European Journal of Cell Biology 89 (2010) 138–144

Contents lists available at ScienceDirect

European Journal of Cell Biology

journal homepage: www.elsevier.de/ejcb

Role of the *GNOM* gene in *Arabidopsis* apical-basal patterning – From mutant phenotype to cellular mechanism of protein action

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ARTICLE INFO

Keywords:
Patterning
ARF-GEF
Membrane traffic
Polarity

ABSTRACT

How the apical-basal axis of polarity is established in embryogenesis is still a mystery in plant development. This axis appeared specifically compromised by mutations in the *Arabidopsis GNOM* gene. Surprisingly, *GNOM* encodes an ARF guanine-nucleotide exchange factor (ARF-GEF) that regulates the formation of vesicles in membrane trafficking. In-depth functional analysis of GNOM and its closest relative, GNOM-LIKE 1 (GNL1), has provided a mechanistic explanation for the development-specific role of a seemingly mundane trafficking regulator. The current model proposes that GNOM is specifically involved in the endosomal recycling of the auxin-efflux carrier PIN1 to the basal plasma membrane in provascular cells, which in turn is required for the accumulation of the plant hormone auxin at the future root pole through polar auxin transport. Thus, the analysis of GNOM highlights the importance of cell-biological processes for a mechanistic understanding of development.

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Introduction

This is the story of a unique gene of *Arabidopsis thaliana* named *GNOM* the analysis of which began some twenty years ago. The starting point was the isolation of a large number of EMS-induced mutants that shared a striking, though somewhat variable, phenotype. The extreme expression of the *gnom* phenotype was a ball-shaped seedling that appeared to lack the apical-basal body axis (Mayer et al., 1991, 1993). While molecular cloning of the *GNOM* gene was underway, genetic and phenotypic analysis of the mutant alleles supported the notion of a pivotal role for GNOM in early embryogenesis but also highlighted an intriguing complexity of gene function, the mechanistic basis of which only became clear in the past few years.

The DNA sequence of the *GNOM* gene revealed a limited similarity of its deduced product to the secretory protein Sec7p of the yeast, *Saccharomyces cerevisiae*, but provided no clue as to the molecular function of the protein in the context of embryo development (Shevell et al., 1994; Busch et al., 1996). However, sequencing of the *S. cerevisiae* genome yielded two open reading frames (ORFs) that showed more sequence similarity to GNOM than did Sec7p (Busch et al., 1996). Interestingly, one of the two yeast ORFs was subsequently isolated as a multi-copy suppressor of a dominant-negative *arf2* mutant. Moreover, the protein encoded by that ORF was shown to mediate guanine-nucleotide exchange on an Arf GTPase and was thus named Gea1p for Guanine-nucleotide exchange on arf (Peyroche et al., 1996). Interestingly, Gea1p activity was sensitive to the fungal toxin brefeldin A (BFA) that is nowadays one of the most popular drugs in plant research. Gea1p was systematically analysed by in vitro mutagenesis for mutant variants that were no longer sensitive to BFA (Peyroche et al., 1999). Remarkably, homologous mutations in the other two ARF-GEFs, Gea2p and Sec7p, had similar effects such that the triple mutant yeast were completely BFA-resistant, suggesting that BFA is a "clean" drug that specifically inhibits sensitive ARF-GEFs. Structural analysis confirmed that BFA fits into a pocket at the interface between ARF-GEF and ARF, acting as a molecular glue (Renault et al., 2003; Mossessova et al., 2003).

Another line of research revealed that tampering with auxin transport or action in *Brassica juncea* embryos generated variable seedling defects that closely resembled *gnom* mutant seedling phenotypes (Hadfi et al., 1998), raising the possibility that GNOM action was somehow related to an ill-defined role of the phytohormone auxin in embryogenesis. At the same time, the *PINFORMED 1* (*PIN1*) gene of *Arabidopsis* had been isolated on the basis of its mutant phenotype suggesting an auxin-related post-embryonic developmental defect (Gälweiler et al., 1998). Moreover, indirect immunofluorescence with an antiserum raised against recombinant PIN1 protein revealed a polar localisation of this membrane protein to the basal surface of vascular cells in stem segments of adult plants. Thus, PIN1 presented itself as a

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doi:10.1016/j.ejcb.2009.11.020

potentially useful marker with which to probe cell polarity in *gnom* mutant embryos.

The seemingly unrelated observations mentioned so far provided different pieces of the puzzle of how GNOM might act to establish or maintain the apical-basal axis of polarity in *Arabidopsis* embryogenesis. In rather simple and vague terms, the primary role of GNOM might be related to some aspect of subcellular membrane trafficking that is necessary for cell or tissue polarity in embryogenesis and possibly also later on. This was the implicit working hypothesis that has guided our mechanistic studies of GNOM action within the Collaborative Research Programme SFB 446.

Origin: screen for embryo patterning mutants

In the 1950s, Andreas Müller performed the first systematic screen for embryo-lethal and abnormal-seedling mutants in *Arabidopsis thaliana* in order to characterise the mutagenic activity of chemical compounds (Müller, 1963). This system was then used by David W. Meinke to identify mutations interfering with embryo development (Meinke and Sussex, 1979). Meinke (1985) described an "embryo-lethal" mutant 112A-2A later renamed *emb30* that lacked both cotyledons and root, and this mutant was unable to differentiate a root during growth from immature embryos or after callus formation (Baus et al., 1986). Both genetic complementation analysis of *gnom* and *emb30* mutants and the molecular cloning of the two genes affected revealed their identity (Mayer et al., 1993; Shevell et al., 1994; Busch et al., 1996). A somewhat different forward-genetics approach was pursued by our group in order to identify genes relevant for pattern formation in *Arabidopsis* embryogenesis (Jürgens et al., 1991; Mayer et al., 1991). Performing a saturation screen for EMS mutants with specifically altered seedling body organisation, we aimed to identify all embryo patterning genes that could be revealed by mutant phenotype. Among other genes, *GNOM* was identified by at least 24 mutant alleles that shared the same embryo and seedling phenotype, suggesting that the developmental abnormalities described were caused by the complete loss of gene function and that *GNOM* was thus not required for general housekeeping activities (Fig. 1) (Mayer et al., 1991, 1993). Rather, *gnom* mutant embryos developed abnormally from the zygote stage and as development progressed, displayed abnormal cell division patterns that reflected their failure to initiate the root meristem and the cotyledon primordia (Fig. 1) (Mayer et al., 1993). Subsequently, a molecular marker for the apical end of the embryo, *LTP1::GUS*, revealed variable apical-basal polarity of *gnom* mutant embryos, being expressed apically, basally, uniformly or not at all (Vroemen et al., 1996). Thus, by all available criteria, *GNOM* qualified as a developmental gene required for establishing a stable apical-basal axis of polarity during embryogenesis.

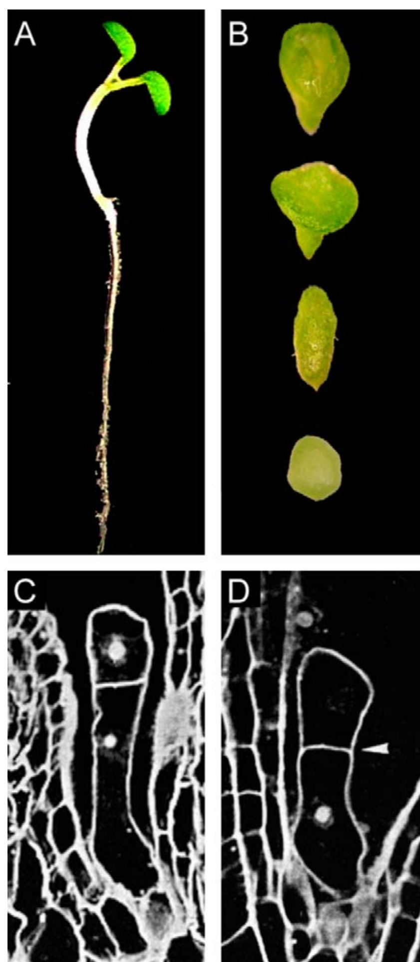

Fig. 1. *gnom* mutant phenotype. (A, C) Wild-type, (B, D) *gnom*. (A, B) Seedling, (C, D) One-cell stage of embryogenesis. Modified after (Mayer et al., 1993).

Genetic complexity of *gnom* mutant alleles

The genetic analysis of an initial set of 24 strong alleles revealed complex complementation behaviour of *gnom* mutants (Mayer et al., 1993). Thus, different alleles were grouped into any one of three classes A–C according to their ability to complement each other. Most alleles were grouped in class A because they complemented 2 class-B alleles partially. In contrast, 2 other alleles did not complement either and thus represented class C of presumably complete loss-of-function alleles (Fig. 2; Table 1). Partial complementation meant that *gnom-A/gnom-B* trans-heterozygous seedlings displayed a short root, in contrast to the *gnom* homozygous seedlings of each parental line. Although no complete complementation was observed among the initial set of mutant alleles, the classification of alleles was later confirmed with 2 additional class-A alleles that nearly completely complemented class-B alleles, resulting in fertile adult plants (Busch et al., 1996; Anders et al., 2008). Molecular analysis of most strong alleles revealed premature stop-codon mutations in class-A alleles such that no mutant protein accumulated, except B4049 and *gnom*-G568R. The latter two class-A alleles and all 3 class-B alleles had missense mutations, resulting in full-length mutant protein with single amino acid substitutions (Table 1) (Busch et al., 1996; Steinmann et al., 1999; Anders et al., 2008). Class-C alleles all lacked mutant protein, although their primary mutational changes were heterogeneous including premature stop-codon and frameshift mutations, small or large deletions. It is still not known why some alleles with a premature stop-codon mutation displayed partial complementation (class A) whereas others did not (class C), although both groups of mutants did not accumulate detectable mutant protein. Interestingly, all 6 fully complementing alleles have missense substitutions in the catalytic SEC7 domain (see below). By reference to the structure of the SEC7 domain of the small ARF-GEF ARNO (Cherfils et al., 1998; Mossessova et al., 1998), 4 class-B alleles had identical mutations of E658K in the catalytic glutamate finger abolishing catalytic activity, whereas 2 class-B alleles had substitutions of amino acid residues (G568R, G579R) in a functionally uncharacterised part of the SEC7 domain away from the catalytic centre (Busch et al., 1996; Anders et al., 2008). That different parts of the SEC7 domain being only 80 amino acids

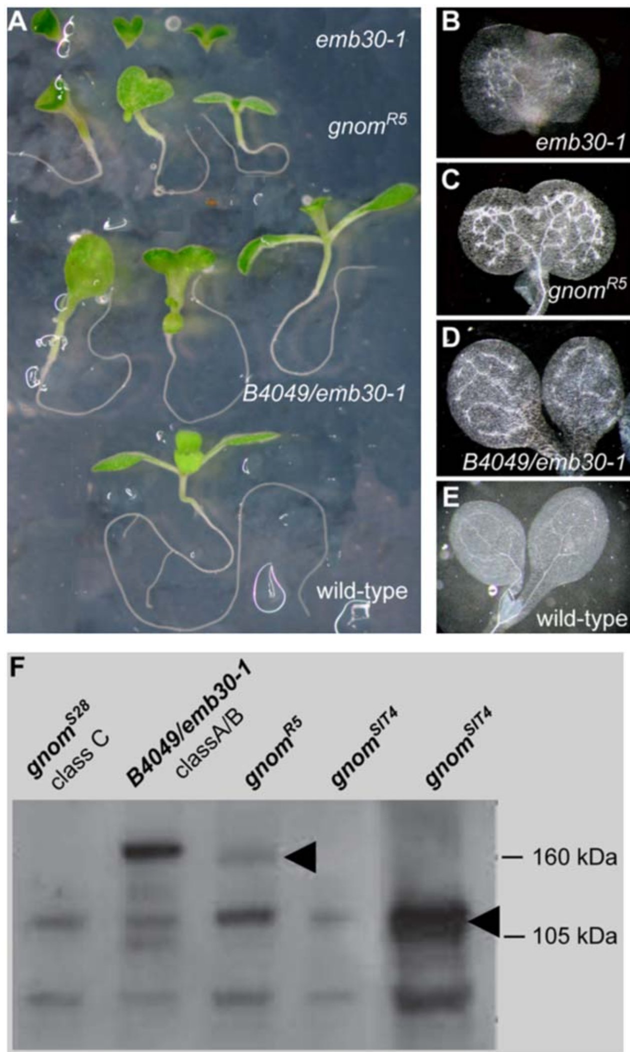

Fig. 2. Strong, weak and complementing *gnom* alleles. (A–E) Phenotypes of strong (*emb30-1*), weak (*gnom*^{R5}) and complementing (*B4049/emb30-1*) *gnom* alleles in comparison to wild type. (A) Seedlings, (B–E) leaf vasculature. (F) Western blot of strong (*gnom*^{S28}) (class-C allele), complementing (*B4049/emb30-1*; class-A and class-B allele, respectively) and weak (*gnom*^{R5}, *gnom*^{SIT4}) alleles. *Note*: *B4049/emb30-1* express mutant full-length protein, *gnom*^{S28} no GNOM protein and weak *gnom* alleles truncated proteins (black arrowheads). The weak alleles accumulate less GNOM protein (much more protein has been loaded in the right-most lane than in the other lanes to reveal the truncated GNOM protein). Modified after (Geldner et al., 2004).

apart were involved in two distinct functions was rather surprising and remained a mystery until recently (see below). Nonetheless, the full complementation of two sets of inactive alleles also suggested that GNOM acts as an oligomeric protein made up of two or more identical subunits (see below).

In addition to the strong alleles, several weak *gnom* alleles were also isolated. Because of their different mutant phenotypes, the weak alleles were only recognised as *gnom* alleles after they had been mapped to the genomic interval harbouring the *GNOM* gene and then tested for complementation of strong *gnom* alleles (Geldner et al., 2004; Miyazawa et al., 2009b). Two alleles, R5 and SIT4, were isolated on the basis of their variably compromised seedling phenotype including the failure to initiate lateral roots (Geldner et al., 2004). Molecularly, these alleles have mutations that truncate the GNOM protein downstream of the SEC7 domain whereas stop-codon mutations of strong alleles are all located in or upstream of the SEC7 domain (Table 1). The deduced GNOM protein of SIT4 is truncated at amino acid residue 983 (HDS1 domain, see below), and the R5 allele has a frameshift mutation in codon 1369 that causes truncation after additional 51 incorrect amino acid residues. Interestingly, both mutants accumulate only low levels of GNOM protein, suggesting that C-terminal truncation interferes with protein stability. Indeed, we isolated a suppressor of R5, su(R5)182, that enabled the development of fertile, almost normal-looking, though genetically mutant plants. This suppressor increased the level of the truncated protein to nearly wild-type protein level, which surprisingly resulted from an additional stop-codon mutation in codon 1315 of the *GNOM* coding sequence, truncating the protein even further (Fig. 2; Table 1) (Heinrich et al., unpublished observation).

Another *gnom* allele does not cause any morphological or gravitropic defect, in contrast to all other known *gnom* alleles. This unusual allele named *mizu-kussei 2* (*miz2*) was isolated as a viable and fertile mutant deficient in root hydrotropism (Miyazawa et al., 2009a). *miz2* has a missense mutation that changes a GNOM-specific amino acid residue, G951E, in the HDS1 domain (see below).

Unexpected SECrets revealed by molecular cloning and functional analysis

The *GNOM* gene encodes a 1451 amino acids long protein with a central domain related to the yeast secretory protein Sec7p, which is now known to be the catalytic domain and was therefore named SEC7 domain (Shevell et al., 1994; Busch et al., 1996). Expression of a *GNOM* cDNA was shown to partially complement

Table 1
gnom alleles.

Allele	Mutagen	Accession[a]	Class[b]	Mutation[c] protein (CDS)	Protein[d] (Western blot)
4-13	EMS	Ler	A	R647* (CGA > TGA)	No signal
B3888	EMS	Col	(not A)	n.a.	No signal
B4049	EMS	Col	A	G579R (GGG > AGG)	Full-length protein
B5387	EMS	Col	C	n.a.	No signal
B7305	EMS	Col	A	W52* (TGG > TGA)	No signal
B8208	EMS	Col	n.a.	n.a.	Full-length protein
B8437	EMS	Col	C	n.a.	No signal
B9171	EMS	Col	(not A)	n.a.	No signal
EK44	EMS	Ler	n.a.	n.a.	No signal
emb30-1	EMS	Col	B	E658K (GAA > AAA)	Full-length protein
G14-109	γ-rays	Col	C	n.a.	No signal
G19-81	γ-rays	Col	C	n.a.	No signal
G33-36	γ-rays	Col	C	2nd intron, deletion and rearrangement	No signal
G60	EMS	Ler	A	n.a.	No signal
G568R	EMS	Ler (*cuc1*)	A	G568R (GGA > AGA)	Full-length protein
R5-33	EMS	Ler	weak	S1369Δ (AGC > A–C)	No/weak signal (ca. 155 kDa)

Table 1 (*continued*)

Allele	Mutagen	Accession[a]	Class[b]	Mutation[c] protein (CDS)	Protein[d] (Western blot)
R48	EMS	Ler	A	n.a.	No signal
R304	EMS	Ler	A	n.a.	No signal
R310	EMS	Ler	A	n.a.	No signal
S28	EMS	Ler	C	2nd intron splice acceptor site (G > A)	No signal
SIT475	EMS	Ler	weak	Q984* (CAA > TAA)	No/weak signal (ca. 110 kDa)
su(R5)182	EMS	Ler	(normal)	R1315* (CGA > TGA)+S1369Δ (AGC > A–C)	Truncated protein (ca. 145 kDa)
T97	EMS	Ler	A	n.a.	No signal
T339	EMS	Ler	A	n.a.	No signal
T340	EMS	Ler	n.a.	n.a.	No signal
T345	EMS	Ler	A	W81* (TGG > TGA)	No signal
T391	EMS	Ler	B	E658K (GAA > AAA)	Full-length protein
T424	EMS	Ler	A	n.a.	No signal
TA477	Ac/Ds	C24	C	aa599-601(ACF)Δ (9-bp deletion)	Full-length protein
U23	EMS	Ler	A	n.a.	No signal
U40	EMS	Ler	A	n.a.	No signal
U75	EMS	Ler	A	n.a.	No signal
U87	EMS	Ler	B	E658K (GAA > AAA)	Full-length protein
U147	EMS	Ler	A	W551* (TGG > TGA)	n.a.
U207	EMS	Ler	C	Q596* (CAA > TAA)	n.a.
U221	EMS	Ler	A	W538* (TGG > TGA)	n.a.
U223	EMS	Ler	A	W538* (TGG > TGA)	n.a.
U228	EMS	Ler	A	n.a.	No signal
U243	EMS	Ler	A	n.a.	No signal
U255	EMS	Ler	A	C600Δ (TGC > TG-)	n.a.
U263	EMS	Ler	A	W551* (TGG > TGA)	n.a.
U323	EMS	Ler	A	n.a.	No signal

Based on data from Mayer et al. (1993), Busch et al. (1996), Steinmann et al. (1999), Geldner et al. (2004) and Heinrich et al. (unpublished data). For details, see text. n.a., not analysed.

[a] Col, Columbia; Ler, Landsberg erecta.
[b] class-A alleles complement class-B alleles; class-C alleles do not complement.
[c] asterisk, stop-codon mutation; Δ, basepair deletion.
[d] Full-length protein is 165 kDa large; no signal, not detectable in Western blot with anti-GNSec7 antiserum.

the temperature-sensitive ARF guanine-nucleotide exchange factor (ARF-GEF) mutant *gea1-19 gea2Δ* of yeast (Steinmann et al., 1999). In addition, full-length GNOM protein was able to stimulate GDP-GTP exchange in vitro on mammalian ARF1 GTPase in a BFA-sensitive manner. Thus, GNOM is a BFA-sensitive ARF-GEF. However, both its subcellular site of action and its ARF substrate were unknown.

GNOM belongs to the large ARF-GEF family comprising two conserved subfamilies, GBF1/Gea1,2p/GNOM (GGG) and BIG/Sec7p (BIG), that have representatives in animals, plants and fungi (Cox et al., 2004; Mouratou et al., 2005; Anders and Jürgens, 2008). However, there was initially no functional evidence for any domain of large ARF-GEFs except the catalytic SEC7 domain (Fig. 3). As discussed above, the full complementation of different *gnom* alleles suggested dimerisation or oligomerisation of GNOM protein, which was confirmed by yeast two-hybrid interaction and pull-down assays that defined an N-terminal dimerisation and cyclophilin-binding (DCB) domain (Grebe et al., 2000). Sequence comparison of ARF-GEFs from various organisms then led to the recognition of conserved domains most of which had not been functionally defined: DCB, HUS (homology upstream of SEC7), SEC7, HDS1-3 (homology downstream of SEC7). The DCB domain was functionally analysed in yeast and mammalian ARF-GEFs (Ramaen et al., 2007).

In addition to large ARF-GEFs, which are conserved across the eukaryotic kingdoms, small and medium-sized ARF-GEFs occur in animals and fungi but are absent from plants. In contrast to the latter, large ARF-GEFs lack a sequence-specific membrane-association domain, although the GDP–GTP exchange on ARF takes place on membranes (Casanova, 2007). Furthermore, although being a peripheral membrane protein, GNOM was shown to firmly associate with membranes, and BFA treatment

Fig. 3. Domain organisation of large ARF-GEFs. Large ARF-GEFs share a common domain architecture comprising an N-terminal dimerisation and cyclophilin-binding domain (DCB), a homology upstream of SEC7 domain (HUS), the central catalytic SEC7 domain (SEC7) and three C-terminal homology downstream of SEC7 domains (HDS1-3).

increased the fraction of membrane-associated GNOM substantially (Steinmann et al., 1999). To explore the molecular basis for the membrane association of GNOM, various combinations of protein fragments were analysed in yeast two-hybrid interaction assays as well as co-immunoprecipitation and immunlocalisation studies of transgenic plants (Anders et al., 2008). These studies identified three activities of GNOM protein in vivo: dimerisation via DCB–DCB interaction, heterotypic interaction between the DCB domain and the remainder of the protein, and membrane association involving heterotypic interaction. This analysis revealed why the *emb30* allele is fully complemented by *B4049*. The non-functional protein encoded by *B4049* cannot perform the heterotypic interaction and thus fails to associate with membranes. In contrast, the SEC7 domain of B4049 is still catalytically active and thus can form functional dimers with the catalytically inactive protein encoded by *emb30* through homotypic interaction via their DCB domains (Anders et al., 2008). These results were taken to suggest that membrane association of GNOM requires a specific protein conformation that is caused by heterotypic domain interaction. Once associated with the membrane, GNOM undergoes a conformational change that activates

GDP–GTP exchange on ARF. It should be noted that heterotypic domain interaction was also detected in mammalian ARF-GEFs, although this was confined to the DCB and HUS domains, with no evidence for the involvement of the SEC7 domain (Ramaen et al., 2007). The reason for this difference to GNOM is not clear.

An unusual member of a conserved family of eukaryotic membrane trafficking regulators

Although GNOM is a member of the GGG subfamily of large ARF-GEFs, its role in membrane trafficking differs from that of other members such as mammalian GBF1. Whereas the latter acts in COPI-mediated retrograde traffic from the Golgi apparatus to the endoplasmic reticulum (ER), GNOM was shown to play an essential role in the regulation of PIN1 recycling from endosomes to the basal plasma membrane (Geldner et al., 2003). PIN1 was originally used as a marker for cell polarity in order to examine *gnom* mutant embryos for defects in cell polarity. Surprisingly, PIN1 was not polarly localised in the very early stages of embryogenesis but only started to accumulate at the basal end of provascular cells at the early-globular stage of wild-type embryogenesis, and this coordinated polarisation did not occur in *gnom* mutant embryos (Steinmann et al., 1999; Friml et al., 2003). Since GNOM is a BFA-sensitive ARF-GEF, seedling roots were treated with BFA to determine whether the polar accumulation of PIN1 at the basal end of vascular cells was altered (Geldner et al., 2001). PIN1 disappeared from the basal plasma membrane and accumulated in so-called BFA compartments. Upon BFA washout, the original distribution of PIN1 was restored, even if protein biosynthesis was inhibited, suggesting that PIN1 cycles continuously between the plasma membrane and some endomembrane compartment, and that a BFA-sensitive ARF-GEF mediates recycling to the plasma membrane (Geldner et al., 2001). Following the yeast model, GNOM was rendered BFA-resistant by specific amino acid exchange. Transgenic plants expressing the engineered BFA-resistant variant of GNOM were viable in the absence of endogenous GNOM protein, indicating functionality of BFA-resistant GNOM. Furthermore, PIN1 recycling was no longer inhibited by BFA treatment in seedling roots expressing BFA-resistant GNOM (Geldner et al., 2003). PIN1 had been implicated in auxin efflux from the cell and has more recently been shown to be an auxin efflux carrier (Gälweiler et al., 1998; Petrásek et al., 2006). We thus tested whether GNOM plays a role in mediating polar auxin transport and in auxin-dependent processes such as root gravitropism and formation of lateral roots. All these processes are inhibited by BFA in wild type but were rendered insensitive in plants expressing the engineered BFA-resistant variant of GNOM (Geldner et al., 2003). In addition, lateral root initiation was abolished in mutant seedlings carrying the weak *gnom* allele R5. Although the pericycle cells were still responsive to auxin, they were unable to reorientate their plane of division, which is required for lateral root initiation (Geldner et al., 2004). These data suggest a close link between GNOM action and polar auxin transport in development, which is also supported by the observation that a *pin1 pin3 pin4 pin7* quadruple mutant displayed *gnom*-like embryo and seedling phenotypes (Friml et al., 2003). Nonetheless, GNOM has also been implicated in root hydrotropism, e.g. bending towards higher moisture, which differs mechanistically in some respect from root gravitropism (Miyazawa et al., 2009a, b).

The advantage of having close relatives

As mentioned above, GNOM is an unusual member of the GGG subfamily of large ARF-GEFs in that it is not essential for viability of cultured cells but rather performs a development-specific recycling function (Steinmann et al., 1999). However, the *Arabidopsis* genome encodes two additional GGG-type ARF-GEFs named GNOM-LIKE 1 (GNL1) and GNL2, the amino acid sequences of which are 61% and 48% identical to GNOM, respectively. Interestingly, GNL1 localises to Golgi stacks, co-localising with the COPI subunit γ-COP (Fig. 4) (Richter et al., 2007). Surprisingly, a *gnl1* knockout mutation is not lethal but rather causes a bushy-plant phenotype and slightly reduced transmission through the pollen, suggesting that some other ARF-GEF might be functionally redundant with GNL1. Indeed, BFA treatment of *gnl1* mutant seedlings prevented membrane association of γ-COP and resulted in Golgi-ER fusion, suggesting that GNL1 is BFA-resistant and that the presumed functionally redundant ARF-GEF is BFA-sensitive (Richter et al., 2007). In an independent approach, Teh and Moore (2007) isolated EMS-induced *gnl1* alleles in a screen for mutants that accumulated secretory GFP (secGFP) in intracellular membrane compartments upon BFA treatment, and one of these *gnl1* alleles also gave a bushy-plant phenotype. The BFA sensitivity of *gnl1* mutant seedlings was rescued by introducing the engineered BFA-resistant variant of GNOM, which restored membrane recruitment of γ-COP. The *gnl1 gnom* double mutant is gametophytic-lethal, revealing the (expected) consequence of inhibiting ER-Golgi traffic in *Arabidopsis* (Richter et al., 2007). Thus, GNL1 and GNOM jointly perform the essential ancestral eukaryotic GGG-type ARF-GEF function in Golgi-ER retrograde

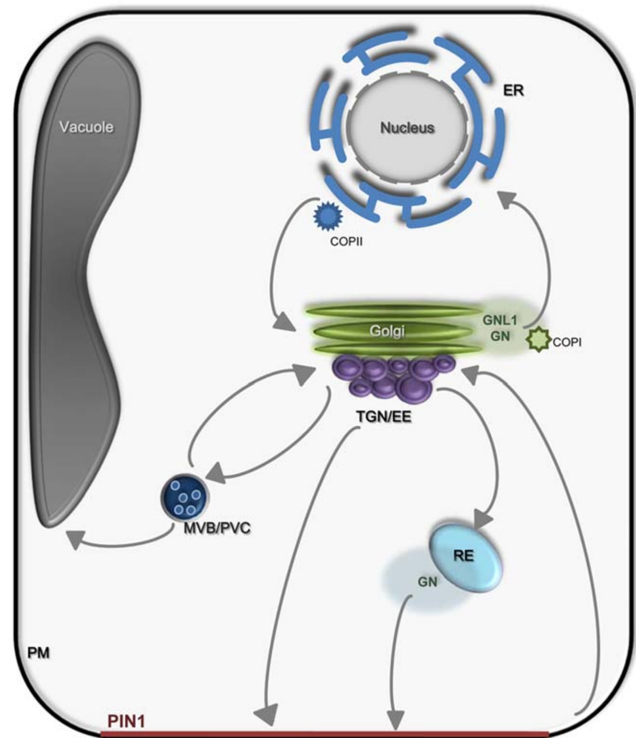

Fig. 4. Trafficking pathways regulated by GNOM and GNL1. Simplified scheme of vesicle trafficking pathways. Secretory and membrane proteins are synthesised at the ER (blue) and passed on to the Golgi apparatus (green) by anterograde trafficking in COPII-coated vesicles. The retrograde route from the Golgi apparatus to the ER is regulated by the ARF-GEFs GNOM (GN) and GNL1, which regulate the recruitment of COPI coats to the Golgi membrane. On the secretory route, proteins are transported to the sorting station, the trans-Golgi network (TGN; lilac). From there, proteins are either transported to the vacuole (grey) via multivesicular bodies (MVB; also called prevacuolar compartment, PVC, which corresponds to a late endosome; deep blue) or trafficked to the plasma membrane (PM). Plasma membrane proteins like the auxin-efflux carrier PIN1 (red), which accumulates at the basal PM at steady state, are continually internalised and trafficked to the TGN, which resembles the early endosome (EE) in plants. From the TGN, PIN1 is recycled to the plasma membrane via the recycling endosome (RE; light blue). This pathway is regulated by the ARF-GEF GNOM.

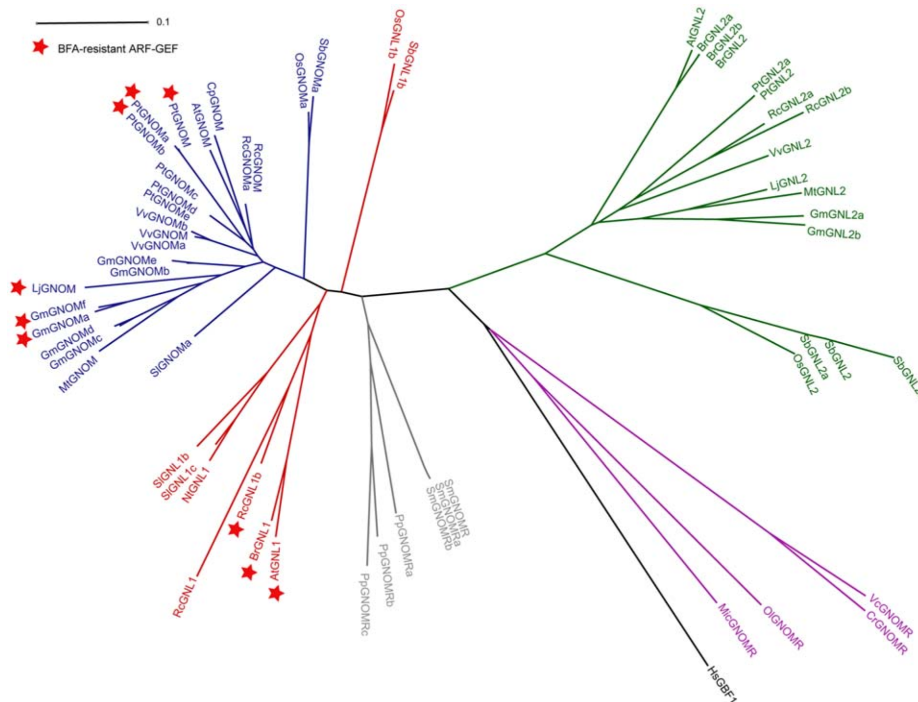

Fig. 5. Phylogenetic tree of GGG-type ARF-GEFs. The tree is based on the analysis of large ARF-GEFs from algae (*Chlamydomonas reinhardtii* (Cr), *Micromonas* spec. (Mic), *Ostreococcus lucimarinus* (Ol) and *Volvox carteri* (Vc)), lower plants (*Physcomitrella patens* (Pp), *Selaginella moellendorfii* (Sm)) and the higher plants *Arabidopsis thaliana* (At), *Brassica rapa* (Br), rice (Os), papaya (Cp), *Medicago truncula*ta (Mt), *Vitis vinifera* (Vv), *Lotus japonicus* (Lj), Sorghum (Sb), *Ricinus communis* (Rc), tomato (Sl), soybean (Gm) and poplar (Pt). Human GBF1 (HsGBF1) was used as outgroup. BFA resistance (red asterisk) or BFA sensitivity of ARF-GEFs was predicted on the basis of critical amino acid residues in the catalytic SEC7 domain (Peyroche et al., 1999; Geldner et al., 2003). Sequences were analysed with ClustalW, and dendroscope was used for generating the tree (Huson et al., 2007). Algae and lower plants only encode GNOM-RELATED (GNOMR) proteins but no orthologues of GNOM, GNL1 or GNL2.

traffic previously described in yeast and mammals (Fig. 4) (Casanova, 2007). Although GNOM can functionally replace GNL1, GNOM has not been clearly localised to Golgi stacks, possibly because its local concentration is at the limit of detection. The converse does not apply: GNL1 cannot replace GNOM, and PIN1 recycling and auxin-dependent processes such as gravitropism and lateral root initiation exclusively depend on GNOM activity whereas root growth requires secretory traffic and is jointly supported by GNOM and GNL1 (Fig. 4) (Richter et al., 2007).

An evolutionary aside

Large ARF-GEFs are conserved across eukaryotes (Anders and Jürgens, 2008). However, flowering plants have several isoforms of GGG-type ARF-GEFs, in contrast to animals, fungi and lower plants, which might suggest functional diversification or specialisation during evolution of the plant lineage (Fig. 5). Whereas only one member is found in lower plants there are (mostly) three functionally distinct members (GNOM, GNL1 and GNL2) in flowering plants (Richter et al., 2007).

Whether a large ARF-GEF is sensitive or resistant to BFA can usually be predicted from the occurrence of specific amino acid residues in the SEC7 domain, e.g. M696 in BFA-sensitive GNOM but M696L in engineered BFA-resistant GNOM (Geldner et al., 2003). Conversely, L696 confers BFA-resistance in GNL1 whereas L696M rendered GNL1 BFA-sensitive (Richter et al., 2007). If GNL1 were naturally BFA-sensitive the role of GNOM in endosomal recycling would have been very difficult to analyse. Whether a particular ARF-GEF or its orthologue in another plant species happens to be sensitive or resistant to BFA appears to be a freak of evolution (Fig. 5). For example, tobacco cells respond to BFA treatment by readily dissociating the COPI subunit γ-COP from the Golgi membrane (Ritzenthaler et al., 2002). This suggests BFA sensitivity of tobacco GNL1, which is consistent with M683 in the putative NtGNL1 (Wang et al., 2008). Thus, a comparable genetic analysis of GNOM in tobacco would have yielded a different result.

Model of GNOM function

GNOM appears to be a plant-specific, evolutionarily derived ARF-GEF that plays a non-redundant and thus essential role in endosomal recycling of PIN1 (and PIN2) to the basal plasma membrane. This cell-biological function forms the basis for (most of) the developmental role of GNOM in establishing apical-basal polarity during embryogenesis. It is important to note that a prerequisite for this developmental specificity of GNOM action is the existence of GNL1, which takes care of the ancestral function of GGG-type ARF-GEFs in Golgi-ER retrograde trafficking. In the absence of GNL1, *gnom* mutants would be gametophytic lethal, masking the specific role of GNOM in endosomal recycling. How GNOM performs this regulatory role mechanistically remains a challenge for the future. Specifically, identifying its ARF substrate(s) as well as the associated cargo-selective coat proteins might help to understand how GNOM distinguishes its derived endosomal recycling function from its ancestral Golgi-ER retrograde trafficking function.

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