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An *Arabidopsis* Minute-like phenotype caused by a semi-dominant mutation in a *RIBOSOMAL PROTEIN S5* gene

Dolf Weijers, Marry Franke-van Dijk, Robert-Jan Vencken, Ab Quint, Paul Hooykaas and Remko Offringa*

Institute of Molecular Plant Sciences, Leiden University, Clusius Laboratory, Wassenaarseweg 64, 2333 AL, Leiden, The Netherlands
*Author for correspondence (e-mail: offringa@rulbim.leidenuniv.nl)

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SUMMARY

Mutations in ribosomal protein (*RP*) genes in *Drosophila* lead to strong developmental phenotypes, expressed in the semi-dominant *Minute* syndrome. In plants, however, mutations in *RP* genes have so far only been reported to result in recessive developmental phenotypes. We present the analysis of an *Arabidopsis* promoter-trap line, in which a T-DNA insertion in an *RPS5* gene (*AtRPS5A*) causes semi-dominant developmental phenotypes. Most cell-division processes are delayed or disturbed in the heterozygous mutant, and development is completely arrested at an early embryonic stage in the homozygous mutant. By analogy with *Drosophila rp* mutants, we have named this mutant *Arabidopsis Minute-like 1* (*aml1*). As with other *Arabidopsis* RPs, RPS5 is represented by a small gene family, but in contrast to other described plant RPs, this family comprises only two members. The *AtRPS5A* gene (mutated in *aml1*) is strongly expressed in dividing cells, whereas expression of the second *RPS5* gene, *AtRPS5B*, is lower than that of *AtRPS5A*, and is correlated with cell differentiation rather than cell division. From expression analyses we conclude that *AtRPS5A* is the most abundantly expressed *RPS5* gene in *Arabidopsis*. The *Minute-like* defects in the *aml1* mutant provide the first evidence that ribosome insufficiency leads to similar consequences in both plants and insects, and emphasize the general importance of efficient protein translation for cell proliferation in higher eukaryotes.

Key words: *Arabidopsis*, Semi-dominant mutation, Cell division, Ribosomal protein, *Minute* syndrome, Haplo-insufficiency

INTRODUCTION

The carriers of protein translation in eukaryotic cells, the ribosomes, have a complex structure consisting of two subunits – the 60S (Large) and 40S (Small) subunit – that are each built from a considerable number of ribosomal proteins (RPs) and ribosomal RNAs (Moore, 1998). From one of the most well-studied models for ribosome structure and constitution, the rat, we now know that eukaryotic ribosomes consist of 82 different, highly conserved proteins (Wool et al., 1995).

The importance of the protein translation machinery in a given process can be studied by analysing the effect of a single *rp* mutation, as the absence of a single RP prevents assembly of the corresponding ribosomal subunit (Volarević et al., 2000). Notably, apart from expected defects such as growth arrest or delay, mutations in *RP* genes often result in specific defects in the development of an organism (Wool, 1996). In some cases, *RP* gene mutations affect DNA replication, RNA processing and DNA repair (Wool, 1996), suggesting roles for RPs that are additional to protein translation.

One of the most prominent examples of specific developmental defects due to *RP* gene mutations is the *Minute* syndrome in *Drosophila*. The *Minute* class of mutants was described, as early as in 1919 (Lambertsson, 1998), and today over 50 different *Minute* loci have been mapped. The *Minute* syndrome is characterised by semi-dominant phenotypes, such as delay in larval development, smaller body size and several pleiotropic morphological aberrations (e.g. short thoracic bristles), and recessive embryo lethality. Kongsuwan and co-workers (Kongsuwan et al., 1985) first showed that a *Minute* phenotype was caused by a deletion of a *RP* gene, and now, at least 11 *Minute* loci have been assigned to *RP* genes (Lambertsson, 1998), demonstrating that ribosome function is essential during specific stages of fly development and that individual RPs can easily become rate-limiting for various processes.

Only few mutations in *RP* genes have been described in the plant *Arabidopsis thaliana*. Two such mutations were identified during screens for either aberrant seedling phenotypes (van Lijsebettens et al., 1994) or altered sensitivity to genotoxic stress (Revenkova et al., 1999). A third *rp* mutant, identified through sequence analyses of *Ds* transposon insertion sites, also appeared to show specific defects in seedling development (Ito et al., 2000). Strikingly, all three of the above *rp* phenotypes were found to be recessive. Semi-dominant phenotypes, as described for the *Drosophila Minute* mutants, have not been reported in plants. This might be a consequence of the fact that *RP* genes are single copy in *Drosophila* (Kay and Jacobs-Lorena, 1987), while in *Arabidopsis* each RP is represented by a small gene family

(Cooke et al., 1997; Revenkova et al., 1999). Alternatively, plants may have evolved mechanisms to avoid the deleterious effect of ribosome insufficiency.

We report the identification and further characterisation of a semi-dominant mutation in an *Arabidopsis RPS5* gene. We named the mutant *Arabidopsis Minute-like 1* (*aml1*), because the semi-dominant growth retardation and floral and vascular defects and the recessive embryo lethality are analogous to defects observed in the *Drosophila Minute* mutants. Our findings show a striking similarity between the effect of ribosome insufficiency in flies and in plants and show that ribosomes can be limiting for plant growth and development.

MATERIALS AND METHODS

Plant material, plant growth and transformation

Arabidopsis thaliana ecotype C24 or Columbia seeds were surface sterilised by incubation for 1 minute in 70% ethanol, 15 minutes in 2% hypochlorite/0.01% Tween-20 and four rinses with sterile water. Seeds were imbibed for 2 to 4 days at 4°C in the dark before germination in regeneration rooms (21°C, 16 hour light/8 hour dark, 4000 lux) on solidified M-A medium (Masson and Paszkowski, 1992). After approximately 2 weeks, seedlings were transferred to soil and grown in growth chambers (21°C, 60% relative humidity, 16 hour light/8 hour dark, 4000 lux) for seed set. Ploidy levels were analysed by counting the number of plastids per guard cell in epidermal strips. The presence of the *826* T-DNA was selected by germination on hygromycin-containing medium (Duchefa, 20 mg/l). *Arabidopsis* plants were transformed with *Agrobacterium tumefaciens* using the 'floral-dip' method (Clough and Bent, 1998) with minor modifications. Transgenic plants were selected on medium containing 30 mg/l phosphinotricin in the case of p*AtRPS5A*::*GUS* and p*AtRPS5B*::G*US* or 75 mg/l kanamycin and 20 mg/l hygromycin in the case of the complementation construct.

Microscopy and histology

For whole-mount embryo analysis, siliques were slit open longitudinally on both sides of the septum with a hypodermic needle, fixed in a mixture of ethanol and acetic acid (3:1) for 1 hour and mounted in a drop of clearing solution (chloral hydrate: water: glycerol, 8:3:1). Embryos were viewed with a Zeiss Axioplan II microscope equipped with differential interference contrast (DIC) optics. For cytology, siliques, roots or flower buds were fixed overnight at 4°C in 4% paraformaldehyde in a 0.1 M sodium cacodylate-buffer (pH 7.2), rinsed twice in the same buffer and dehydrated through a graded ethanol series before embedding in Technovit 7100 (Heraeus Kulzer, Germany). Sections (4 μm) were stained with 0.1% Toluidine Blue. For localisation of GUS activity, tissues were fixed for 1 hour in 90% acetone at –20°C. Tissues were then washed twice for 5 minutes in washing buffer (0.1 M phosphate, pH 7.0, 10 mM EDTA, 2 mM $K_3Fe(CN)_6$) under vacuum. Subsequently, staining buffer (0.1 M phosphate, pH 7.0, 10 mM EDTA, 1 mM $K_3Fe(CN)_6$, 1 mM $K_4Fe(CN)_6{\cdot}3H_2O$, 1 mg/ml X-GLUC) was infiltrated by a brief vacuum treatment and specimens were incubated from 5 minutes (p*AtRPS5A*::*GUS* seedlings) to overnight (*aml1* mutant or *pRPS5B:GUS* seedlings) at 37°C. GUS-stained tissues were fixed in a 3:1 mixture of ethanol and acetic acid and cleared and mounted in a drop of clearing solution. All microscopic images were recorded using a Sony DKC-5000 digital camera, and compiled using Adobe Photoshop 5.5.

Measurements and morphometric analyses

For cotyledon- and root measurements, seeds of the hemizygous *aml1* mutant were germinated in a near-vertical position on MA medium without antibiotic selection. Four or 7 days after germination, seedlings were stained for GUS activity. Root and cotyledon size of GUS-positive and GUS-negative seedlings was measured from digital recordings using Scion Image software and statistical analysis (Student's *t*-test) was performed in Microsoft Excel. To quantify vascular patterning in the mutant, the same seedlings were observed under dark-field illumination.

Molecular cloning

Molecular cloning was performed following standard procedures (Sambrook et al., 1989). Inverse PCR (I-PCR) was performed on *Nco*I-digested chromosomal DNA from *826* hemizygous plants using PCR primers in the GUS gene as described by Offringa and van der Lee (Offringa and van der Lee, 1995). EST clones homologous to the I-PCR fragment sequence (Accession Numbers H35978 and N37914) were obtained from the Arabidopsis Biological Research Centre (Columbus, OH). For promoter isolation, 1.7 kb fragments containing the promoter region of the *AtRPS5A* or *AtRPS5B* gene were amplified by PCR and fused to the e*GFP:GUS::intron* reporter gene (Quaedvlieg et al., 1998) in pGPTV-BAR (Becker et al., 1992) to yield *AtRPS5A::GUS* and *AtRPS5B::GUS*. Primers for PCR were based on genome sequences deposited in the GenBank database. For complementation of *aml1*, we amplified a 4.2 kb region spanning the entire *AtRPS5A* gene, including 2 kb promoter sequence from C24 genomic DNA using the following primers: RPS5AcompF GCGCAGATCTGTAGACTGTTGCTTCTC and RPS5AcompR AGCAGGAGATCTATCAGTGCAGTCTG. The entire fragment was ligated into pMOG800 (MOGEN International N.V., Leiden, The Netherlands). To detect complementation, we used primers I (GCTCACCAACTCTCTCATGATGCACG), II (GAGTGTTGTATG-TACGTGTGTTTGACTTGG) and III (TGCCGTAATGAGTGAC-CGCATCG), and NPTII3 (TTGTCAAGACCGACCTGTCC) and NPTII6 (CACCATGATATTCGGCAAGC) in PCR reactions on DNA isolated from primary transformants with the MOG-*RPS5A* construct. Binary plasmids were transformed to *A. tumefaciens* strain LBA1115 by electroporation (den Dulk-Ras and Hooykaas, 1993). Sequence analysis was performed at Eurogentec (Belgium). DNA and protein sequences were analysed using the Vector NTI 5.5 software package (Informax, Bethesda, MD).

RT-PCR

Total RNA was extracted from 2-week-old seedlings according to Chang et al. (Chang et al., 1993). For RT-PCR, 10 μg of total RNA was treated with 50 U of DNAseI (Roche Biochemicals, The Netherlands) in DNAseI assay buffer (40 mM Tris-HCl, pH 7.5, 6 mM $MgCl_2$, 1 U RNAguard (Roche Diagnostics, The Netherlands) during 1 hour at 37°C. The RNA was then purified by chloroform extraction and precipitated (0.5 volume 7.5 M NH_4OAc, two volumes ethanol) at –80°C for 1 hour. The RNA pellet was resuspended in 9 μl RNAse free water and 1 μl oligo $d(T)_{15}$ (0.5 μg/μl, Roche Diagnostics, The Netherlands). After 5 minutes at 70°C, the RT mix (1× buffer concentration (supplied with enzyme), 0.5 mM dNTPs, 5 U MmuLV-RT (Roche Diagnostics, The Netherlands), 1 U RNAguard) was added and kept at 42°C for 1 hour. The cDNA was then purified by phenol/chloroform and chloroform extractions and precipitated with 0.4 volume 7.5 M NH_4OAc and two volumes ethanol. One-tenth of the total cDNA pool was used in all reactions. Fragments were amplified from these cDNA pools (or 10 ng C24 chromosomal DNA as a control for the primer pairs) using Taq polymerase (Roche Diagnostics, The Netherlands) according to the manufacturers procedures. Primer pairs were as follows: for *AtRPS5A*, 826g21F – CTCTCATTCGCGCGACGCAAACG and 826constR – GGGTT-CAAGTCAGACAAGAGGTGG; and for *AtRPS5B*, 826e11F – CGGCTAAAGATCCCTACTTCTCTCG and 826constR. PCR conditions were as follows: 4 minutes at 95°C, 30 cycles of 15 seconds at 94°C, 30 seconds at 60°C and 1 minute at 72°C, followed by 5 minutes at 72°C.

Whole-mount in situ hybridisation

In-situ hybridisation on 5-day-old seedlings was performed as described (J. Friml, PhD thesis, University of Cologne, 2000) with slight modifications. For auxin-induction, seedlings were treated with 50 μM 1-NAA during 16 hours in liquid M-A medium (Masson and Paszkowski, 1992) in the dark. To detect *RPS5A* mRNA specifically, a 150 bp fragment of the *AtRPS5A* cDNA was PCR-amplified from the cloned cDNA (RPS5A.F CGGATCCGTTTTCTCTGCCGTC and RPS5A.R GGAATTCGATGTCTGTGACCGTAACG) and cloned into pBluescript SK+ and KS+ vectors. Digoxigenin-labelled sense- or antisense RNA probes were synthesised using T7 RNA-polymerase (Promega, Leiden, The Netherlands) according the manufacturers procedures. For hybridisation we used 100 ng probe per ml hybridisation volume.

RESULTS

A promoter trap line that marks cell division

In an effort to identify new auxin-responsive genes, we screened a collection of transgenic *Arabidopsis* plant lines harbouring a promoter-trap T-DNA construct with the *GUS* gene as a reporter for promoter activity (Goddijn et al., 1993). Line 553-826 (hereafter referred to as line 826) was selected because seedlings showed auxin-responsive GUS activity in the root pericycle (R. O. and P. H., unpublished). In untreated seedlings, GUS activity was found in the apical meristems of both shoot and root (Fig. 1A-C), in lateral root primordia (Fig. 1D), young leaf primordia (Fig. 1B) and in the vascular tissue of cotyledons (Fig. 1E). This basal expression pattern was not enhanced by auxin (data not shown) and as auxin-induced expression was confined to a cell layer in which cell division is activated by auxin, we concluded that the *GUS* gene in the 826 line is regulated by a cell division-related, rather than by an auxin-specific pathway. This conclusion is supported by the fact that GUS activity was detectable in the most distal cells that represent the division zone of the root tip, but not in the mitotically inactive quiescent center (Fig. 1C) (Dolan et al., 1993).

The *826* T-DNA insertion is linked to a semi-dominant delay in embryo development, recessive embryo lethality and reduced gamete viability

Line 826 was tetraploid and contained four T-DNA loci. The line was backcrossed three times with the C24 wild type. A diploid plant, hemizygous for a single T-DNA insert (hereafter referred to as the *826* T-DNA), but which displayed the same GUS pattern as the original line, was selected. When seeds from this diploid 826 line were selected for the hygromycin resistance marker on the T-DNA, we observed a ratio of 0.62:1 resistant versus sensitive seedlings (n=1821). Because this ratio strongly deviated from the expected 3:1 ratio, we analysed embryo development to determine whether selective seed abortion contributed to the low number of seeds containing the *826* T-DNA. Embryo development clearly differed between wild-type and line 826 embryos. Most embryos in a wild-type silique appeared similar in developmental stage (data not shown). By contrast, three developmental classes of embryos were found in each silique of line 826. During early embryo development, siliques contained globular stage embryos, early dermatogen stage embryos, and two-cell stage embryos. Later, torpedo stage embryos, heart stage embryos and early globular stage embryos were found (Fig. 2A). Late siliques contained nearly mature embryos, bent-cotyledon stage embryos and arrested globular stage embryos (Fig. 2B,C). Cells of these arrested embryos were swollen (Fig. 2Aiii) and showed subcellular structures typical for mature embryos (Fig. 2D). Morphology of the endosperm appeared normal even in ovules that contained the most developmentally retarded class of embryos (data not shown), indicating that endosperm development is not severely affected in line 826.

To test if the arrested embryos in line 826 represent the individuals homozygous for the *826* T-DNA, progeny from 31 hygromycin-resistant plants were analysed for segregation of the T-DNA. All 31 plants segregated for the *826* T-DNA. Based on this, we concluded that the development of homozygous progeny did not progress to the seedling stage, but arrested prematurely during seed development. To determine linkage between presence of the *826* T-DNA and the mutant embryo phenotypes, GUS activity was analysed in embryos. Irrespective of the developmental stage, GUS activity was never detected in the most developmentally advanced embryos, whereas all GUS-positive embryos were retarded in their growth (Fig. 2E). Moreover, GUS activity in the most developmentally retarded class was always higher than in the intermediate class of embryos (compare Fig. 2Eii with 2Eiii), confirming the developmental separation of homo- and hemizygotes for the *826* T-DNA, and strongly suggesting that the T-DNA insertion in line 826 causes semi-dominant delay and recessive arrest of embryogenesis.

Recessive embryo lethality has been observed in several other *Arabidopsis* mutants (Patton et al., 1991; Castle et al., 1993; Yadegari et al., 1994; Uwer et al., 1998; Albert et al., 1999). Still, the 0.62:1 ratio observed in the hemizygous line 826 deviates strongly from the 2:1 segregation ratio expected in the case of embryo lethality. To determine if transmission of the *826* T-DNA to the next generation was affected by the viability of mutant gametes, we performed reciprocal crosses between the diploid, hemizygous 826 line and C24 wild-type plants, and scored for segregation of the T-DNA locus in the resulting progeny. When using 826 plants as a male parent, we found 15% of the progeny to be hygromycin resistant (n=41). In the case of full viability of the male gamete, one would expect this to be 50%. Thus, we conclude that the viability of pollen that carries the *826* T-DNA is decreased by approximately 70%. Using 826 plants as a female parent, we found 26% of the progeny to be hygromycin resistant (n=312). The viability of mutant female gametes is thus reduced by 50%. From these data the segregation ratio expected for a self-pollination was calculated to be 0.53:1 (resistant versus sensitive), which closely approaches the observed 0.62:1 ratio. The *GUS* gene in line 826 is expressed in pollen (not shown), implying that the decreased pollen viability is a result of the presence of the *826* T-DNA.

Semi-dominant defects during post-embryonic development in line 826

We noticed that the hygromycin-resistant progeny of the diploid, hemizygous 826 line showed a variety of semi-dominant phenotypes. Seedlings carrying the T-DNA, although variable in size (Fig. 1A), were generally smaller than wild-type seedlings. We observed an average 20% reduction in cotyledon length, 4 days after germination, and a 40%

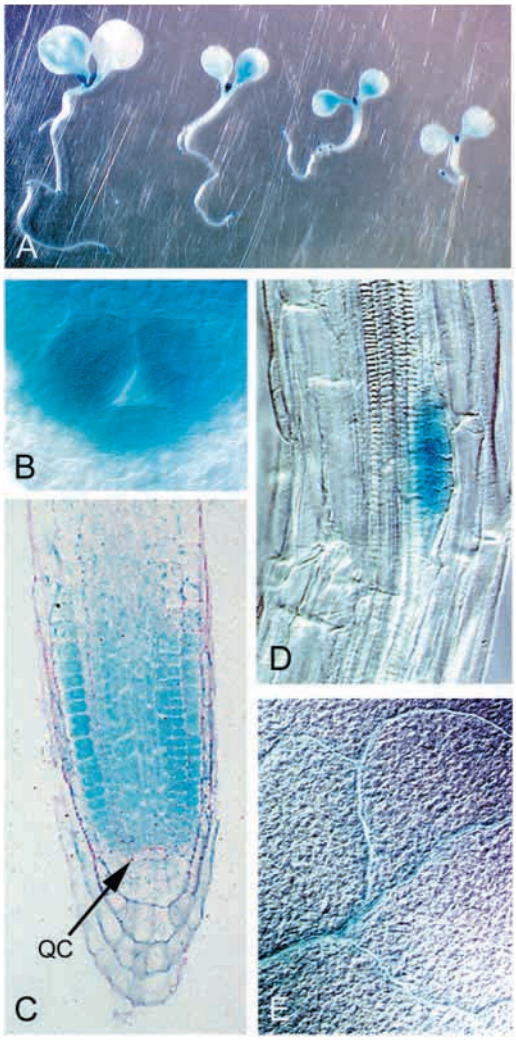


Fig. 1. GUS expression in the 826 line is confined to cell division foci. (A) Expression of the *GUS* gene in 826 seedlings is found in the apical regions of both shoot and root. (B) GUS activity is found in the SAM and leaf primordia, but not the surrounding tissues. (C) The division zone of the main root, but not the quiescent center (QC), is marked by strong GUS expression. (D) Lateral root primordia are marked by GUS activity. (E) GUS expression in the vascular tissue of cotyledons.

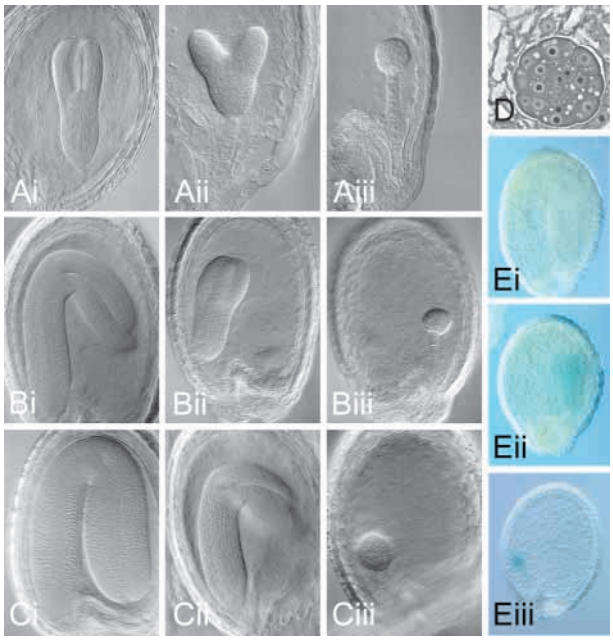


Fig. 2. Embryo development is delayed in 826 hemizygotes and arrests prematurely in 826 homozygotes. Whole-mount analysis of developing seeds from plants hemizygous for the *826* T-DNA. (A-C) Representative seeds from three siliques in subsequent stages of development. Indices (i-iii) indicate the different classes of embryos found in the same silique. When development of wild-type embryos is at the torpedo (Ai), bent-cotyledon (Bi) or mature stage (Ci), *826* hemizygous embryos have only reached the heart (Aii), torpedo (Bii) or bent-cotyledon stage (Cii). At all later time points, homozygous 826 embryos remain arrested at the globular stage (Aiii, Biii, Ciii). The arrested embryos display storage structures typical for late wild-type embryo development (D). Histochemical staining of seeds from the same silique reveals that the GUS activity correlates with the delay in embryo development. GUS expression is absent in mature embryos (Ei), whereas weak and strong expression is observed in respectively delayed torpedo stage (Eii) and arrested globular stage embryos (Eiii).

reduction of root length, one week after germination (Table 1). Hypocotyl length in 826 seedlings did not differ significantly from the wild type. It was shown that hypocotyl growth in seedlings is achieved by cell-elongation, and not by cell division (Gendreau et al., 1997). We therefore suspect that only cell division related processes are disturbed in 826 seedlings. We did not however, observe a significant difference in lateral root number (data not shown).

Cotyledons of the 826 line appeared rounded in shape (Figs 1A, 3A). To analyse the nature of this shape change, we studied venation patterns in mutant and wild-type seedlings in more detail. Vascular development in wild-type cotyledons is highly reproducible, resulting in a recognisable pattern (for a detailed description see Berleth et al. (Berleth et al., 2000) and references therein). Nonetheless, slight differences in developmental stages among individual seedlings cause stochastic variation of vascular pattern complexity (Fig. 3B). In 826 hemizygous seedlings, the vascular strands were often shorter and poorly interconnected (Fig. 3A), frequently leading to aberrant, simplified patterns. The lack of interconnections between xylem strands correlated with the absence of pro-vascular strands (data not shown), indicating a defect in cell division rather than vascular tissue differentiation. In general, vascular patterning was less complex in the *826* hemizygotes (Fig. 3B). The decrease in cotyledon size is correlated with a simplification of the vascular pattern. However, this does not answer the question of whether cotyledons are smaller due to incomplete vascular tissue development, or whether vascular tissue formation is compromised because cotyledons are smaller. In any case, the defect in vascular pattern correlates with the observed expression of the G*US* gene in the vascular tissue in cotyledons (Fig. 1E). Similar expression was also seen with the promoter of the *Arabidopsis CDC2A* gene, which marks cells competent for cell division (Hemerly et al., 1993). No obvious defects were observed in other leaf types such as rosette leaves, bracts, sepals, petals and silique valves.

Plants hemizygous for the *826* T-DNA developed normal

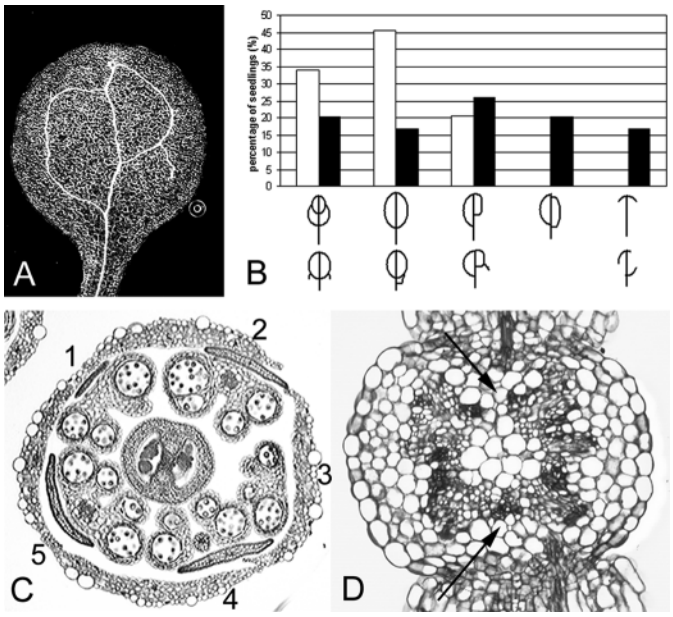


Fig. 3. Vascular and floral defects in hemizygous 826 plants. (A) Abnormal venation pattern in a cotyledon of a plant hemizygous for the *826* T-DNA. Note that the central vein is short and a secondary vein is interrupted. (B) Vascular pattern complexity in the most advanced cotyledon of wild-type (GUS negative, white bars) and hemizygous 826 (GUS positive, black bars) seedlings. The *y*-axis shows the percentage of seedlings falling into the categories represented on the *x*-axis. (C) Hemizygous 826 flowers carry only four to five stamens, as clearly shown in a cross-section of a flower bud from an 826 hemizygous plant. (D) The floral defect originates from the floral meristem, as indicated by the absence of one vascular strand (compare top with bottom arrow) in a cross-section through the receptacle of a young flower.

rosettes and flowering stems, although flowering appeared to be slightly delayed (not shown). The stamen number in mutant flowers was often reduced to four or five instead of six (5.2±0.7, *n*=37; Fig. 3C). The fact that vascular strands were missing in the receptacle (Fig. 3D) indicates that the phenotype can be traced back to the floral meristem, and is caused by a defect in primordium initiation rather than in primordium outgrowth. GUS expression in mature plants was observed in flower primordia but not in leaves, stems or mature floral organs. Thus, there is a striking correlation between the spatial *GUS* gene expression and sites where mutant phenotypes are observed.

Arabidopis Minute-like phenotypes caused by a mutation in a *RIBOSOMAL PROTEIN S5* gene

The 826 line provides a unique example of the coincidence of semi-dominant and recessive phenotypes due to defects in developmental processes that involve cell division. To understand the genetic lesion that affects such diverse developmental processes, we analysed the molecular nature of the *826* T-DNA insertion locus. Inverse-PCR was used to amplify a 450 bp fragment flanking the T-DNA insertion. Sequence analysis and database searches revealed that the *826* T-DNA had inserted into the fifth exon of a *RIBOSOMAL PROTEIN S5* (*RPS5)* gene. The last 11 codons of the *RPS5* gene were replaced by a spacer of the same length, resulting in a translational fusion between the RPS5 and GUS proteins (Fig. 4A,B). RPS5 is a structural component of the 40S small ribosomal subunit and is believed to function in binding of the eIF-3 (Westerman and Nygård, 1983; Tolan et al., 1983). To show unequivocally that the insertion of the T-DNA in the *RPS5* gene caused the mutant phenotypes, we transformed hemizygous 826 plants by floral dip with a T-DNA construct harbouring the wild-type *RPS5* locus, and used embryo abortion as an unambiguous phenotype to detect complementation. We first tested 96 individual hygromycin-resistant seedlings from the 826 line in a duplex-PCR for the presence of a wild-type locus and the T-DNA insertion in the *RPS5* gene (Fig. 4B). All of these 96 seedlings were hemizygous for the T-DNA insertion (data not shown) and hereby we confirmed our earlier conclusion that viable homozygotes are not recovered. As cells in the female germline are target for in planta *Agrobacterium* transformation (Desfeux et al., 2000), we reasoned that, in case of complementation, the introduction of a wild-type *RPS5* gene copy in a mutant female gamete, followed by fertilisation of this egg-cell by mutant pollen, should yield a viable embryo, homozygous for the *826* T-DNA insertion. In a population of 10 primary transformants that were selected for the presence of both the *826* T-DNA and the complementation construct, we found one that was homozygous for the *826* T-DNA, while the other nine were hemizygous (Fig. 4C). The complementation of the embryo-arrest phenotype by a wild-type *RPS5* copy and the strict correlation between the observed semi-dominant phenotypes and *RPS5:GUS* expression pattern lead us to conclude that the phenotypes in line 826 are caused by a T-DNA insertion in the *RPS5* gene. The mutant phenotypes caused by disruption of this *RPS5* gene in *Arabidopsis* are analogous to the situation in *Drosophila*, where mutations in *RP* genes result in the *Minute* syndrome. The *Minute* syndrome is characterised by

Table 1. Organ size of *Arabidopsis* wild-type and hemizygous 826 seedlings

		+/+ (GUS negative)		+/826(GUS positive)		
		Length (mm)±s.e.m.	*n*	Length (mm)±s.e.m.	*n*	Reduction (%)
Root length*	4 dag	3.46±0.16	43	3.06±0.12	46	11.6§
	7 dag	9.76±0.49	113	5.79±0.04	49	40.7§
Cotyledon length‡	4 dag	1.19±0.02	90	0.97±0.03	104	18.5§
Hypocotyl length	4 dag	2.22±0.05	45	2.17±0.07	52	–

GUS negative (+/+) and GUS positive (+/826) seedlings were measured after staining.
*Root length was measured in seedlings either 4 or 7 days after germination (dag).
‡Cotyledon length was measured in the proximal-distal axis of both cotyledons of each seedling.
§The means were significantly different when tested with Student's *t*-test ($P<0.05$).
n, number of measurements; s.e.m., standard error of the mean.

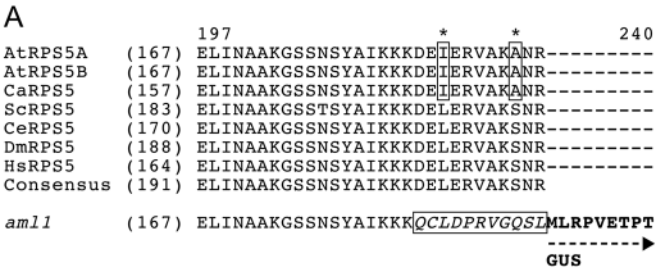
recessive embryo lethality, decreased gamete viability and several semi-dominant defects, such as delay in development and reduced body size (Lambertsson, 1998). The similarity between the *Drosophila Minute* mutants and our mutant line prompted us to rename our 826 line *aml1* for *Arabidopsis Minute-like 1*.

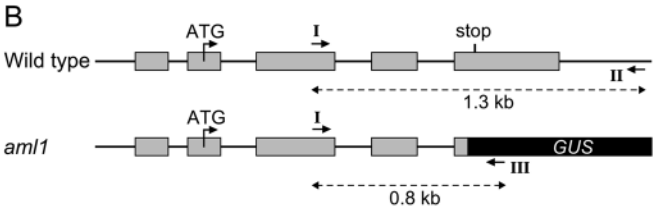
RPS5 is represented by a small gene family in *Arabidopsis*

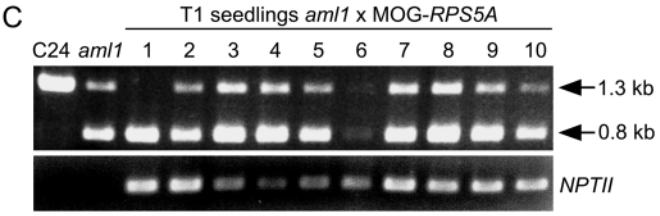
A search of the TIGR *Arabidopsis* gene index (http://www.tigr.org/tdb/agi) with the *AtRPS5* gene sequence revealed the presence of a second class of *RPS5* ESTs in *Arabidopsis*. The initially identified gene will be referred to as *AtRPS5A*, and the second copy will be referred to as *AtRPS5B*. We sequenced full-length cDNAs of both *RPS5* genes and found that they were 78% identical at the nucleotide level and contained an open reading frame of 621 bp, encoding a protein of 207 amino acids with a predicted molecular mass of 22.9 kDa. The two predicted proteins show 94% identity. Alignment of a number of RPS5 sequences from different organisms revealed many invariant amino acids, as well as a few plant-specific residues (Fig. 4A). Only two of the amino acid substitutions between the two family members are at residues that are otherwise conserved in eukaryotic RPS5 proteins, and the two proteins are thus very likely to be functionally redundant.

Southern analysis and a survey of the completed sequence of the *Arabidopsis* genome confirmed the presence of two gene copies in *Arabidopsis*. In the *aml1* mutant, a third hybridising band was observed, which represents the T-DNA insertion in *AtRPS5A* (not shown). The *AtRPS5A* gene resides on P1 clone MEC18 (Accession Number, AP002040) and maps at 18 cM on chromosome III. The *AtRPS5B* gene is located on BAC F3G5 (Accession Number, AC005896) and maps at 69.0 cM on chromosome II. No other mutants with similar phenotypes to *aml1* were found to map near these two loci.

The gene structure of the two *RPS5* copies is identical. Both contain five exons and four introns (ranging from 79-385 bp), the first of which is located 4 bp upstream of the translational start codon and the other three within the coding region (Fig. 4B). Both the sequence (49% identity) and length of the introns (Fig. 4D) are conserved between the two gene copies. The transcripts share a number of characteristics with mammalian RP transcripts (Wool et al., 1995). Three pyrimidine stretches are found within the 5′-UTR of the mRNA (at –34, –46 and –53 bp relative to the ATG in *AtRPS5A*; –29, –35 and –59 in *AtRPS5B*). The translation initiation context is G(T/C)CAUGG, the termination codon is UAA and both transcripts have an A/T-rich, poly-adenylated 3′-UTR (71% A/T in *AtRPS5A* and 68% A/T in *AtRPS5B*). This shows that not only the amino acid sequence, but also mRNA characteristics are strongly conserved between different







D

Exon	*AtRPS5A* (bp)	*AtRPS5B* (bp)	Intron	*AtRPS5A* (bp)	*AtRPS5B* (bp)
1	54	54	1	83	77
2	99	99	2	385	342
3	272	272	3	87	97
4	206	206	4	78	92
5	204	176			

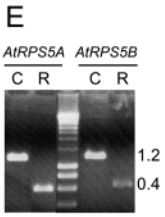


Fig. 4. Molecular characteristics of the *Arabidopsis RPS5* gene family. (A) Alignment of the extreme C terminus of RPS5 proteins from different species shows a strong conservation of primary structure. AtRPS5A, GenBank ID N37913; AtRPS5B, GenBank ID H35978; CaRPS5, *Cicer arietinum* (GenBank ID AJ005346); ScRPS5, *Saccharomyces cerevisiae* (GenBank ID X89368); CeRPS5, *Caenorhabditis elegans* (GenBank ID P49041); DmRPS5, *Drosophila melanogaster* (GenBank ID U48394); and HsRPS5, *Homo sapiens* (GenBank ID MN001009). The fusion of RPS5A and GUS in line 826 (*aml1*) is shown in the bottom row with the connecting border sequence in italics and the GUS sequence in bold. The number between brackets indicates the position in the protein relative to the N terminus. The asterisks indicate plant-specific residues. (B) Gene structure of *AtRPS5A* and the *aml1* allele. The *AtRPS5A* gene contains five exons, interrupted by four introns. Arrows I, II and III indicate the positions of PCR-primers. (C) Complementation of the *aml1* mutant with a wild-type *RPS5A* gene copy. The top panel shows the result of a duplex-PCR to detect the wild-type (1.3 kb) and *aml1* (0.8 kb) alleles of the *RPS5A* gene in seedlings. From left to right: C24 wild-type plants, the *aml1* mutant and ten independent primary transformants with the MOG-*RPS5A* construct. Plant 1 is homozygous for the *aml1* allele. Bottom panel shows the presence of the *NPTII* gene located on the MOG-*RPS5A* construct. (D) Intron and exon sizes (numbering is in the 5′ to 3′ direction of the genes) are extremely conserved between *AtRPS5A* and *AtRPS5B*. (E) RT-PCR analysis using *AtRPS5* and *AtRPS5B* gene-specific primer sets. *AtRPS5A* primers (left panel) amplify a 0.4 kb fragment from seedling RNA (R) to higher levels than do *AtRPS5B* specific primers (R, right panel). Control PCR reactions using chromosomal DNA (C) indicate equal efficiency of both primer pairs.

species. In both genes, a conserved motif is present directly upstream of the transcribed region that is referred to as the *telo* box. This box is found in many plant genes that are involved in protein synthesis and is thought to act in concert with the *Tef1* box to direct expression of genes to foci of cell division in plants (Regad et al., 1995; Tremousaygue et al., 1999). The *Tef1* box consensus sequence was found in the promoter sequence of the *AtRPS5A* gene. Only a weak similarity to the *Tef1* box consensus sequence is present in the promoter of *AtRPS5B*.

AtRPS5A and *AtRPS5B* are differentially expressed

Despite the presence of two expressed *RPS5* family members in *Arabidopsis*, a mutation in only one gene does result in semi-dominant *Minute*-like phenotypes. This suggests that *AtRPS5A* and *AtRPS5B* are differentially expressed and prompted us to compare the expression pattern of both genes.

RT-PCR analysis with primer sets specific for either of the genes revealed that *AtRPS5A* is expressed several-fold stronger than *AtRPS5B* in seedlings (Fig. 4E). The RT-PCR data are supported by the occurrence of 36 independent ESTs representing *AtRPS5A* and only 12 ESTs representing *AtRPS5B* in the *Arabidopsis* gene index (AtGI).

AtRPS5A expression

The strict correlation between the *Minute*-like phenotypes in the *aml1* mutant and the expression pattern of the *RPS5:GUS* fusion gene suggested that this fusion gene perfectly reproduces the expression pattern of the *AtRPS5A* gene. To confirm this, we performed whole-mount in situ hybridisation on wild-type seedlings using a probe specific for the *AtRPS5A* gene. The antisense probe detected a strong hybridisation signal in young leaf primordia (Fig. 5A,B) and the shoot apical meristem (Fig. 5B). Furthermore we found hybridisation signal in the vasculature of cotyledons (Fig. 5C). In roots we found strong signals in the distal tip (Fig. 5D,E). Upon closer inspection, the region that shows this signal appears to be the division zone of the root meristem. In accordance with the cell-division-correlated expression, the QC cells do not show a detectable signal (Fig. 5E). Upon treatment for 16 hours with the synthetic auxin 1-NAA we found hybridisation signals in lateral root primordia (Fig. 5F). As hybridisation using a sense control probe lacked the signals described above (Fig. 5G), these expression signals confirm the pattern of *RPS5:GUS* activity detected in the *aml1* mutant (Fig. 1; data not shown). We therefore conclude that the *RPS5A:GUS* gene expression genuinely reflects the expression of the *AtRPS5A* gene.

To address the spatiotemporal regulation of *AtRPS5A* gene transcription in the absence of the *aml1* mutation, we constructed a promoter::reporter gene fusion. GUS activity in p*AtRPS5A::GUS* seedlings could already be detected after 10 minutes of histochemical staining at the sites of cell division, i.e. in the shoot apical meristem, the youngest leaf primordia and the division zone of the root meristem (Fig. 6A,C). Strong expression was also found in lateral root primordia, where intense GUS staining was already visible during the first division of a single pericycle cell (data not shown). Weaker GUS activity was detected in non-dividing pericycle cells (Fig. 6B) that are considered to be competent for cell division (Hemerly et al., 1993; Doerner et al., 1996). Expression of GUS was also seen in all axillary meristems, floral meristems and flower primordia, and typically faded as organs matured. In general, the pattern of p*AtRPS5A::GUS* expression was identical to the *AtRPS5A* mRNA distribution pattern and to that of the *RPS5A:GUS* fusion in the *aml1* mutant (Figs 1, 5). Apparently, no *cis*-regulatory elements other than the promoter region are necessary to provide the complete *AtRPS5A* gene expression pattern.

AtRPS5B expression

As the gene structure of the two *AtRPS5* genes in *Arabidopsis* is highly homologous, we reasoned that, as with *AtRPS5A*, the promoter activity of *AtRPS5B* would reflect its mRNA expression pattern. As predicted by the RT-PCR analysis, *GUS* reporter gene expression in p*AtRPS5B::GUS* lines was much weaker than in p*AtRPS5A::GUS* lines. The sites of p*AtRPS5A::GUS* and p*AtRPS5B::GUS* expression partially overlapped, but while *AtRPS5A* is expressed in dividing cells (Figs 1, 5, 6A-C), *AtRPS5B* expression seems correlated with differentiation and elongation of cells in young growing tissues (Fig. 6D-F). Expression of *AtRPS5B* in the root was found in epidermal cells at the tip, several cells proximal from the initials (Fig. 6D), and was later observed in the trichoblast cell files and in root hairs (Fig. 6H), cell types where *AtRPS5A* expression is undetectable (Fig. 6G). In an in situ hybridisation experiment using a probe that recognised both mRNAs, we could reproduce this trichoblast-specific *AtRPS5B* expression (data not shown). Weaker expression of *pAtRPS5B::GUS* was found in developing lateral root primordia and in the shoot (Fig. 6F). Expression is absent from the pericycle layer (Fig. 6E), as opposed to that of p*AtRPS5A::GUS* (Fig. 6B). In contrast to *AtRPS5A*, expression of *AtRPS5B* was not observed in the SAM, but rather in the distal parts (and trichome cells) of young leaf primordia (Fig. 6F).

In the *pAtRPS5A::GUS* lines, GUS expression was found in the embryo from the zygote stage onward (Fig. 7A-E). GUS expression increased in strength until the octant stage and was strongly reduced after the late heart stage (Fig. 7B-E). Already during the first stages of embryo development, GUS expression was detected in the dividing endosperm (Fig. 7A-C). This activity decreased with the progression of endosperm development (Fig. 7C,D). *pAtRPS5B::GUS* lines showed only weak GUS expression at early stages of embryogenesis (Fig. 7G,H). During the transition to the heart stage, GUS activity increased and became preferentially localised to the inner cell layers, excluding the apical protoderm (Fig. 7I,J). During later stages, this pattern progressed, marking the inner cell types of the embryo axis and the cotyledons at late-torpedo stage (Fig. 7K). No GUS activity was found in the endosperm of *pAtRPS5B::GUS* lines until the globular stage of embryo development (Fig. 7G-J). The differential expression patterns of *AtRPS5A* and *AtRPS5B* in the embryo are schematically summarised in Fig. 7F.

GUS expression in both promoter::*GUS* lines was induced by treatment with IAA in the pericycle and in the newly formed lateral root primordia. Slight auxin-induction of both genes was confirmed in a micro-array experiment (B. van der Zaal, unpublished). The finding that lateral root initiation is marked by the expression of both gene copies explains why we did not observe a clear defect in lateral root formation in the heterozygous *aml1* mutant. GUS expression was not induced

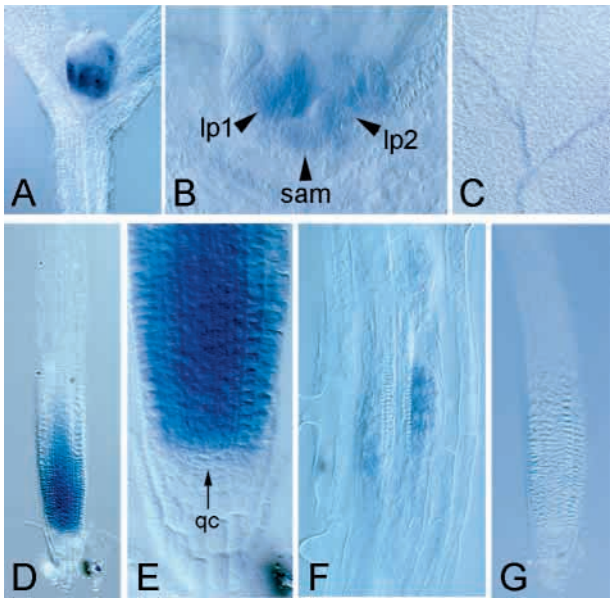


Fig. 5. Localisation of *AtRPS5A* mRNA by in situ hybridisation. Five-day-old wild-type seedlings were hybridised with an *AtRPS5A*-specific antisense (A-F) or sense (G) probe. Untreated seedlings (A-E,G) as well as NAA-treated seedlings (F) were used. Expression is detected in young leaf primordia (A). (B) A close-up of the apical shoot region of a seedling. Expression is confined to the leaf primordia (lp1 and lp2) and the shoot apical meristem (sam). A significant signal is detected in the vasculature of cotyledons (C), and the primary root tip (D). (E) A close-up of a root apical region. A strong signal is present in all dividing cells, but not in the quiescent center (qc). NAA-induced lateral root primordia stain strongly for the *AtRPS5A* signal (F). (G) The absence of a signal in a sense-probe control on an untreated seedling root.

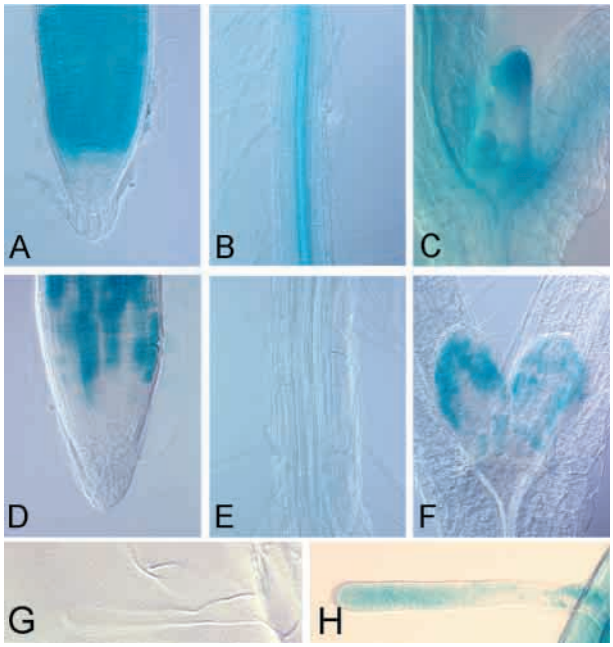


Fig. 6. *AtRPS5A* and *AtRPS5B* are differentially expressed in seedlings. Four-day-old seedlings from *pAtRPS5A::GUS* (A-C) and p*AtRPS5B::GUS* (D-F) plants were stained for GUS activity during 10 minutes and 2 hours, respectively. The *AtRPS5A* promoter is strongly active in the division zone of the primary root tip (A) and the SAM and leaf primordia (C), and is weaker in the pericycle of the root (B). p*AtRPS5B* is active in the epidermal cells of the primary root tip (D), in a zone more distal from the tip than p*AtRPS5A*. Activity is almost absent in the root pericycle (E), but present in lateral root primordia (not shown). GUS activity is also found in the distal, differentiating cells of the shoot primordium, but absent from the SAM (F). *AtRPS5B* (H), but not *AtRPS5A* (G) is expressed in root hairs.

by 1 or 10 μM zeatin, 10 μM NPA, or by mechanical wounding (data not shown).

DISCUSSION

A semi-dominant mutation in a plant ribosomal protein gene

Protein synthesis by the translational carriers, the ribosomes, is an essential feature of living cells. Mutations in genes encoding the structural components of ribosomes, the ribosomal proteins and ribosomal RNAs, have been described in various organisms. In addition to growth arrest or delay, these mutations often induce developmental aberrations (Wool, 1996). In *Drosophila*, such defects are expressed in the *Minute* syndrome, a semi-dominant phenotype affecting multiple developmental processes (Lambertsson, 1998).

Diverse mutant screens in *Arabidopsis thaliana* have identified mutations in several *RP* genes. The first to be identified was a recessive mutation in the *RPS18A* gene, which results in plants with pointed leaves (van Lijsebettens et al., 1995). The second *RP* gene to be identified by a mutation in *Arabidopsis* was the *AtRPS27A* gene. A recessive mutation in this gene caused plants to be more sensitive to a genotoxic stress treatment (Revenkova et al., 1999). Recently, Ito et al. (Ito et al., 2000) used a reverse genetics approach to identify a recessive mutation in the *AtRPS13A* gene. As with *AtRPS18A*, this mutation results in pointed leaves, and in addition, has impaired root growth and trichome branching.

Considering the importance of protein translation in general developmental processes and the fact that disruption of *RP* genes in *Drosophila* often results in semi-dominant phenotypes, the question arises why mutations in the *Arabidopsis RP* genes discussed above do not result in a semi-dominant phenotype. A possible explanation comes from the observation that in *Drosophila*, RPs are mostly represented by a single gene (Kay and Jacobs-Lorena, 1987). It was proposed that the *Minute* syndrome is caused by haplo-insufficiency (Sæbøe-Larssen et al., 1998), i.e. if one functional gene copy per diploid genome is insufficient for complete gene function, this results in a loss-of-function phenotype. Evidence for this interpretation comes from the finding that a number of *Minute* mutants were shown to have a 50% decrease of mRNA levels in the heterozygote, and that there is a proportional relationship between the amount of *RP* mRNA and the severity of the *Minute* phenotype (Sæbøe-Larssen and Lambertsson, 1996; Lambertsson, 1998). Additional support for the haplo-insufficiency hypothesis comes from experiments in which partial *Minute* mutants were obtained in *Drosophila* when the *RPA1* or *Rp49* mRNA was over-expressed in antisense orientation (Qian et al., 1988; Patel and Jacobs-Lorena, 1992).

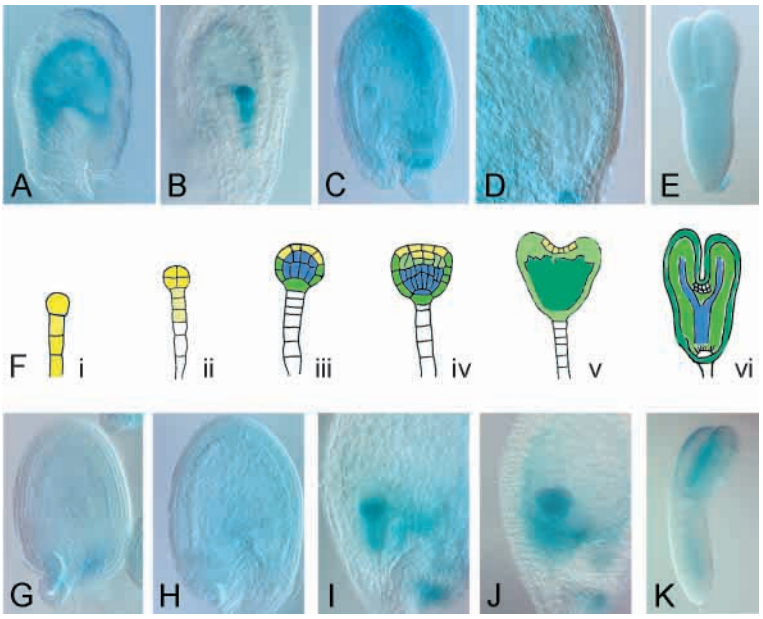


Fig. 7. The *AtRPS5A* and *AtRPS5B* genes are differentially expressed during embryogenesis. Siliques of *pAtRPS5A::GUS* (A-E) and *pAtRPS5B::GUS* (G-K) plants were stained for GUS activity for 2 and 8 hours, respectively. (A) In p*AtRPS5A::GUS* lines, GUS activity is already observed at the 2-cell stage in both embryo and endosperm. GUS activity remains strong through the octant (B) and globular stage (C), and decreases after the transition (D) and late torpedo stage (E). Activity is strongest in the peripheral cell layer (C-E). Expression of p*AtRPS5B::GUS* in embryos is weak until the globular stage (G,H), but increases during the late globular (I), heart (J) and late torpedo (K) stages. Note that expression is strongest in the inner cell layers (I-K), and is visible in the provascular tissue (K). (F) Schematic representation of the differential expression pattern of *AtRPS5A* (yellow) and *AtRPS5B* (blue). Places where both genes are expressed are marked with green. (i) 2-cell, (ii) octant, (iii) globular, (iv) transition, (v) heart and (vi) torpedo stage.

All three *Arabidopsis RP* genes for which mutants were previously described have at least two additional copies in the *Arabidopsis* genome (van Lijsebettens et al., 1994; Revenkova et al., 1999; Ito et al., 2000) and, at least for *AtRPS18*, there is a large overlap in the expression domain of the different gene copies (van Lijsebettens et al., 1994). Therefore, haplo-insufficiency is not likely to occur in mutants of these genes.

We describe the first mutation in an *Arabidopsis RP* gene, *AtRPS5A*, which results in semi-dominant phenotypes. In contrast to what has been previously concluded, based on EST data analysis (Cooke et al., 1997), we show that *AtRPS5A* belongs to a gene family that comprises only two members in *Arabidopsis*. Detailed analysis of the spatiotemporal expression of both family members by mRNA in situ hybridisation and promoter::*GUS* fusions shows clearly that the two gene copies are differentially regulated. The processes affected in the *aml1* mutant can only be seen in tissues/organs where *AtRPS5A* and *AtRPS5B* expression do not overlap. This closely resembles the situation in *Drosophila Minute* mutants, where for each RP only one functional gene is present in the genome.

Analogous to the situation in *Drosophila*, where mutations in the *RPS5* gene result in a *Minute* phenotype (McKim et al., 1996), our results indicate that the semi-dominant *aml1* phenotypes are caused by haplo-insufficiency. In the complementation experiment we obtained a plant homozygous for the *aml1* mutation, that harboured an extra wild-type gene copy. If the RPS5:GUS would have a dominant-negative effect, the complemented homozygous *aml1* mutant (ratio wild-type:mutant=1:2) would either be lethal, or at least have a more severe phenotype than the hemizygous mutant (ratio wild-type:mutant=1:1). Instead, this plant looked phenotypically similar to the hemizygote (data not shown) and did not grow less vigorously, implying that the semi-dominance of the *aml1* mutation is caused by haplo-insufficiency rather than by dominant-negative effects.

Differential expression of the RPS5 gene copies in *Arabidopsis*

To date the expression of only two plant gene families encoding RPs has been thoroughly analysed. *RPL16* is represented by three gene copies in *Arabidopsis*, one of which shows strong expression in all proliferating tissues, while another is expressed in more specific cell types (Williams and Sussex, 1995). The *AtRPS18* gene family comprises three members, two of which are strongly expressed in a similar pattern (van Lijsebettens et al., 1994). Interestingly, at least one copy of the *AtRPS18* and *AtRPL16* genes shares an almost identical gene expression pattern with *AtRPS5A*. For the *AtRPS5* gene family, *AtRPS5A* can be considered as the major gene copy, which, like *AtRPL16B* and *AtRPS18A* (*PFL1*), is expressed in proliferating cells, i.e. in meristems, pericycle cells and embryos. A single transcription factor or a family of transcription factors with the same DNA-binding specificity could well regulate this common expression. For example, the co-ordinate expression of *RP* genes in yeast is thought to be mediated by RAP1 and ABF1, transcription factors that contain binding sites in the promoters of these genes (Mager and Planta, 1990). One candidate for such a motif in plants is the *Tef1* box. This box was found in the promoter of *AtRPS5A*, while only a weak resemblance to the consensus sequence was found in *AtRPS5B*. The same motif is also present in the promoters of *AtRPS18A* (*PFL1*) (van Lijsebettens et al., 1994), *AtRPL16A* (Williams and Sussex, 1995) and *AtRPL16B* (D. W. and R. O., unpublished) and in a number of other promoters of genes that code for proteins that contribute to the translational apparatus (Regad et al., 1995). Regad et al. (Regad et al., 1995) have shown binding of proteins from nuclear extracts to the *Tef1* box. However, to date no further studies have been published concerning the nature of such a *Tef1* box-binding protein. Considering the exact stoichiometry of ribosome biosynthesis, it is very likely that specific plant transcription factors act coordinately to regulate the expression of at least one copy of each ribosomal protein gene family. Such transcription factors might act as master switches to increase- or decrease the translational status of a cell. Considering the importance of this process in development, the identification and modulation of such master switches should provide tools for the modification of plant growth and stature. Similarly,

enhanced expression of the mitotic cyclin CYC1At in *Arabidopsis* has been shown to accelerate growth and increase biomass (Doerner et al., 1996).

The *Arabidopsis* Minute-like phenotype

The delay in development in *Drosophila Minute* mutants has been shown to be due to a cell-autonomous reduction of the rate of cell division (Morata and Ripoll, 1975). In our studies, *aml1* phenotypes are associated with disturbed cell division rather than with reduced cell growth. One of the best examples is that the only part of the seedling that is not decreased in size in the *aml1* mutant is the hypocotyl. Indeed, it has been shown that elongation growth of the hypocotyl is not dependent on cell division (Gendreau et al., 1997). By analogy, in mouse it was shown that a functional knockout of the *RPS6* gene affected cell division, but not cell expansion (Volarević et al., 2000).

In embryos that are homo- or heterozygous for the *aml1* mutation cell division respectively stops or is delayed. Further, the semi-dominant post-embryonic *aml1* phenotypes, combined with prominent expression of *AtRPS5A* in the root meristem, in cotyledon primordia and the vascular tissue of cotyledons and in floral organs, and the low levels or absence of *AtRPS5B* expression in these organs, indicate that the *aml1* mutation affects cell division.

Expression of *AtRPS5B* is detected in the embryo, but it is significantly weaker than that of *AtRPS5A*. Apparently, the expression of *AtRPS5B* does not supply sufficient RPS5 protein to compensate for the presence of the mutant *aml1* allele, even in the heterozygous state. Strikingly, the homozygous *aml1* embryo still reaches the globular stage of development in the absence of proper RPS5 expression. It is possible that weak expression of *AtRPS5B* until the globular stage may partially rescue the loss of *AtRPS5A* expression. However, even the significantly stronger *AtRPS5B* expression during the globular stage is not able to rescue the *aml1* embryo, making it more likely that the embryo develops until the globular stage because of maternal RPs that are deposited in the egg cell. This explanation is in accordance with the large number of *Arabidopsis* embryo mutants that arrest at the globular stage (Patton et al., 1991; Castle et al., 1993; Yadegari et al., 1994; Uwer et al., 1998; Albert et al., 1999), some of which are also disrupted in housekeeping genes. The analysis of RP accumulation in the gametophyte and embryo is needed to address this question. Preliminary data show that the *AtRPS5B* gene is strongly expressed in the egg cell before fertilization (D. W. and R. O., unpublished).

To our knowledge, this is the first report describing an *Arabidopsis* mutant with a semi-dominant delay in embryo development. In this respect, it will be interesting to determine the *AtRPS5B* mutant phenotype. Based on the differential expression pattern of the two *AtRPS5* gene copies, we predict that a mutation in this *AtRPS5B* gene will lead to recessive phenotypes related to delayed cell growth and differentiation, including the differentiation of root hairs and trichomes.

Conclusion

The analysis of the *Arabidopsis aml1* mutant reveals a striking similarity between plant and insect development in that both systems have an absolute need for a functional and efficient translational machinery. This again shows that basic cellular processes are extremely conserved in species that are evolutionary distant, and that the research on plant cellular processes may provide essential information on the biology of other complex multicellular organisms. Our results show for the first time that expression of genes encoding components in the translational machinery can be rate limiting for plant development. The coordinate expression of such genes may be an interesting target to improve regeneration or plant stature in agronomically important crops.

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