The Plant Cell, Vol. 18, 781–784, April 2006, www.plantcell.org

IN THIS ISSUE

Genetic and Epigenetic Regulation of Embryogenesis

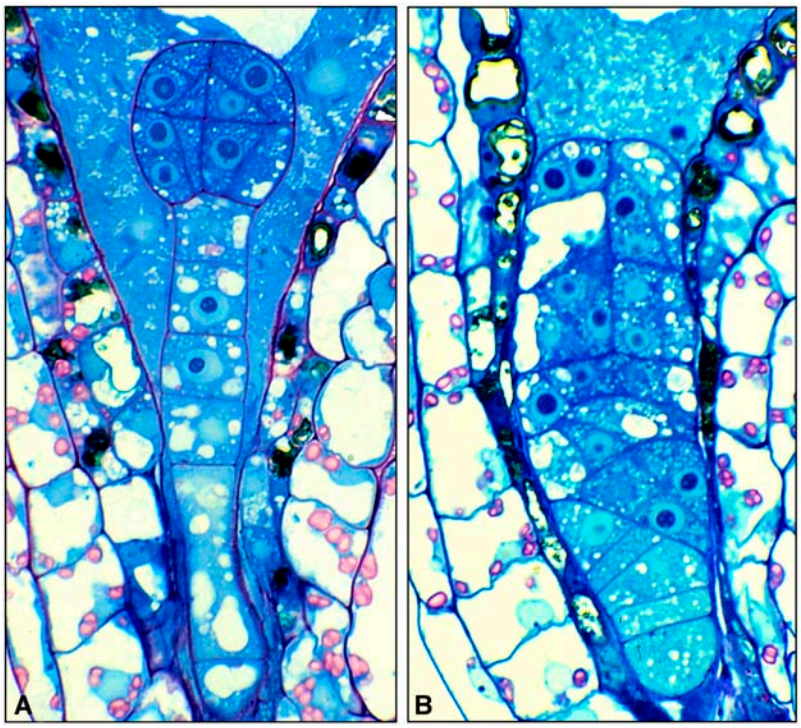
Embryogenesis is a crucial period in the development of eukaryotes. In most plants, embryogenesis begins with an asymmetric cell division that gives rise to a polar embryo having a larger basal cell and a smaller apical cell. The embryo proper develops from the apical cell, and the basal cell develops into the suspensor, which is attached to the ovule and serves as a conduit for nutrient transfer to the developing embryo. The early stages of *Arabidopsis* embryo development have stereotyped cell divisions. Cotyledons then develop and serve to store nutrients for the seedling after germination. During late seed development, the seed desiccates and remains dormant until conditions are right for germination.

Cell division and differentiation during these events follow highly regulated patterns that are influenced by both genetic and epigenetic mechanisms. Mammalian genomes undergo genomic reprogramming during embryogenesis involving global changes in DNA methylation believed to play an important role in developmental regulation of gene expression. DNA methylation is often associated with transcriptionally inactive portions of the genome (heterochromatin), and during early embryogenesis, large portions of the genome undergo a demethylation and subsequent remethylation process that is thought to contribute to chromatin decondensation and transcriptional activation of genes essential for embryo development (Li, 2002). This might have the effect of providing coarse control over gene expression, allowing hundreds of genes specifically required for embryogenesis to be switched on during this critical period and to remain off during other stages of the life cycle. On perhaps a more fundamental level, RNA polymerase II is at the heart of transcriptional machinery that transcribes protein-coding genes into mRNA. The RNA polymerase II machinery contains >85 polypeptides within 10 subcomplexes (Holstege et al., 1998), and the fine control of transcription involves the activity of these and many other interacting factors.

Two articles in this issue of *The Plant Cell* report on genetic and epigenetic aspects of the regulation of gene expression during embryogenesis. **Xiao et al. (pages 805–814)** show that DNA methylation performed by *METHYLTRANSFERASE1* (*MET1*) influences gene expression during embryogenesis in *Arabidopsis* and is critical for normal development of the embryo and seed viability. In another article, **Ding et al. (pages 815–830)** show that the pentatricopeptide repeat (PPR) protein GLUTAMINE-RICH PROTEIN23 (GRP23) is a nuclear protein that interacts with RNA polymerase II and likely functions in regulating gene expression during early embryogenesis.

DNA METHYLATION PLAYS A VITAL ROLE IN PLANT EMBRYOGENESIS

In mammals, cytosine methylation occurs mainly at CpG sites (a cytosine residue linked on its 3′ side to the 5′ side of a guanine residue) and is maintained by DNA methyltransferase1 (Dnmt1), which acts on hemimethylated DNA (double-stranded DNA methylated on only one strand, which occurs following DNA replication). During gametogenesis and embryogenesis, DNA methylation is lost (on both strands of double-stranded DNA) over a large portion of the genome and later in embryo development is reestablished by de novo methyltransferases Dnmt3a and Dnmt3b, which act on fully unmethylated DNA. Both



DNA Methylation in *Arabidopsis* Embryogenesis.

Wild-type **(A)** and *met1* mutant **(B)** embryos 3 d after pollination.

IN THIS ISSUE

maintenance and de novo methylation are essential in animals, as mutations in either the *Dnmt1* or *Dnmt3* gene are embryo lethal (Li et al., 1992; Okano et al., 1999). Imprinting, or the differential expression of gene alleles dependent on the parent of origin, depends on heritable DNA methylation patterns established during gametogenesis in animals, and imprinted genes are protected from demethylation in the global reprogramming during embryogenesis (Li, 2002). In plants, imprinting of certain genes occurs in the endosperm and affects endosperm development (reviewed in Gehring et al., 2004).

Arabidopsis contains at least three classes of DNA methyltransferase genes: *MET1*, which is a homolog of Dnmt1 and encodes the major CpG maintenance methyltransferase; *CHROMOMETHYLASE3* (*CMT3*), which encodes a plant-specific DNA methyltransferase that acts mainly at CpNpG and CpNpN residues; and *DOMAINS REARRANGED1* (*DRM1*) and *DRM2*, which share homology with *Dnmt3* and are thought to function as the major de novo methyltransferases in plants (Cao and Jacobsen, 2002; Cao et al., 2003). Xiao et al. analyzed *met1-6* mutants of *Arabidopsis*, which carry a putative null allele of *MET1*. Homozygous *met1-6* mutant embryos were found to contain a number of defects in cell division of both suspensor and embryo cells beginning at the earliest stages of embryogenesis (see figure). Although the mutant plants produced some nonviable seed in the first generation, most of the seed was viable, suggesting that DNA methylation patterns play a limited role during embryogenesis or that other DNA methyltransferases may able to partially compensate for MET1 loss of function. Analysis of *met1 cmt3* double mutant plants showed that these genes have partially overlapping functions and confirmed that DNA methylation is critical for embryogenesis and seed viability, as the double mutants showed synergistic effects on seed viability and plant growth relative to either of the single mutants.

Xiao et al. noticed that *met1* mutant embryos resembled mutants having defects in establishing auxin gradients (described in Friml et al., 2003) in that the plane of cell divisions and apical-basal polarity appeared to be disrupted. Friml et al. (2003) used the auxin-responsive promoter *DR5rev* linked to green fluorescent protein (GFP) to show that establishing and maintaining auxin gradients is a critical process during early embryogenesis. Xiao et al. found that *DR5*:*GFP* transgene expression was evenly distributed in *met1* mutant embryos, in contrast with the pattern indicative of an auxin gradient in the wild type, suggesting that DNA methylation may be important for setting up and/or maintaining auxin gradients in the developing embryo. They also examined the expression of *PIN1*, which encodes an auxin efflux carrier shown by Friml et al. (2003) to be responsible for establishing auxin gradients in early embryogenesis. *PIN1* expression was found to be relatively evenly distributed throughout the developing embryo in *met1* mutants, in contrast with the pattern observed in wild-type embryos, suggesting that DNA methylation may affect *PIN1* expression. However, DNA methylation (as measured using DNA methylation–sensitive restriction endonucleases and DNA gel blot analysis) could not be detected in the coding, upstream, or downstream regions of *PIN1* in DNA isolated from either *met1* or wild-type seedlings, leading the authors to conclude that DNA methylation might affect *PIN1* expression indirectly.

The authors analyzed gene expression of three other genes that play important roles in specification of cell identity during embryogenesis: *YODA* (*YDA*), which encodes a mitogen-activated protein kinase kinase kinase involved in specifying embryo and suspensor cell identity (Lukowitz et al., 2004), and *WUSCHEL-RELATED HOMEOBOX2* (*WOX2*) and *WOX8*, which are expressed specifically in apical and basal cell lineages, respectively, of developing embryos and encode homeodomain transcription factors that influence cell division (Haecker et al., 2004). *YDA* expression (as measured by steady state mRNA levels) was enhanced, whereas the expression of both *WOX* genes was reduced in seed of *met1-6* mutants relative to the wild type, suggesting that DNA methylation status affects the expression of genes that influence cell identity during embryogenesis. The authors examined methylation status of the *YDA* gene and found that it is methylated in wild-type plants, and this is dependent on MET1 activity, as mutations in *MET1* resulted in loss of DNA methylation at the locus. This might explain why *YDA* expression was elevated in *met1-6* mutant seeds compared with the wild type.

The reduction in *WOX* gene expression was perhaps surprising, as DNA methylation typically is associated with gene silencing, and hypomethylation in the mutant therefore would be expected to result in enhanced gene expression. It has been shown that DNA methylation enhances transcription of mouse *insulin growth factor2*, apparently by blocking the binding of repressors to a specific intragenic region (Murrell et al., 2001). This suggests that DNA methylation exerts a degree of gene-specific control over gene expression during embryogenesis. The methylation status of the *WOX* genes was not examined, but it will be of interest to determine how the decrease in DNA methyltransferase activity in the *met1* mutant embryos leads to decreased transcription of *WOX* genes.

The work of Xiao et al. shows that DNA methylation is critical for embryogenesis in *Arabidopsis* and is involved in regulating gene expression affecting both auxin responsiveness and embryo cell identity.

REGULATION OF RNA POLYMERASE II IN EMBRYOGENESIS

Eukaryotic transcription of protein coding sequences into mRNA is performed by RNA polymerase II. The core of this enzyme supercomplex is essentially the same for the many thousands of genes that are transcribed, and specificity for the regulation of gene expression lies within the multitude of interacting factors and subunits that influence formation and binding of the complex to gene promoter regions (Holstege et al., 1998). Screening of *embryo-defective* (*emb*) and seed coat mutants in *Arabidopsis* has identified ~750 genes that are required for normal seed development (McElver et al., 2001; Tzafrir et al., 2003, 2004). Among an initial set of 250 *EMB* genes, ~5% were predicted to be transcription factors (Tzafrir

IN THIS ISSUE

et al., 2004). The function of most of these genes remains unknown.

Yang et al. isolated an *emb* mutant from a collection of enhancer trap mutants that carry an Ac/Ds transposable element linked to the β-glucuronidase reporter gene (see Springer et al., 1995; Sundaresan et al., 1995). The defect was traced to a single recessive embryo-lethal mutation that caused an arrest of embryo development at the early globular stage (before the 16-cell dermatogen stage). Many of the mutant embryos exhibited aberrant divisions of the embryo proper and suspensor cells, whereas early endosperm development was unaffected. This is one of only a few *emb* mutants characterized that shows defects at this very early stage of embryo development. The mutation was found to be a loss-of-function rather than enhancer insertion in a gene designated *GRP23*, which encodes a polypeptide with a leucine zipper domain, nine PPRs at the N terminus, and a Gln-rich C-terminal domain with an unusual Trp-Gln-Gln (WQQ) repeat.

PPR proteins constitute one the largest protein families in plants, with >400 members in *Arabidopsis*. Evidence is emerging that a number of plant PPR proteins are RNA binding proteins involved in post-transcriptional processes, such as mRNA processing, often in mitochondria and chloroplasts (Lurin et al., 2004). For example, PPR proteins have been identified as fertility restorers of cytoplasmic male sterility in petunia (Bentolila et al., 2002) and rice (Wang et al., 2006). The March issue of *The Plant Cell* highlighted the work of Wang et al. (2006), who showed that cytoplasmic male sterility in Boro II rice is caused by a cytotoxic peptide encoded by an aberrant mitochondrial open reading frame, and fertility restoration in this system depends on PPR proteins that block production of the peptide by cleavage or degradation of its mRNA. Lurin et al. (2004) hypothesized that PPR proteins function as sequence-specific adaptors, a role that is consistent with the large number of proteins in this family.

Ding et al. show that GRP23 is a nuclear PPR protein that interacts physically with subunit III of RNA polymerase II via its C-terminal WQQ domain in experiments with both yeast and plant cells. The gene is expressed at a relatively high level in gametophytes (particularly pollen grains) and young embryos and at low levels in endosperm tissue and in actively dividing cells in meristems of vegetative tissues. GRP23 therefore exhibits clear differences relative to other known PPR proteins, which mostly have been characterized as putative RNA binding proteins expressed at low levels in all tissues and predicted to be targeted to organelles (e.g., Small and Peeters, 2000; Lurin et al., 2004).

Structural sequence features of the gene suggest that *GRP23* is a novel type of basic domain leucine zipper (bZIP) protein, as it has a bZIP domain unrelated to known bZIP proteins in *Arabidopsis*. bZIP domains are known to be involved in protein–protein interactions, forming homodimers or heterodimers via the zipper portion of the domain, and to bind DNA in a sequence-specific manner via the basic part of the domain. PPR motifs are also predicted to be sequence-specific RNA or DNA binding domains, and Ding et al. hypothesize that GRP23 may bind directly to DNA *cis*-regulatory elements through the basic region of the bZIP domain or through the PPR motifs. The interaction of the WQQ domain with RNA polymerase III, together with gene expression patterns and mutant phenotypes, suggest that GRP23 may recruit RNA polymerase II to control the expression of genes essential during early embryogenesis. It will be important to determine the downstream targets of transcriptional control by GRP23, the function of the bZIP domain and PPR motif, and the possibility of direct sequence-specific binding to DNA.

Nancy A. Eckardt
News and Reviews Editor

REFERENCES

Bentolila, S., Alfonso, A.A., and Hanson, M.R. (2002). A pentatricopeptide repeat-containing gene restores fertility to cytoplasmic male-sterile plants. Proc. Natl. Acad. Sci. USA **99,** 10887–10892.

Cao, S., and Jacobsen, S.E. (2002). Role of the Arabidopsis DRM methyltransferases in de novo DNA methylation and gene silencing. Curr. Biol. **12,** 1138–1144.

Cao, X., Aufsatz, W., Zilberman, D., Mette, M.F., Huang, M.S., Matzke, M., and Jacobsen, S.E. (2003). Role of the *DRM* and *CMT3* methyltransferases in RNA-directed DNA methylation. Curr. Biol. **13,** 2212–2217.

Ding, Y.-H., Liu, N.-Y., Tang, Z.-S., Liu, J., and Yang, W.-C. (2006). *Arabidopsis GLUTAMINE-RICH PROTEIN23* is essential for early embryogenesis and encodes a novel nuclear PPR motif protein that interacts with RNA polymerase II subunit III. Plant Cell **18,** 815–830.

Friml, J., Vieten, A., Sauer, M., Weijers, D., Schwarz, H., Hamann, T., Offringa, R., and Jurgens, G. (2003). Efflux-dependent auxin gradients establish the apical-basal axis of Arabidopsis. Nature **426,** 147–153.

Gehring, M., Choi, Y., and Fischer, R.L. (2004). Imprinting and seed development. Plant Cell **16** (suppl.), S203–S213.

Haecker, A., Gross-Hardt, R., Geiges, B., Sarkar, A., Breuninger, H., Herrmann, M., and Laux, T. (2004). Expression dynamics of WOX genes mark cell fate decisions during early embryonic patterning in *Arabidopsis thaliana.* Development **131,** 657–668.

Holstege, F.C.P., Jenning, E.G., Wyrick, J.J., Lee, T.I., Hengartner, C.J., Green, M.R., Golub, T.R., Lander, E.S., and Young, R.A. (1998). Dissecting the regulatory circuitry of a eukaryotic genome. Cell **95,** 717–728.

Li, E. (2002). Chromatin modification and epigenetic reprogramming in mammalian development. Nat. Rev. Genet. **3,** 662–673.

Li, E., Bestor, T.H., and Jaenisch, R. (1992). Targeted mutation of the DNA methyltransferase gene results in embryonic lethality. Cell **69,** 915–926.

Lukowitz, W., Roeder, A., Parmenter, D., and Somerville, C. (2004). A MAPKK kinase gene regulates extra-embryonic cell fate in *Arabidopsis.* Cell **116,** 109–119.

Lurin, C., et al. (2004). Genome-wide analysis of Arabidopsis pentatricopeptide repeat proteins reveals their essential role in organelle biogenesis. Plant Cell **16,** 2089–2103.

McElver, J., et al. (2001). Insertional mutagenesis of genes required for seed development in *Arabidopsis thaliana.* Genetics **159,** 1751–1763.

Murrell, A., Heeson, S., Bowden, L., Constância, M., Dean, W., Kelsey, G., and Reik, W. (2001). An intragenic methylated region in the imprinted *Igf2* gene augments transcription. EMBO Rep. **2,** 1101–1106.

IN THIS ISSUE

Okano, M., Bell, D.W., Haber, D.A., and Li, E. (1999). DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. Cell **99,** 247–257.

Small, I.D., and Peeters, N. (2000). The PPR motif: A TPR-related motif prevalent in plant organellar proteins. Trends Biochem. Sci. **2,** 46–47.

Springer, P.S., McCombie, W.R., Sundaresan, V., and Martienssen, R.A. (1995). Gene trap tagging of *PROLIFERA*, an essential *MCM2-3-5*–like gene in Arabidopsis. Science **268,** 877–880.

Sundaresan, V., Springer, P.S., Volpe, T., Haward, S., Jones, J.D.G., Dean, C., Ma, H., and Martienssen, R.A. (1995). Patterns of gene action in plant development revealed by enhancer trap and gene trap transposable elements. Genes Dev. **9,** 1797–1810.

Tzafrir, I., Dickerman, A., Brazhnik, O., Nguyen, Q., McElver, J., Frye, C., Patton, D., and Meinke, D. (2003). The *Arabidopsis* SeedGenes project. Nucleic Acids Res. **31,** 90–93.

Tzafrir, I., Pena-Muralla, R., Dickerman, A., Berg, M., Rogers, R., Hutchens, S., Sweeney, T.C., McElver, J., Aux, G., Patton, D., and Meinke, D. (2004). Identification of genes required for embryo development in Arabidopsis. Plant Physiol. **135,** 1206–1220.

Wang, Z., et al. (2006). Cytoplasmic male sterility of rice with Boro II cytoplasm is caused by a cytotoxic peptide and is restored by two related PPR motif genes via distinct modes of mRNA silencing. Plant Cell **18,** 676–687.

Xiao, W., Custard, K.D., Brown, R.C., Lemmon, B.E., Harada, J.J., Goldberg, R.B., and Fischer, R.L. (2006). DNA methylation is critical for *Arabidopsis* embryogenesis and seed viability. Plant Cell **18,** 805–814.