

Interaction of the *Arabidopsis* Polycomb group proteins FIE and MEA mediates their common phenotypes

C. Spillane^{*†‡}, C. MacDougall^{‡§}, C. Stock^{‡§}, C. Köhler^{*†}, J-P. Vielle-Calzada^{#¶}, S.M. Nunes[†], U. Grossniklaus^{*†#} and J. Goodrich[§]

Genes of the *FERTILISATION INDEPENDENT SEED* (*FIS*) class regulate cell proliferation during reproductive development in *Arabidopsis* [1–5]. The *FIS* genes *FERTILISATION INDEPENDENT ENDOSPERM* (*FIE*) and *MEDEA* (*MEA*) encode homologs of animal Polycomb group (Pc-G) proteins, transcriptional regulators that modify chromatin structure and are thought to form multimeric complexes [3–11]. To test whether similarities in *fis* mutant phenotypes reflect interactions between their protein products, we characterised *FIE* RNA and protein localisation *in vivo*, and *FIE* protein interactions in yeast and *in vitro*. Expression of *FIE* mRNA overlaps with that of *MEA* during embryo sac and seed development and is unaffected in *mea* mutants. Results from the yeast two-hybrid system and an *in vitro* pull-down assay indicate that *MEA* and *FIE* proteins interact. The relevance of this interaction *in vivo* is supported by the finding that *FIE* and *MEA* co-localise in the nucleus in transfected plant cells. Interaction of *MEA* and *FIE* is mediated by the amino-terminal region of *MEA*. Despite sequence divergence in this domain, *MEA* can interact with its corresponding animal partner Extra sex combs (ESC) in the yeast two-hybrid system. We conclude that *FIE* and *MEA* act together as part of a multimeric complex and that this accounts for the similarities in mutant phenotypes. We propose that an ancient mechanism for chromatin modification has been independently recruited to different developmental processes in the two kingdoms.

Addresses: *Institute of Plant Biology, University of Zürich, CH-8008 Zürich, Switzerland. †Friedrich Miescher Institute, CH-4058 Basel, Switzerland. ‡Institute of Cell and Molecular Biology, University of Edinburgh, Mayfield Road, Edinburgh EH9 3JH, UK. #Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724, USA.

Present address: †CINVESTAV-Irapuato, Plant Biotechnology Unit, CP 36 500, Irapuato GTO, Mexico.

‡These authors contributed equally to this work.

Correspondence: Justin Goodrich
E-mail: Justin.Goodrich@ed.ac.uk

Received: 25 August 2000

Revised: 5 October 2000

Accepted: 5 October 2000

Published: 17 November 2000

Current Biology 2000, 10:1535–1538

0960-9822/00/\$ – see front matter

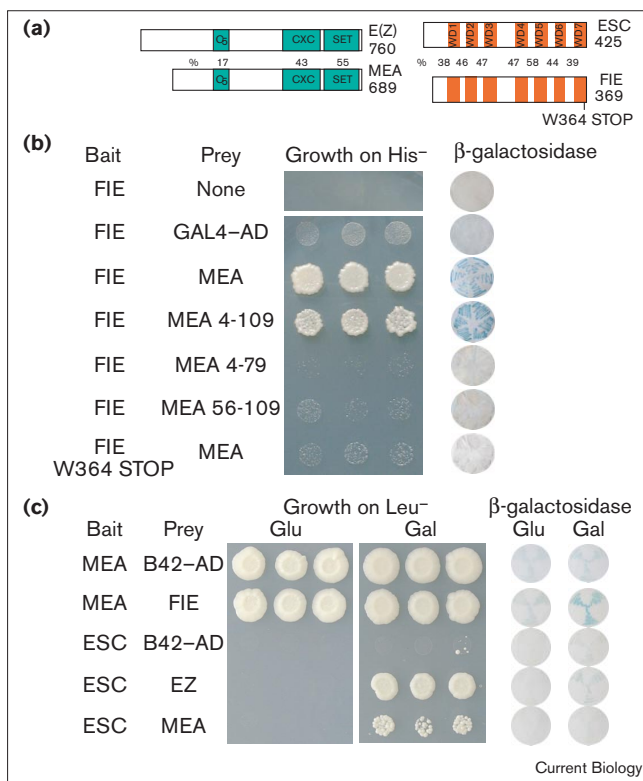
© 2000 Elsevier Science Ltd. All rights reserved.

Results and discussion

MEA and *FIE* encode homologues of the *Drosophila* Enhancer of zeste E(Z) and ESC proteins, respectively (Figure 1a; see also [3–7] and Supplementary material). These are widely conserved and have been shown to co-localize in complexes distinct from the other Pc-G proteins [9–16]. To test whether plant *MEA* and *FIE* could interact, yeast two-hybrid assays were performed. The near full-length proteins were expressed as ‘bait’ fusions to the yeast Gal4 DNA-binding (DB) domain, and as ‘prey’ fusions to the Gal4 transcriptional activation (TA) domain [17] (see Supplementary material). Yeast strains that carried the *FIE* bait construct alone were unable to grow in the absence of histidine and did not express β -galactosidase, indicating that the DB–*FIE* fusion did not activate either reporter gene. In addition, GAL4–TA and *FIE*–DB did not interact in yeast. However, yeast transformants carrying *DB–FIE* and *TA–MEA* expressed both reporters, suggesting that *FIE* and *MEA* interact (Figure 1b). We did not detect a reciprocal interaction when *FIE* was expressed as a prey fusion and *MEA* as a bait (data not shown), presumably because one or both fusions were non-functional. In an alternative two-hybrid system [18], *MEA*–DB constructs on their own were able to weakly activate reporter gene expression, but gave increased β -galactosidase activity in the presence of *FIE*–TA prey (Figure 1c), also consistent with an *FIE*–*MEA* interaction. To test whether mutations that eliminate *FIE* activity *in planta* also affect its interaction with *MEA*, we introduced the *fie-6* mutation into the *FIE*–GAL4–DB bait. *fie-6* confers a strong mutant phenotype equivalent to that of alleles encoding extensive truncations or deletions [7]. It encodes *FIE* W364STOP, which truncates the *FIE* protein by six residues and disrupts the highly conserved WD residues at the carboxy-terminal end of WD repeat motif seven, which are predicted to stabilise the β -propeller structure (for example [8,9,19]). Introduction of the W364STOP mutation into the *FIE* bait prevented its interaction with *MEA* (Figure 1b), consistent with the *FIE*–*MEA* interaction being necessary for *FIE* activity *in planta*.

To map the region of the *MEA* protein required for interaction with *FIE*, a series of carboxy-terminal truncations of *MEA* were expressed as fusions to the Gal4 activation domain. We found that the amino-terminal residues 4–109 were sufficient for interaction with *FIE*. Proteins with more extensive deletions (residues 4–79) were unable to interact with *FIE* (Figure 1b) and a series of amino-terminal

Figure 1



FIE and MEA interact in yeast. **(a)** Alignment of MEA and E(Z), and FIE and ESC proteins. Amino acid identities in conserved domains are indicated. C₅, CYS and SET, conserved cysteine-rich regions and SET domain previously described (for example [4]); WD, WD repeat motif. **(b)** Assays using a Gal4-based yeast two-hybrid system [16]. Three independent transformants are shown for each growth assay on His⁻ media. For β-galactosidase activity assays, six independent transformants were grown on filter paper, lysed, and incubated on media containing X-Gal (see Supplementary material). **(c)** Assays using a LexA-DB-based system [18]. In this system, *LEU2* and *lacZ* (β-galactosidase) reporter genes are transcriptionally dependent on LexA. Expression of the prey construct is galactose inducible, so colonies were grown on glucose- or galactose-containing media to compare reporter gene activation in the presence of bait alone or bait with prey, respectively. Three independent transformants are shown for each assay. E(Z)-DB and MEA-DB alone activated the *LEU2* reporter but gave weaker activation of the β-galactosidase reporter, FIE-DB alone strongly activated both reporters and therefore could not be used in two-hybrid assays.

deletions all tested negative for interaction with FIE. Together, these results suggested that residues 4–109 of MEA were sufficient for interaction with FIE.

An amino-terminal portion of the *Drosophila* E(Z) protein is sufficient for its interaction with ESC [8–10]. This suggested that the interaction between E(Z) and ESC homologues is conserved between plants and animals, and is mediated by amino-terminal regions of E(Z) and MEA. However, there is little sequence similarity between these regions of MEA and E(Z) indicating that the domains mediating the interaction have diverged in the plant and

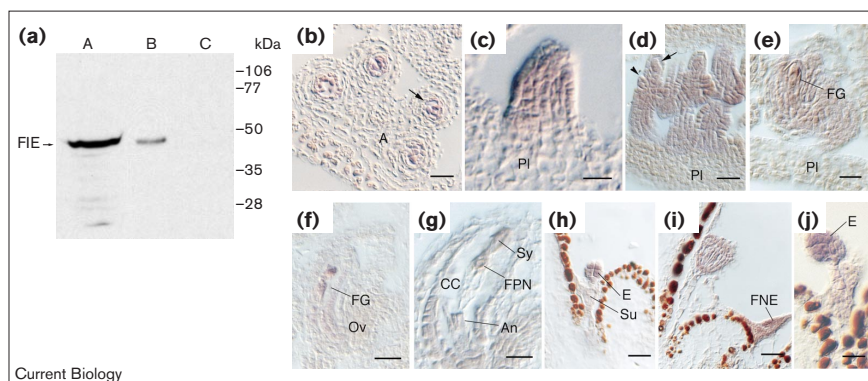
animal lineages. To test whether the animal and plant counterparts could recognise one another in two-hybrid assays (Figure 1c), we used a LexA-DB-based system in which E(Z) and ESC interaction had been demonstrated [9]. In this system, Leu2 and β-galactosidase reporters are transcriptionally dependent upon LexA [18]. TA-E(Z) and TA-MEA preys both interacted with DB-ESC bait and activated the reporters, although the interaction with MEA was weaker than with E(Z) (Figure 1c). Thus MEA and EZ can both interact with ESC, despite their divergent sequences in the amino-terminal region.

To confirm independently that FIE and MEA interact, we performed *in vitro* binding assays (Figure 2a). Full-length MEA protein was expressed as a glutathione-S-transferase (GST) fusion in *Escherichia coli*, purified, and immobilized on glutathione-agarose beads. To test for binding to FIE, extracts were prepared from *E. coli* cells that expressed FIE tagged with Xpress epitope and incubated with equal volumes of beads attached to GST or GST-MEA. Western blots of the bead-associated proteins were probed with anti-Xpress antibodies. As shown in Figure 2, Xpress-FIE protein bound to GST-MEA (lane B) but not to GST alone (lane C), suggesting a direct physical interaction between MEA and FIE proteins. Thus, the MEA and FIE proteins bind to each other *in vitro* as well as in yeast.

If the physical interaction of *FIE* and *MEA* is biologically relevant, their products would be expected to be co-expressed *in vivo*. The *in vivo* expression pattern of *MEA* was described previously [20] and is consistent with its roles in the embryo sac and during zygotic development: before fertilisation *MEA* RNA is expressed in the synergids, the egg and central cell of the embryo sac; after fertilisation, *MEA* RNA is present in both fertilization products of the developing seed, the embryo and endosperm. To determine localisation of *FIE* mRNA *in vivo*, we hybridised digoxigenin-labelled *FIE* antisense (Figure 2b-j) and sense RNA probes (see Supplementary material) to tissue sections of developing reproductive organs *in situ*. *FIE* is initially expressed in the developing anther and the young ovule primordium. In the anther, *FIE* mRNA is abundant in microspore mother cells undergoing meiosis, in microsporocytes and in the tapetum (Figure 2b), but absent from vascular bundles, the connective tissue and the filament. *FIE* mRNA is also absent from pollen grains at subsequent developmental stages (data not shown). In the female reproductive organs, *FIE* mRNA is initially present in all cells of the young ovule primordium before archesporial differentiation (Figure 2c). *FIE* is not expressed in the placenta or the developing carpel. It is abundantly expressed in the ovule sporophytic tissue and the megaspore mother cell before meiosis (Figure 2d). Subsequently, at the initiation of megagametogenesis, *FIE* mRNA levels appear to decrease in the nucellus and integuments but to increase in the developing embryo sac (megagametophyte).

Figure 2

(a) *In vitro* binding of MEA and FIE. Bacterial extract containing Xpress–FIE protein was tested for binding to GST–MEA (lane B) or GST (lane C). Proteins that bound to GST or GST–MEA were separated by SDS–PAGE, transferred to PVDF membrane, and incubated with anti-Xpress antibodies. The input lane (lane A) contains 1.5% of the volume of bacterial extract used in the binding assay. **(b–j)** Localisation of *FIE* mRNA (shown by brown staining) in reproductive organs and developing seeds. **(b)** Young stamens; *FIE* mRNA is localised in the lodicule (arrow) but not the rest of the anther. **(c)** Young ovules before megaspore mother cell differentiation. **(d)** Ovules undergoing meiosis; the arrow indicates the megaspore mother cell and the arrowhead the integument primordia. **(e)** Two-nucleate female gametophyte. **(f)** Eight-nucleate female gametophyte. **(g)** Mature female gametophyte. **(h)** Seed containing an embryo at the octant stage. **(i)** Seed with an embryo at the transition stage. **(j)** Developing



mea/mea seeds. The dark brown cells in (h,i,j), are endothelial cells, which commonly exhibit strong background staining. Control hybridisations using sense *FIE* probes are presented in Supplementary material. A, anther; An, antipodals; CC, central cell; E, embryo proper; FG, female gametophyte;

FNE, free nuclear endosperm; FPN, fused polar nuclei; PT, placental tissue; Su, suspensor. Scale bars represent (b) 12 μ m; (c) 8 μ m; (d) 22 μ m; (e) 15 μ m; (f,g) 10 μ m; (h,i) 30 μ m; (j) 25 μ m.

At the two-nucleated stage, *FIE* mRNA is abundant in the developing megagametophyte (Figure 2e). At maturity, expression is restricted to all cells of the megagametophyte, the endothelium and the persistent nucellar cells located at the chalazal region of the ovule (Figure 2f,g). After fertilisation, during early seed development, *FIE* is expressed in both embryo and endosperm, but not in the developing seed coat. At the octant stage of embryo development, *FIE* mRNA is localised in the embryo proper but not the suspensor (Figure 2h,i). In the developing endosperm, *FIE* mRNA is weakly expressed in free endosperm nuclei covering the mycophylar region of the embryo sac, but is abundant in large free nuclear nodules present in the chalazal region of the developing seed (Figure 2i). *FIE* mRNA is absent from the seed coat or other maternal tissues from developing siliques.

The distribution of *FIE* mRNA in the megagametophyte and in developing seeds is therefore consistent with its biological function of repressing central cell proliferation before fertilisation, and controlling early embryo/endosperm development. Furthermore, the expression patterns of *FIE* and *MEA* [20] overlap in the central, egg and synergid cells of the embryo sac, and during early embryo and endosperm development (except for the suspensor), indicating the potential for their protein products to interact.

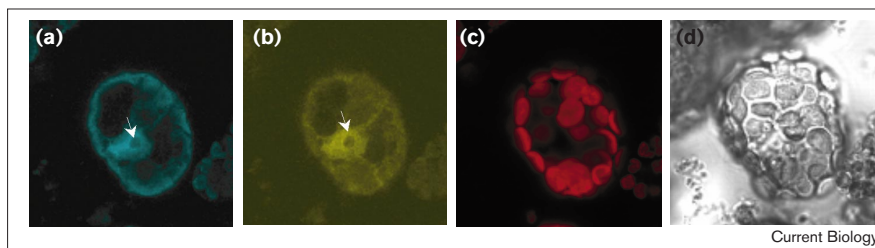
To test whether *MEA* might regulate *FIE* transcription, we characterised *FIE* expression in developing siliques of self-pollinated homozygous *mea/mea* plants (produced by embryo rescue from *mea* seed as described in [20]). We compared wild-type and *mea* mature ovules before and after fertilisation. For all stages of development examined,

no differences were observed for the patterns of *FIE* mRNA accumulation (Figure 2j). This suggests that *MEA* does not regulate *FIE* at the transcriptional level.

To investigate the intracellular localisation of MEA and FIE proteins we performed transient expression experiments in cowpea protoplasts, using reporter gene constructs that expressed MEA or FIE as amino-terminal fusions to yellow fluorescent protein (YFP) or cyan fluorescent protein (CFP). Cotransfections of cowpea protoplast cells with *FIE*–CFP (Figure 3a) and *MEA*–YFP (Figure 3b) indicated that FIE and MEA co-localised to the nucleus and were both consistently excluded from the nucleolus. Nuclear localisation of both MEA and FIE was also observed in single transfections and for the reciprocal constructs FIE–YFP and MEA–CFP. The *in vivo* co-localisation of MEA and FIE proteins in the nucleus of plant cells, in addition to the demonstration of their physical interactions in yeast and *in vitro* (Figures 1,2), is consistent with a MEA–FIE Pc-G protein complex occurring *in vivo*.

The *FIE* and *MEA* genes confer similar mutant phenotypes in both unfertilized embryo sacs and developing seeds; that is, fertilisation-independent endosperm proliferation and the abortion of fertilised seeds that carry a maternally inherited mutant *mea* or *fie* allele. We have shown that MEA and FIE interact in yeast and *in vitro*, and that the *fie-6* mutation which drastically reduces *FIE* activity *in vivo* also eliminates interaction of FIE and MEA. Furthermore, *FIE* and *MEA* RNA expression overlaps *in vivo* and the proteins co-localise within the nucleus *in planta*. We conclude that the similarity of the *fie* and *mea* phenotypes reflects the interaction of their gene products

Figure 3



Cowpea protoplast co-transfected with pFIE-CFP and pMEA-YFP shows co-localisation of FIE-CFP and MEA-YFP fusion proteins. (a) FIE-CFP; (b) MEA-YFP; (c) chloroplast autofluorescence; (d) phase-contrast microscopy. White arrows indicate the nucleolus. Co-localized FIE-CFP and MEA-YFP in the cytoplasm may be related to high levels of expression from the 35S promoter.

as part of a multiprotein complex. By analogy with animal systems [11] it is likely that the FIE-MEA complex modifies higher-order chromatin structure around their target genes to confer a transcriptionally inactive state.

The ESC and E(Z) proteins are more widely conserved than other members of the *Drosophila* and vertebrate Pc-G [12,13], suggesting that these two proteins are of more ancient origin than other Pc-G proteins. Because plant and animals are thought to have evolved multicellularity independently, an ancient Pc-G-dependent mechanism for chromatin modification has been recruited to independent developmental processes in the two kingdoms. Elucidation of the FIE/MEA regulatory network will test whether different target genes have been acquired in the two lineages.

Note added in proof

Luo *et al.* have also recently reported that FIE and MEA interact in two-hybrid assays [21].

Supplementary material

Supplementary material including a detailed description of Materials and methods and additional Results is available at <http://current-biology.com/supmat/supmatin.htm>.

Acknowledgements

We thank Jeff Simon and Rick Jones for clones and information, Renate Schmidt for locating the *FIE* cDNA on the *Arabidopsis* physical map, Bill Crosby and Roger Brent for yeast two-hybrid vectors and technical advice and Santiago Mora-Garcia for constructs for FIE protein expression. We thank Khalid Shah, Dorus Gadella and Sacco de Vries (Wageningen Agricultural University) for invaluable assistance with the fluorescence microscopy analysis. U.G. acknowledges support by the Novartis Research Foundation, IAESTE, and the Kanton Zürich. C.K. and J-P. V-C. were supported by EMBO Long Term and Swiss National Science Foundation Fellowships, respectively, and U.G. is a Searle Scholar. J.G. is a Royal Society University research fellow. C.M.D. and C.S. were funded by a CAD award and a PhD studentship from the BBSRC, respectively.

References

- Chaudhury AM, Ming L, Miller C, Craig S, Dennis ES, Peacock WJ: Fertilization-independent seed development in *Arabidopsis thaliana*. *Proc Natl Acad Sci USA* 1997, **94**:4223-4228.
- Ohad N, Margossian Y, Hsu Y-C, Williams C, Repetti P, Fischer RL: A mutation that allows endosperm development without fertilisation. *Proc Natl Acad Sci USA* 1996, **93**:5319-5324.
- Grossniklaus U, Vielle-Calzada JP: ...response: parental conflict and infanticide during embryogenesis. *Trends Plant Sci* 1998, **3**:328.
- Grossniklaus U, Vielle-Calzada JP, Hoepfner MA, Gagliano WB: Maternal control of embryogenesis by *MEDEA*, a Polycomb group gene in *Arabidopsis*. *Science* 1998, **280**:446-450.
- Kiyosue T, Ohad N, Yadegari R, Hannon M, Dinneny J, Wells D, *et al.*: Control of fertilization-independent endosperm development by the *MEDEA* polycomb gene *Arabidopsis*. *Proc Natl Acad Sci USA* 1999, **96**:4186-4191.
- Luo M, Bilodeau P, Koltunow A, Dennis ES, Peacock WJ, Chaudhury AM: Genes controlling fertilization-independent seed development in *Arabidopsis thaliana*. *Proc Natl Acad Sci USA* 1999, **96**:296-301.
- Ohad N, Yadegari R, Margossian L, Hannon M, Michaeli D, Harada JJ, *et al.*: Mutations in *FIE*, a WD polycomb group gene, allow endosperm development without fertilization. *Plant Cell* 1999, **11**:407-415.
- Tie F, Furuyama T, Harte PJ: The *Drosophila* Polycomb Group proteins ESC and E(Z) bind directly to each other and co-localize at multiple chromosomal sites. *Development* 1998, **125**:3483-3496.
- Jones CA, Ng J, Peterson AJ, Morgan K, Simon J, Jones RS: The *Drosophila* ESC and E(Z) proteins are direct partners in Polycomb group-mediated repression. *Mol Cell Biol* 1998, **18**:2825-2834.
- Shao Z, Raible F, Mollaaghababa R, Guyon JR, Wu C-T, Bender W, Kingston RE: Stabilisation of chromatin structure by PRC1, a Polycomb complex. *Cell* 1999, **98**:37-46.
- van der Vlag J, Otte AP: Transcriptional repression mediated by the human polycomb-group protein EED involves histone deacetylation. *Nat Genet* 1999, **23**:474-478.
- Korf I, Fan Y, Strome S: The Polycomb group in *Caenorhabditis elegans* and maternal control of germline development. *Development* 1998, **125**:2469-2478.
- Holdeman R, Nehrt S, Strome S: MES-2, a maternal protein essential for viability of the germline in *Caenorhabditis elegans*, is homologous to a *Drosophila* Polycomb group protein. *Development* 1998, **125**:2457-2467.
- Van Lohuizen M, Tijms M, Voncken JW, Schumacher A, Magnuson T, Wientjens E: Interaction of the mouse Polycomb-group (Pc-G) proteins *Enx1* and *Enx2* with *Eed*: indication for separate Pc-G complexes. *Mol Cell Biol* 1998, **18**:3527-3579.
- Ng J, Hart CM, Morgan K, Simon JA: A *Drosophila* ESC-E(Z) protein complex is distinct from other polycomb group complexes and contains covalently modified ESC. *Mol Cell Biol* 2000, **20**:3069-3078.
- Sewalt R, van der Vlag J, Gunster MJ, Hamer KM, den Blaauwen JL, Satijn DPE, *et al.*: Characterization of interactions between the mammalian polycomb-group proteins *Enx1/E(Z)H2* and *EED* suggests the existence of different mammalian polycomb-group protein complexes. *Mol Cell Biol* 1998, **18**:3586-3595.
- Kohalmi SE, Reader LJV, Samach A, Nowak J, Haughn GW, Crosby WL: Identification and characterization of protein interactions using the yeast two-hybrid system. *Plant Mol Biol* 1998, **M1**:1-30.
- Golemis EA, Gyuris J, Brent R: Interaction trap/two-hybrid system to identify interacting proteins. In *Current Protocols in Molecular Biology*. Edited by Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K. John Wiley: New York; 1996:20.1.1-20.1-23.
- Ng J, Li R, Morgan K, Simon J: Evolutionary conservation and predicted structure of the *Drosophila* extra sex combs repressor protein. *Mol Cell Biol* 1997, **17**:6663-6672.
- Vielle-Calzada JP, Thomas J, Spillane C, Coluccio A, Hoepfner MA, Grossniklaus U: Maintenance of genomic imprinting at the *Arabidopsis* *MEDEA* locus requires zygotic *DDM1* activity. *Genes Dev* 1999, **13**:2971-2982.
- Luo M, Bilodeau P, Dennis ES, Peacock WJ, Chaudhury A: Expression and parent-of-origin effects for *FIS2*, *MEA*, and *FIE* in the endosperm and embryo of developing *Arabidopsis* seeds. *Proc Natl Acad Sci USA* 2000 **97**:10637-10642.