



RESEARCH PAPER

BIGYIN*, an orthologue of human and yeast *FIS1* genes functions in the control of mitochondrial size and number in *Arabidopsis thaliana

Iain Scott, Alyson K. Tobin and David C. Logan*

School of Biology, Sir Harold Mitchell Building, University of St Andrews, St Andrews, Fife KY16 9TH, UK

Received 3 October 2005; Accepted 13 December 2005

Abstract

Reverse-genetics was used to evaluate the role of an *Arabidopsis* homologue of the human and yeast *FIS1* genes, which are both involved in mitochondrial fission. Two independent T-DNA insertion mutants of gene At3g57090 were identified and genetically transformed to express mitochondria-targeted GFP to enable visualization of mitochondria *in vivo*. Plants homozygous for either of the recessive T-DNA mutant alleles, termed *bigyin1-1* (*bgy1-1*) and *bigyin1-2* (*bgy1-2*), displayed an abnormal mitochondrial morphology. Disruption of *BIGYIN* leads to a reduced number of mitochondria per cell, coupled to a large increase in the size of individual mitochondria, relative to wild-type. It is concluded that *BIGYIN* is an *Arabidopsis* *FIS* orthologue and is part of the *Arabidopsis* mitochondrial division apparatus.

Key words: Dynamin, *FIS1*, mitochondria, mitochondrial division, morphology, organelle fission.

Introduction

Mitochondria undergo continual cycles of division and fusion. These two antagonistic processes, which operate concurrently, regulate the number, size, and shape of mitochondria in a cell (Sesaki and Jensen, 1999). The process of division is of particular importance since mitochondria cannot be created *de novo*, and must therefore be formed by the division of an existing organelle. In yeast and humans, several of the genetic components that control mitochondrial division and fusion have been identified and their function elucidated (for a review see Logan, 2003; Okamoto and Shaw, 2005).

For example, in budding yeast (*Saccharomyces cerevisiae*) three genes encode the primary components of the mitochondrial division machinery. *DNM1* encodes a protein that is structurally similar to the dynamin-related GTPase proteins involved in membrane scission during endocytosis (Hermann *et al.*, 1997; Otsuga *et al.*, 1998). Yeast Dnm1p is targeted to mitochondrial division sites and is believed to act as a mechano-enzyme, constricting and/or severing the mitochondrial membranes. This dynamin-related protein requires two other interacting proteins to effect yeast mitochondrial division. Firstly, Mdv1p which, like Dnm1p, localizes to the outer mitochondrial membrane at division sites (Fekkes *et al.*, 2000; Mozdy *et al.*, 2000; Tieu and Nunnari, 2000; Cerveny *et al.*, 2001). Secondly, Fis1p which is evenly distributed across the mitochondrial outer surface (Mozdy *et al.*, 2000; Tieu and Nunnari, 2000). These three proteins form a complex on the yeast outer mitochondrial membrane and function in concert to effect division of the organelle (Cerveny and Jensen, 2003). All three genes are required for effective mitochondrial division in yeast, since knocking out any one of the three leads to a similar abnormal mitochondrial phenotype (a net-like sheet of mitochondrial tubules). In humans, normal mitochondrial division requires the dynamin-related *DRP1* and *hFIS1*, which function analogously to their yeast orthologues, *DNM1* and *FIS1* (Smirmova *et al.*, 2001; James *et al.*, 2003; Youle and Karbowski, 2005). Searching the databases using the BLAST algorithm fails to identify cognate homologues of *MDVI* in multicellular organisms: limited protein level similarity is restricted to members of the WD-40 repeat family of proteins (Logan, 2003; Stojanovski *et al.*, 2004).

Research into the mitochondrial division apparatus in higher plants is at a rudimentary stage. Recently, it has been shown that two non-redundant *Arabidopsis thaliana*

* To whom correspondence should be addressed. E-mail: david.logan@st-andrews.ac.uk

Dnm1p/DRP1 homologues are involved in mitochondrial division. Disruptions in either DRP3A or DRP3B lead to an altered mitochondrial phenotype where the organelles are both larger and fewer in number, suggesting both proteins are required for the normal division of mitochondria (Arimura and Tsutsumi, 2002; Arimura *et al.*, 2004; Logan *et al.*, 2004). The identification of two mutant alleles of an *Arabidopsis hFIS1/FIS1* orthologue (At3g57090) is reported here. These mutants, which were named *bigyin1-1* and *bigyin1-2*, have an aberrant mitochondrial phenotype characterized by an increase in the size of individual mitochondria and a concomitant decrease in the number of mitochondria per cell. These results demonstrate that *BIGYIN* is an *Arabidopsis hFIS1/FIS1* orthologue and is the first member of only the second protein family known to be involved in plant mitochondrial division.

Materials and methods

Plant materials and growth conditions

Searches of the *Arabidopsis* genome, using the BLASTP algorithm (Gish and States, 1993) and yeast Fis1p and human hFis1 protein sequences, identified two homologues: At3g57090 (yeast 7e-7, human 6e-8) and At5g12390 (yeast 7e-6, human 2e-8) (Logan, 2003). The SAIL (Sessions *et al.*, 2002) and SALK (Alonso *et al.*, 2003) T-DNA insertion-line databases were searched to identify independent lines with an insertion in At3g57090. SAIL_1171_G11 is predicted to contain a T-DNA insertion in the first exon (8e-45) and SALK_086794 is predicted to contain an insertion in the final (fifth) exon (5e-15) (Fig. 1). SAIL seed was obtained from Syngenta Biotechnology Inc. (Research Triangle Park, NC, USA) and SALK seed from the Nottingham *Arabidopsis* Stock Centre (NASC, University of Nottingham, UK). Mitochondrial-GFP wild-type (Col-0 background) seed used in the study was of line 43C5 (Logan and Leaver, 2000). Seeds were surface-sterilized and germinated on MS-agar plates (Murashige and Skoog, 1962) containing 8% (w/v) agar (Type M, Sigma Chemical Co., St Louis, USA), 2% (w/v) sucrose, and 0.5% (w/v) MES pH 5.8. For maximum synchronous germination, plates were kept in the dark at 4 °C for 3 d before transfer to a controlled environment growth room (16/8 h day/night, 25 °C). After a further 2 weeks, seedlings were transplanted to compost (Levington F2, Scotts, Marysville, USA) and grown in a greenhouse under supplementary lighting and constant 25 °C.

GFP transformation

Bulked seed from each insertion line were sown on a 2:1 mixture of compost/vermiculite in separate pots and germinated in the green-

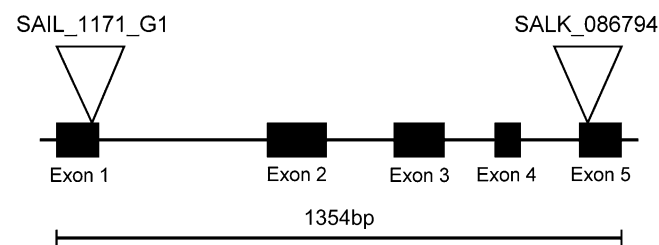


Fig. 1. T-DNA insertions in the coding sequence of *BIGYIN*. The SAIL_1171_G11 (*bigyin1-1*) allele is predicted to contain a T-DNA insertion in the first exon of *BIGYIN*, while the SALK_086794 (*bigyin1-2*) allele is predicted to contain an insertion in the final (fifth) exon.

house. At 3-weeks-old, these plants were genetically transformed to express mitochondria-targeted GFP (mito-GFP) using the *Agrobacterium* floral dip method (Clough and Bent, 1998). SAIL_1171_G11 was generated using pCSA110 (Sessions *et al.*, 2002) conferring resistance to glufosinate ammonium (BASTA) *in planta* and so was transformed with the mito-GFP vector pBINmgfp5-atpase, which confers resistance to kanamycin (Logan and Leaver, 2000). SALK_086794 was generated with pROK2 (Alonso *et al.*, 2003) conferring resistance to kanamycin *in planta* and so was transformed with the mito-GFP vector pMLBARTmgfp5-atpase, which confers resistance to BASTA (Logan *et al.*, 2004). Mito-GFP T₁ seedlings of SAIL_1171_G11 were first selected by germination on MS-agar plates containing 50 mg l⁻¹ kanamycin, while mito-GFP T₁ seed of SALK_086794 were sown on compost and the seedlings sprayed twice with 120 mg l⁻¹ BASTA (when 2 and 3-weeks-old). Resistant seedlings were screened by epifluorescence microscopy for expression of mito-GFP.

PCR analysis of putative insertion mutants

Both SAIL and SALK lines contain T-DNA insertions at known sites in the *Arabidopsis* genome and the SIGnAL iSECT tool (<http://signal.salk.edu/isects.html>) creates custom gene-specific forward and reverse PCR primers to check the nature of an insertion (absent, hemizygous, homozygous) in any individual plant. For SAIL_1171_G11, gene-specific forward (5'-GTAAACATC-TAATCGTGAAAGAT-3') and reverse (5'-TTCCAAGCAGAA-CACGAAAAC-3') primers flanking the insertion site were used in combination with a primer to the T-DNA left border (LB3 5'-TAGCATCTGAATTCATAACCAATCTCGATACAC-3') to confirm the nature of the insertion (Sessions *et al.*, 2002). For SALK_086794, the gene-specific LP (5'-TTCGTTGACTTGGC-CATTTAC-3') and RP (5'-ATCGAGGTT-TCATCCACTTC-3') primers were used in conjunction with the T-DNA left border primer LbB1 (5'-GCGTGGACCGCTTGCTGCAACT-3') (Alonso *et al.*, 2003).

Microscopy and image analysis

GFP-positive plants were examined using either an Olympus BX-40 (Olympus Optical Co., UK) or Zeiss Axioskop 2 (Carl Zeiss Ltd., UK) epifluorescent microscope fitted with cubes for GFP (Olympus model 41001, excitation 455–495 nm, emission 510–555 nm; Zeiss model 488013, excitation 470–520 nm, emission 505–530 nm). Visualization of mitochondria was performed at ×1000 using an oil-immersion objective (Olympus ×100 Universal Plan Fluorite, numerical aperture=1.3; Zeiss ×100 Plan-Apochromat, numerical aperture=1.4). Epifluorescent micrographs were captured using a monochrome digital camera (F-View, Soft Imaging System GmbH, Munster, Germany) coupled to a PC running the analySIS software package (Soft Imaging System GmbH, Germany) for image analysis and storage.

Protoplasts were isolated from 2-week-old seedlings under aseptic conditions. Briefly, leaf blades were dissected, chopped, and incubated overnight at room temperature in a standard enzyme solution (0.33% w/v cellulase, 0.17% w/v pectinase, 3 mM MES, 7 mM CaCl₂ in 0.4 M mannitol). The digested material was sequentially filtered through 100 μm and 40 μm nylon mesh, harvested at 50 g and resuspended in 0.5 M mannitol to an approximate concentration of 1 × 10⁶ protoplasts ml⁻¹. Aliquots of the protoplast suspensions were mounted on a microscope slide under a glass cover-slip immediately prior to microscopy. Single images were captured of each of 25 intact protoplasts, chosen at random, from each of the three experimental lines. The plan area of individual mitochondria and their number per protoplast-field-of-view were measured in mito-GFP-positive wild-type, SAIL_1171_G11 and SALK_086794 protoplasts using the analySIS software package.

Results and discussion

Arabidopsis homologues of yeast FIS1 and human hFIS1

The *Arabidopsis thaliana* genome contains two homologues of the yeast *FIS1* and human *hFIS1* genes. Analysis of their protein sequence using the EMBOSS pairwise alignment program (<http://www.ebi.ac.uk/emboss/align/index.html>) shows that At3g57090 shares highest similarity with hFis1 (26.7% identity, 48.3% similarity) and other *FIS1*-type genes from multicellular organisms, such as the *Caenorhabditis elegans* *FIS-2* gene (locus NM_001029389) (Fig. 2). Conversely, At5g12390 shares highest similarity with yeast Fis1p (27.0% identity, 43.8% similarity) (Fig. 2). As the *Arabidopsis* gene symbol *FIS* is already in use, At3g57090 was named *BIGYIN*, on the basis of the mitochondrial phenotype in the T-DNA mutants.

In silico analysis of the protein structure of *BIGYIN* or At5g12390 using InterProScan (<http://www.ebi.ac.uk/cgi-bin/iprscan/iprscan>) reveals a conserved tetratricopeptide repeat (TPR)-like binding domain (residues 51–139 of *BIGYIN*), common to all Fis1-type proteins (Suzuki *et al.*, 2003, 2005). In addition, all Fis1-type proteins contain a single C-terminal putative transmembrane domain (residues 142–164 in *BIGYIN*) with a topology predicted to leave the N-terminal region exposed to the cytoplasm (Mozdy *et al.*, 2000; Tieu and Nunnari, 2000; Suzuki *et al.*, 2003; Yoon *et al.*, 2003). The C-terminal structure of hFis1 has been demonstrated to be essential for mitochondrial localization: the transmembrane region is located

within the outer-mitochondrial membrane with the C-terminal tail localized in the intermembrane space (Yoon *et al.*, 2003). The cytosolic N-terminal region of hFis1, containing the conserved TPR motifs, has been shown to participate in the interaction with the dynamin-like protein, DLP1, or a DLP1-containing complex (Yu *et al.*, 2005).

The second *FIS1/hFIS1* homologue in *Arabidopsis* (At5g12390) sets it apart from yeast and humans, which contain only a single copy of a *FIS1*-type gene. However, the presence of multiple homologues of mitochondrial division genes appears to be a feature of the *Arabidopsis* genome. For example, while yeast, humans, and nematodes have a single dynamin-related gene involved in mitochondrial fission, there are at least two dynamin-related genes associated with this function in *Arabidopsis* (Arimura and Tsutsumi, 2002; Arimura *et al.*, 2004; Logan *et al.*, 2004). A similar analysis of the role of At5g12390 in mitochondrial fission is currently hampered by the lack of T-DNA mutants of this gene. Future research using alternative reverse genetics approaches will focus on the role of At5g12390 in mitochondrial dynamics and should reveal any redundancy between the two *Arabidopsis* Fis1-type genes.

There are fewer, but larger, mitochondria in the bigyin mutant

Mitochondria in yeast *FIS1* gene knockouts do not have a normal branched-tubular structure, but instead form a mitochondrial net. A similar phenotype was observed in knock-downs of *hFIS1* in COS-7 cells, although some mitochondria formed extended tubules. These phenotypes are thought

BIGYIN	M---D--AKIGQFFDSVGTFFSGS-----DKIPWCDGDVIAGCEREVREATDSGTEDLKK
at5g12390	M---D--AAIGKVFDSVSDFFSFGAASASADEFPPLCSDIISGCE---KELAEAQDEGRKK
Fis1p	MTKVDFWPTLKDAYEP-----LYP---QQLLEIL---RQQVVSEGGP-TATI
hFis1	M-----EAVLNELVSV-----EDLLKFEK---KFQSEKAAGSVSK
FIS-2	M---DYGTILEERTNP-----AVLMNARE---QYMRQCARGDPSA
	* . : . . : : : .
BIGYIN	ECLMRLSWALVHSRQTEDVQRGIAMLEASLESSAPPLEDREKLYLLAVGYRSGNYSRSR
at5g12390	ECIMRLSWALVHSMKPSDIQRGIAMLEALVVNDTSAMKLRKLYLLALGYRSGDFRSR
Fis1p	QSRFNWGLIKSTDVNDERLGVKILTDIYKEAES--RRRECLYYLTIGCYKLGEYSMAK
hFis1	STQFEYAWCLVRSKYNDDIRKGI VLLLEEL-PKGSKEEQRDYVFLAVGNRYLKEYEKAL
FIS-2	ASTFAFAHAMIGSKNKLDVKEGIVCLEKLLRDEEDRTSKRNVVYVYLAHAHARIKQYDLAL
	: : :: * * : * : * * : * : : * : : : : : :
BIGYIN	QLVDRCIEMQADWRQALVKKTIEDKITKDGVIIGITAT-AFGAVGL---IAGGIVAAM
at5g12390	DCIERCLEVEPEGQAQALKKAIEDRIVKDGVIIGIIVT-AVGVVAG---IAAAILRS-
Fis1p	RYVDTLFEHERNNKQVGALKSMVEDKIQKETLKGVVVAGGVLAVAVASV----F----L
hFis1	KYVRGLLQTEPQNNQAKELERLIDKAMKKDGLVGMIVGGMALGVAGLAGLIGLAVSKSK
FIS-2	GYIDVLLDAEGDNQQAATLKESIKSAMTHDGLIGAAIVGGGALALAGLVAI-----FSM
	: : : : * . * : : : : : * : . . .
BIGYIN	SRKK-
at5g12390	----
Fis1p	RNKRR
hFis1	S----
FIS-2	SRK--

Fig. 2. Sequence homology between *BIGYIN* and other FIS-1-type proteins. *BIGYIN* has a higher sequence similarity to the FIS-1-type proteins from multicellular organisms, such as human (hFis1) and *Caenorhabditis elegans* (FIS-2), than to yeast (Fis1p). The regions of highest similarity represent the TPR-like binding domain (residues 51–139 in *BIGYIN*) and the C-terminal transmembrane domain (residues 142–164 in *BIGYIN*).

to result from reduced mitochondrial fission caused by an inability to recruit Dnm1p/Drp1 (Mozdy *et al.*, 2000; Tieu and Nunnari, 2000; Stojanovski *et al.*, 2004). To determine if BIGYIN is a functional orthologue of Fis1p/hFis1, the mitochondrial phenotype of two independent homozygous T-DNA mutants was analysed, SAIL_1171_G11_mito-GFP and SALK_086794_mito-GFP, bearing insertions within locus At3g57090 (Fig. 3). The mean mitochondrial plan area in wild-type plants was $0.366 \mu\text{m}^2 \pm 0.003 \text{ SE}$ ($n=1793$; Fig. 3B). In plants homozygous for either the SAIL or SALK mutant allele, named *bigyin1-1* (*bgy1-1*) and *bigyin1-2* (*bgy1-2*), respectively, mitochondrial size was greatly increased (Fig. 3B). In *bigyin1-1* (SAIL_1171_G11_mito-GFP), the mean mitochondrial plan area was $0.629 \mu\text{m}^2 \pm 0.012 \text{ SE}$ ($n=1065$), while in *bigyin1-2* (SALK_086794_mito-GFP), the mean plan area was $0.710 \mu\text{m}^2 \pm 0.018 \text{ SE}$ ($n=1018$) (Fig. 3B). In addition to an increase in mitochondrial plan area in *bigyin* mutants, there is a concomitant decrease in the number of mitochondria per cell. Wild-type protoplasts contained an average of $70.8 \pm 6.2 \text{ SE}$ mitochondria per protoplast-field-of-view, compared with means of $42.7 \pm 4.9 \text{ SE}$ and $39.7 \pm 4.8 \text{ SE}$ for *bigyin1-1* and *bigyin1-2*, respectively (Fig. 3C). The net effect of the increase in size of individual mitochondria, coupled to the decrease in number of mitochondria per cell in *bigyin*, relative to wild-type, is that the total mitochondrial area per protoplast varies little between lines. For example, the average total mitochondrial plan area for wild-type is $25.6 \mu\text{m}^2$, compared with $27 \mu\text{m}^2$ and $28.4 \mu\text{m}^2$ for *bigyin1-1* and *bigyin1-2*, respectively. This result suggests that the total mitochondrial volume per cell is homeostatically controlled, under conditions whereby the normal equilibrium of mitochondrial fission and fusion is shifted towards fusion, and that BIGYIN is not necessary for the maintenance of total mitochondrial volume. A similar homeostatic control mechanism for mitochondrial volume was implied in an analysis of mitochondria in developing barley or wheat leaves: the volume fraction of mitochondria within mature mesophyll cells was found to be maintained at 0.6–1% of total cell volume (Bowsher and Tobin, 2001). Unfortunately, although it is well accepted that the equilibrium between mitochondrial fission and fusion controls mitochondrial shape, size, and number we can find no other analysis in the literature where the effect of a shift of this equilibrium on mitochondrial area, volume or mass has been quantified. However, similar correlations between organelle plan area and number have been reported in chloroplast *arc* mutants, where reductions in the number of chloroplasts per cell are linked to increases in organelle plan area (Pyke and Leech, 1991; Pyke and Leech, 1994; Aldridge *et al.*, 2005). The mitochondrial phenotype of *bigyin* is similar to that in mutants of the *Arabidopsis* dynamin-like genes *DRP3A* (Arimura *et al.*, 2004; Logan *et al.*, 2004) and *DRP3B* (Arimura and Tsutsumi, 2002; I Scott, AK Tobin, DC Logan, unpublished results) and is indicative of a disruption of normal mitochondrial fission.

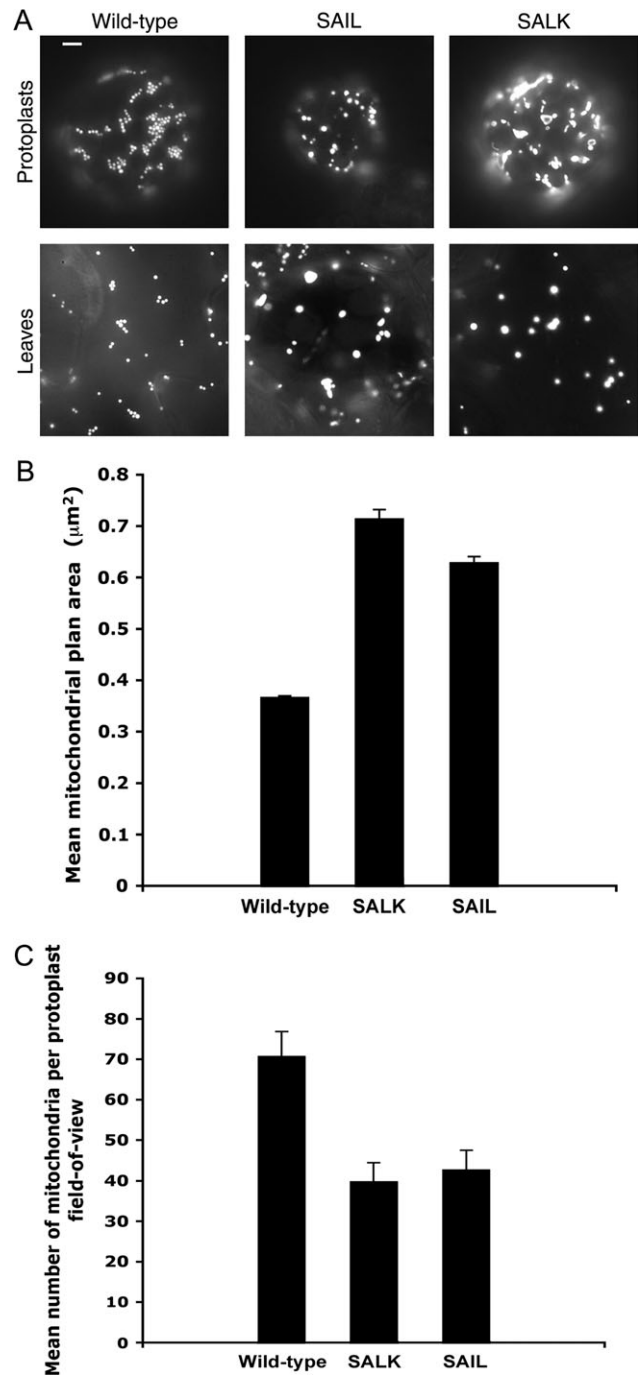


Fig. 3. (A) Mitochondrial morphology in wild-type and *bigyin* plants. Wild-type mitochondria are pleomorphic, although typically spherical or sausage-shaped. The SAIL (*bigyin1-1*) and SALK (*bigyin1-2*) T-DNA insertion mutants have a greatly reduced number of mitochondria per cell relative to wild type, but the size of individual mitochondria in the mutants is proportionately larger. Scale bar=10 μm . (B) Mean mitochondrial plan area in wild-type and *bigyin* protoplasts. Single images were captured of 25 wild-type, SAIL (*bigyin1-1*) and SALK (*bigyin1-2*) protoplasts and the plan area of all mitochondria measured. Means \pm SE are calculated from $n=1793$, 1065, and 1018 mitochondria for wild-type, SAIL, and SALK protoplasts, respectively. (C) Mean number of mitochondria per protoplast-field-of-view in wild-type and *bigyin* protoplasts. Single images were captured of 25 wild-type, SAIL (*bigyin1-1*) and SALK (*bigyin1-2*) protoplasts and the mean number \pm SE of mitochondria per protoplast-field-of-view was calculated.

BIGYIN is a functional homologue of FIS1/hFIS1

Segregation analyses were performed to confirm whether or not the aberrant mitochondrial morphology phenotype was due to a homozygous T-DNA insertion in the *BIGYIN* gene. The mitochondrial morphology of 104 T₂ individuals of line SAIL_1171_G11_mito-GFP was analysed by epifluorescent microscopy and 28 were identified as having an abnormal mitochondrial morphology phenotype. Statistical analysis of the result, using the chi-square (χ^2) test, shows that the observed segregation ratio of 2.7:1 (wild-type:mutant) concurs closely with the 3:1 Mendelian segregation ratio expected for a single, recessive nuclear gene ($P > 0.6$, $df=1$, $n=104$). PCR analysis of the 104 T₂ plants showed that all 28 individuals with aberrant mitochondrial morphology were homozygous for the mutant allele. The remaining 76 plants with wild-type mitochondrial morphology were either homozygous wild-type or hemizygous for the T-DNA insertion. A similar mitochondrial morphology analysis of the offspring from a T1 SAL-K_086794_mito-GFP hemizygote revealed a segregation ratio of 2.6:1 (wild-type:mutant), which also correlated with the expected 3:1 Mendelian segregation ratio for a single, recessive nuclear gene (χ^2 test, $P > 0.6$, $df=1$, $n=58$). It is concluded that *BIGYIN* is an *Arabidopsis* functional orthologue of yeast *FIS1* and human *hFIS1* and is involved in mitochondrial fission.

Acknowledgements

We thank Harry Hodge for technical assistance in the laboratory, growth rooms, and greenhouse. We thank Syngenta Biotechnology Inc., Triangle Park, North Carolina USA and the Nottingham *Arabidopsis* Stock Centre (NASC, University of Nottingham, UK) for provision of the SAIL and SALK lines, respectively. This work was funded by a UK Biotechnology and Biological Sciences Research Council studentship to IS and research grants (G15541 and BBC0001291) to AKT and DCL.

References

- Aldridge C, Maple J, Moller SG. 2005. The molecular biology of plastid division in higher plants. *Journal of Experimental Botany* **56**, 1061–1077.
- Alonso JM, Stepanova AN, Leisse TJ, et al. 2003. Genome-wide insertional mutagenesis of *Arabidopsis thaliana*. *Science* **301**, 653–657.
- Arimura S, Aida GP, Fujimoto M, Nakazono M, Tsutsumi N. 2004. *Arabidopsis* dynamin-like protein 2a (ADL2a), like ADL2b, is involved in plant mitochondrial division. *Plant and Cell Physiology* **45**, 236–242.
- Arimura S, Tsutsumi N. 2002. A dynamin-like protein (ADL2b), rather than FtsZ, is involved in *Arabidopsis* mitochondrial division. *Proceedings of the National Academy of Sciences, USA* **99**, 5727–5731.
- Bowsher CG, Tobin AK. 2001. Compartmentation of metabolism within mitochondria and plastids. *Journal of Experimental Botany* **52**, 513–527.
- Cerveny KL, Jensen RE. 2003. The WD-repeats of Net2p interact with Dnm1p and Fis1p to regulate division of mitochondria. *Molecular Biology of the Cell* **14**, 4126–4139.
- Cerveny KL, McCaffery JM, Jensen RE. 2001. Division of mitochondria requires a novel DNM1-interacting protein, Net2p. *Molecular Biology of the Cell* **12**, 309–321.
- Clough SJ, Bent AF. 1998. Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *The Plant Journal* **16**, 735–743.
- Fekkes P, Shepard KA, Yaffe MP. 2000. Gag3p, an outer membrane protein required for fission of mitochondrial tubules. *Journal of Cell Biology* **151**, 333–340.
- Gish W, States DJ. 1993. Identification of protein coding regions by database similarity search. *Nature Genetics* **3**, 266–272.
- Hermann GJ, King EJ, Shaw JM. 1997. The yeast gene, MDM20, is necessary for mitochondrial inheritance and organization of the actin cytoskeleton. *Journal of Cell Biology* **137**, 141–153.
- James DI, Parone PA, Mattenberger Y, Martinou JC. 2003. hFis1, a novel component of the mammalian mitochondrial fission machinery. *Journal of Biological Chemistry* **278**, 36373–36379.
- Logan DC. 2003. Mitochondrial dynamics. *New Phytologist* **160**, 463–478.
- Logan DC, Leaver CJ. 2000. Mitochondria-targeted GFP highlights the heterogeneity of mitochondrial shape, size and movement within living plant cells. *Journal of Experimental Botany* **51**, 865–871.
- Logan DC, Scott I, Tobin AK. 2004. ADL2a, like ADL2b, is involved in the control of higher plant mitochondrial morphology. *Journal of Experimental Botany* **55**, 783–785.
- Mozdy AD, McCaffery JM, Shaw JM. 2000. Dnm1p GTPase-mediated mitochondrial fission is a multi-step process requiring the novel integral membrane component Fis1p. *Journal of Cell Biology* **151**, 367–380.
- Murashige T, Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* **15**, 473–497.
- Okamoto K, Shaw JM. 2005. Mitochondrial morphology and dynamics in yeast and multicellular eukaryotes. *Annual Review of Genetics* **10.1146/annurev.genet.38.072902.093019**.
- Otsuga D, Keegan BR, Brisch E, Thatcher JW, Hermann GJ, Bleazard W, Shaw JM. 1998. The dynamin-related GTPase, Dnm1p, controls mitochondrial morphology in yeast. *Journal of Cell Biology* **143**, 333–349.
- Pye KA, Leech RM. 1991. Rapid image-analysis screening-procedure for identifying chloroplast number mutants in mesophyll-cells of *Arabidopsis thaliana* (L.) Heynh. *Plant Physiology* **96**, 1193–1195.
- Pye KA, Leech RM. 1994. A genetic analysis of chloroplast division and expansion in *Arabidopsis thaliana*. *Plant Physiology* **104**, 201–207.
- Sesaki H, Jensen RE. 1999. Division versus fusion: Dnm1p and Fzo1p antagonistically regulate mitochondrial shape. *Journal of Cell Biology* **147**, 699–706.
- Sessions A, Burke E, Presting G, et al. 2002. A high-throughput *Arabidopsis* reverse genetics system. *The Plant Cell* **14**, 2985–2994.
- Smirnova E, Griparic L, Shurland DL, van der Bliek AM. 2001. Dynamin-related protein Drp1 is required for mitochondrial division in mammalian cells. *Molecular Biology of the Cell* **12**, 2245–2256.
- Stojanovski D, Koutsopoulos OS, Okamoto K, Ryan MT. 2004. Levels of human Fis1 at the mitochondrial outer membrane regulate mitochondrial morphology. *Journal of Cell Science* **117**, 1201–1210.
- Suzuki M, Jeong SY, Karbowski M, Youle RJ, Tjandra N. 2003. The solution structure of human mitochondria fission protein

- Fis1 reveals a novel TPR-like helix bundle. *Journal of Molecular Biology* **334**, 445–458.
- Suzuki M, Neutzner A, Tjandra N, Youle RJ.** 2005. Novel structure of the N-terminus in yeast Fis1 correlates with a specialized function in mitochondrial fission. *Journal of Biological Chemistry* **280**, 21444–21452.
- Tieu Q, Nunnari J.** 2000. Mdv1p is a WD repeat protein that interacts with the dynamin-related GTPase, Dnm1p, to trigger mitochondrial division. *Journal of Cell Biology* **143**, 353–365.
- Yoon Y, Krueger EW, Oswald BJ, McNiven MA.** 2003. The mitochondrial protein hFis1 regulates mitochondrial fission in mammalian cells through an interaction with the dynamin-like protein DLP1. *Molecular and Cellular Biology* **23**, 5409–5420.
- Youle RJ, Karbowski M.** 2005. Mitochondrial fission in apoptosis. *Nature Reviews Molecular Cell Biology* **6**, 657–663.
- Yu T, Fox RJ, Burwell LS, Yoon Y.** 2005. Regulation of mitochondrial fission and apoptosis by the mitochondrial outer membrane protein hFis1. *Journal of Cell Science* **118**, 4141–4151.