cell fractionation studies indicated that in pollen tubes Rop is present both in a cytosolic and in a membrane-bound fraction. Rop was also shown to be a substrate for geranylgeranylation, but not farnesylation [248]. Microinjected anti-Rop antibodies inhibited pollen tube elongation, confirming a role for Rop in tip growth [249]. The inhibitory effects of microinjected anti-Rop antibodies were enhanced in low Ca^{2+} or subinhibitory concentrations of caffeine, which is known to dissipate Ca^{2+} gradients in plants. Microinjection of the Rho GTPase inhibitor C3 ADP-ribosyltransferase inhibited cytoplasmic streaming, resulting in cessation of pollen tube growth. Since microinjected antibody did not inhibit cytoplasmic streaming, it was proposed that Rop is not sensitive to this enzyme and that the effects of C3 exoenzyme are caused by the inhibition of an unidentified C3 exoenzyme-sensitive Rho GTPase [249]. Because no clear evidence exists for cortical actin at the pollen tube tip, it was proposed that Rop might regulate a Ca^{2+}-dependent pathway involved in vesicle docking and fusion. Other indications for the role of Rop GTPases were provided by gene expression studies in developing cotton fibers. Cotton fibers are single cells which elongate for 3 weeks, producing a primary cell wall. At the transition to secondary cell wall synthesis, the pattern of cortical microtubules changes and this cytoskeletal rearrangement might be regulated by actin reorganization [250]. The mRNA levels of two different *Rop* genes peaked during the transition phase, which is consistent with a role for Rop proteins in regulating actin polymerization [241]. Possible functions for Rop proteins, analogous to the activation of the membrane NADPH oxidase complex by animal Rac2 [222, 224, 251] or β-1,3-glucan synthase by yeast Rho1p [252–254], have also been proposed [241]. However, because Rops are not true homologs of either animal Rac2 or yeast Rho1p, the involvement of plant Rops in these functions remains to be seen. The resemblance of plant RHO GTPases to *Dictyostelium* RacE, which is specifically required for cytokinesis [245, 255], might also indicate a possible function for these molecules in plant cell division. Two reports have suggested a role for small GTPases in activation of plant NADPH oxidase. The animal NADPH oxidase complex is made up of several components, one of which is $gp91^{phox}$. The complex produces superoxide anions, resulting in oxidative bursting. A similar complex has been described in plants [256] and plant homologs of $gp91^{phox}$ were recently identified [257, 258]. Components of the plant NADPH oxidase complex and a small GTPase immunologically related to mammalian Rac2 translocate to the membrane upon elicitor treatment of tomato plant cells [259]. In tobacco, the localization of this putative Rac2 was also affected by fungal elicitors [260]. Since real homologs of mammalian Rac2 have not been found in plants, it is possible that another, functionally distinct Rac-like GTPase was detected by the antiserum used in these experiments.

Ran GTPases-control: elements of nuclear protein import and export

General. The Ran GTPases and their regulatory factors, the Ran-binding proteins (RanBP) [261, 262], RCC1 (a guanine nucleotide-exchange factor) [263] and RanGAP (a GTPase-activating protein; [264]), play a key role in the nuclear protein import system [262, 265–268]. The nuclear import of proteins with a nuclear localization signal (NLS), a sequence containing one or more basic clusters of amino acids, starts with binding to the importin α/β complex [269] followed by interaction of importin β with the nuclear pore complex and translocation of importin α/β together with the NLS substrate into the nucleus. Although we are still far from understanding all the molecular details of the mechanism of nuclear import, it is clear that Ran fulfills at least two distinct functions in this process. First, Ran′s GTP cycle probably drives translocation of cytoplasmic proteins through the nuclear pore complex into the nucleus: GDP Ran apparently interacts with the nuclear pore complex, followed by nucleotide exchange and GTP hydrolysis, but does not seem to require binding to importin β [270, 271]. Second, Ran seems to regulate the interaction between importin α and importin ß, resulting in disassembly of this complex on the nuclear side of the nuclear pore complex and thereby terminating the translocation [271]. In view of the basic importance of the flux of macromolecules across the nucleus-cytosol boundary, it is not surprising that members of the Ran superfamily and their accessory factors are highly conserved between plants and animals.

Genes. Plant *Ran* genes from *Arabidopsis*, tomato, tobacco and *Vicia faba* have high homology to each other (94% identity) and to yeast and mammalian proteins (75% identity; see table 1). *Arabidopsis* cDNAs and genomic clones corresponding to three different genes encode nearly identical proteins of about 221 amino acids differing only at their C termini [272]. The *AtRan1* and *AtRan2* genes are located close to each other in head-to-tail orientation. Using these genes as a bait in a yeast two-hybrid screen, *AtRanBP1a* and *AtRanBP1b* were isolated and characterized [272]. Four members of

the Ran family were isolated from tobacco (*NtRan1–4*); two of these cDNAs were full-length and showed 60% similarity to yeast and mammalian RanBPs. Three *Ran* genes were isolated from tomato [273].

Expression and functional information. Expression of Ran GTPases was found in all tissues analyzed, with highest expression levels in mitotically active meristematic tissues (i.e. root tip, developing embryos; see table 1). Analysis of transgenic *Arabidopsis* lines containing *AtRan* promoters fused to the *GUS* reporter gene showed that the *Arabidopsis Ran* genes were differentially expressed: *AtRan1* showed highest expression levels, *AtRan2* moderate levels, whereas *AtRan3* was expressed about 20-fold lower than *AtRan1*. These results were confirmed by RT-PCR quantification. By in situ hybridization analysis, Haizel et al. [272] showed that the expression of *AtRan1* and *AtRanBP1a* was coordinated and highest in meristematic tissues. These results were consistent with the findings by Ach and Gruissem [273], showing that mRNA levels of both genes decreased in mature leaves. Therefore it was concluded that the GTPase cycle of Ran proteins is likely regulated by accessory proteins controlling cell cycle progression. In contrast, it was observed that tomato *LeRan* gene expression did not decrease during fruit development despite a strong reduction in cell division activity [273].

All tested plant Ran GTPases complemented the yeast cell cycle regulatory mutant *pim1-46*, a temperature-sensitive mutant that prematurely initiates mitosis but fails to complete chromosomal DNA replication [274]. Interaction of both the GTP-locked and wild-type forms of AtRan1 with AtRanBP1 was demonstrated by in vitro binding studies using GST-AtRanBP1a. The Ran-binding domain (RBD) of AtRanBP1 and its C-terminus were both shown to be required for interaction [272]. In order to study the role of plant Ran proteins directly, an in vitro system was established using evacuolated tobacco BY-2 suspension protoplasts [276]. It was shown that GTP is specifically important for nuclear import, as GTPγS but not ATPγS blocked the import of fluorescently labelled protein into the plant nucleus. Furthermore, plant nuclear protein import seemed to be insensitive to NEM or iodoacetic acid, in contrast to the mammalian system. In addition, the majority of NtRan-A1 was in the plant cytoplasm and not in the nucleus, where the majority of human Ran proteins are located.

Perspective

Over the last decade we learned that plants use the GTPase switch to choreograph numerous cellular pathways as diverse as growth control, translational control, vesicular transport, cytoskeletal organization and nuclear import. Many plant GTPases have been cloned, showing high sequence conservation in the GTPase domains. However, several genes are difficult to assign clearly to specific GTPase subfamilies (for example the Rop proteins). Moreover, genetic approaches and systematic genome-sequencing initiatives identified several genes with novel arrangements of GTPase domains or even entirely novel classes [277, 278]. This indicates that plant evolution made efficient use of GTPase domains as 'tinkering' to link these elements with new functions. In most cases, however, current functional analysis of plant GTPases has been limited to complementation of corresponding yeast genes or to ectopic expression in transgenic plants. To get a full understanding of the important role of plant GTPases in regulation of cell signalling or regulation of metabolic processes, we urgently need a detailed analysis of mutants (loss or gain-of-function mutations). Particularly the use of loss-of-function (knockout) mutations caused either by agrobacterial T-DNA or transposon insertion will be important to overcome limitations in antisense approaches such as gene redundancy, incomplete antisense inhibition or gene silencing [279, 280]. We expect that through the combined efforts and the interchange of ideas from different fields, a detailed understanding of how plant GTPases work is in sight. We expect that knowledge of these molecular machines will lead to practical applications in engineering regulatory pathways in plants.

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