Genomic imprinting: Seeds of conflict
Santiago Mora-Garcia and Justin Goodrich

Recent studies have shown that the Arabidopsis MEDEA gene is imprinted, so that paternally and maternally inherited alleles are differentially expressed during seed development. Furthermore, a chromatin remodelling factor has been implicated as a novel trans-acting regulator of imprinting.

Address: Institute of Cell and Molecular Biology, University of Edinburgh, King's Buildings, Edinburgh EH9 3JH, UK. E-mail: Justin.Goodrich@ed.ac.uk

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In Greek mythology, Medea betrays her homeland to assist her lover, the adventurer Jason, to obtain the Golden fleece. In exile she bears him two children, but later discovers that Jason plans to marry the King of Corinth's daughter, so that their future children will become heirs to the throne. Seeking revenge, Medea poisons the bride and slaughters her children, leaving Jason with no chances of descent. Alluding to this classical example of infanticide, several genes with maternal effects upon embryo viability have been named after Medea. Recent studies [1,2] of the Arabidopsis MEDEA (MEA) gene have provided insights into the process of genomic imprinting in plants.

For most genes, paternally and maternally inherited copies of a given allele are expressed identically. In mammals, however, a handful of genes are subject to imprinting, so that only one copy is transcriptionally active, the other being silenced in a parent-of-origin-specific fashion. In many cases, imprinted genes have major effects upon embryo viability, and it is therefore puzzling why imprinting should occur, as it renders the organism functionally haploid for these loci. Perhaps the most elegant attempt to explain the evolution of imprinting is the parental conflict hypothesis of Haig and Westoby [3].

In flowering plants, as in mammals, the mother nourishes growing embryos for an extensive period after fertilisation, whereas the father experiences negligible costs. In cases where the progeny of a given female have different fathers, as in polygamous species, parental interests in the progeny conflict. The mother has an equal genetic stake in each embryo and is best served to allocate equal resources to each. By contrast, each father is better served if his particular embryos grow faster and extract a greater share of resources from the mother than do their siblings, in which he has no genetic stake. Such conflicts were suggested [3] to favour the evolution of differential gene expression, so that genes that restrict zygote growth become paternally silenced, whereas genes promoting growth would be silent from maternal alleles.

So far, evidence for imprinting in plants has largely been indirect and inferred from genome dosage effects. Consistent with the predictions of the parental conflict theory, crosses between Arabidopsis lines of different ploidy have shown that increases in paternal genome dosage give larger seeds, whereas seeds with increased maternal genome dosage are smaller [4]. The two recent studies [1,2] have taken an important step forward, demonstrating parent-specific gene expression in developing seeds.

In plants, gametes are produced in haploid multicellular structures called gametophytes. Pollen grains are the male gametophytes and contain two haploid sperm cells; female gametophytes, known as embryo sacs, are produced within carpels in the centre of flowers and in Arabidopsis comprise seven cells. Two of these, the egg cell and the central cell, participate in a double fertilisation unique to higher plants. The egg cell, fertilised by one sperm cell, will form the diploid embryo. The central cell, which is diploid as it forms from the fusion of two nuclei of the gametophyte, is fertilised by a second sperm cell to give rise to a triploid structure, the endosperm, with a crucial role in the transmission of nutrients and signals between the mother and the embryo (Figure 1). The ratio of two maternal to one paternal genomes in the endosperm seems critical; other combinations tend to lead to aborted seed development. In contrast, dosage alterations in the embryo seem to be less harmful [5]. This observation is cited as evidence for imprinting occurring chiefly in the endosperm.

A handful of mutations that act on the female gametophyte are known in Arabidopsis. In one case, half of the seeds set by plants heterozygous for the mutation are aborted with arrested embryos that show cell overproliferation and scanty endosperm. The mutation was named medea (mea-1), after Jason's filicidal lover, when Grossniklaus et al. [6] demonstrated that viability depended only on seeds inheriting at least one wild-type allele from the mother, regardless of the dosage of wild-type paternal MEA alleles present. One possibility is that maternal expression of MEA in the female gametophyte is required before fertilisation for normal embryo development; for example if MEA product is loaded into the egg and/or central cell. Alternatively, the MEA locus may be imprinted, so that paternal MEA alleles are silent during zygotic development.
Vieille-Calzada et al. [1] have now characterised MEA mRNA distribution during gametophyte and early seed development. MEA is expressed maternally in the embryo sac, most strongly in the central cell, but also in the egg cell. Following fertilisation, MEA mRNA was detected both in the developing endosperm and the embryo, the persistence and levels of expression suggesting zygotic transcription, not mere carry-over from the maternal load. More importantly, they were able to detect nascent MEA transcripts in the central cell nucleus: two signal dots were visible, presumably corresponding to the products from the two MEA copies in this diploid cell. Fusion of the pollen nucleus did not add another transcription focus, so that only two dots were observed in the derived triploid endosperm cells. This showed that MEA was expressed zygotically from the onset of endosperm development, and that the paternal allele was likely silenced. But because transcription foci could only be resolved at very early stages, the question remained as to how long imprinting persisted, and whether it also occurred in the embryo.

Kinoshita et al. [2] identified a sequence polymorphism that made it possible to distinguish the MEA transcript from two different Arabidopsis races in polymerase chain reaction (PCR)-based assays. After making reciprocal crosses between the two lines, they were able to detect MEA transcripts in the central cell nucleus: two signal dots were visible, presumably corresponding to the products from the two MEA copies in this diploid cell. Fusion of the pollen nucleus did not add another transcription focus, so that only two dots were observed in the derived triploid endosperm cells. This showed that MEA was expressed zygotically from the onset of endosperm development, and that the paternal allele was likely silenced. But because transcription foci could only be resolved at very early stages, the question remained as to how long imprinting persisted, and whether it also occurred in the embryo.

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Means of such imprints that restrict zygote growth is consistent with the predictions of the parental conflict hypothesis. The results of both groups [1,2] indicate that MEA is imprinted in the endosperm, but they disagree on whether this also occurs in the embryo. One possible explanation for the discrepancy is that different developmental stages were analysed: dissection is not possible in seeds as young as those used by Vieille-Calzada et al. [1]. This may be a critical time of embryo development where the parental contribution needs to be silenced, but is activated shortly after. These results deserve further analysis, because they define whether differential expression is relevant in the embryo, or only in the endosperm. Immature mea-1 homozygous embryos can be rescued from seeds before they abort and cultured in vitro to give rise to phenotypically normal, but female sterile, plants [6]. This suggests that the effects of mea mutations on the embryo are at least partly indirect, probably resulting from defects in the endosperm. One way to resolve this issue may be to test whether expression of MEA from endosperm-specific promoters rescues the embryo in a mea mutant background.

The parental conflict hypothesis predicts that the selection pressure for imprinting will depend on reproductive behaviour. Because Arabidopsis self-pollinates, all the offspring of a plant have the same father, and the parental conflict should be relaxed. Vieille-Calzada et al. [1] recovered a few viable seeds when pollen from different races was used to fertilise mea-1 homozygous plants. Kinoshita et al. [2],
working with just two races, detected late expression of the paternal allele in embryo sacs when crossed in one direction, but not in the other. Perhaps imprinting in Arabidopsis is an evolutionary relic inherited from outbred ancestors, and is in the process of breaking down, with slight differences among races.

This is the first time that a gene relevant to seed development has been shown to be imprinted. However, the fact that MEA is also expressed maternally before fertilisation raises the question of whether imprinting is relevant to the maternal mutant phenotype. The effects of mea mutations upon embryo and endosperm development might simply reflect a lack of previous MEA expression in the female gametophyte. Certainly maternal MEA expression has functional consequences, because mea mutant embryo sacs, unlike wild-type embryo sacs, undergo limited endosperm, and sometimes embryo [7], development in the absence of fertilisation.

To answer this question, Vielle-Calzada et al. [1] looked for factors that could weaken paternal silencing in the zygote. Differential methylation has been correlated with imprinting in mammals. Several screens in Arabidopsis for hypomethylated DNA [8] or reactivation of silent transgenes [9] recurrently found mutations in the gene DDM1 (for ‘decreased DNA methylation’). Unlike mammals, for which methylation defects are lethal, Arabidopsis ddm1 maternal allele in the endosperms of mature seeds when crossed has shown that repression is initiated by transcription factors which are transiently expressed at a specific stage of development, but is later taken over by Polycomb-group proteins, which lock mitotically stable states of repression. The best understood role of Polycomb-group proteins in animals is to maintain the repressed state of homeotic genes in cell lineages. Studies with Drosophila have shown that repression is initiated by Polycomb-group proteins, which lock mitotically stable states of repression in the seed is yet to be resolved.

The correlation of genomic imprinting and methylation observed in mammals seems to apply to plants, too. The protein product of DDM1 unexpectedly belongs to the SWI/SNF family of DNA-dependent ATPases, which alter DNA-nucleosome interactions and catalyse chromatin remodelling [10]. DDM1 may help make DNA more accessible to methyltransferases, especially in tightly packed regions of chromatin. Single-copy genes are less prone to demethylation, probably because they are in more relaxed chromatin domains. But like repetitive DNA sequences, MEA is affected in the first generation of ddm1 homoygotes. What does this say about the chromatin state around this imprinted gene? It will be interesting to study the methylation state of MEA in accessible tissues, such as pollen or developing seeds, as well as to conduct similar experiments with other available lines that have reduced methylation levels. Other mea alleles that are available produce different, even opposite, phenotypes [11]; it will be necessary to study the behaviour of the full allelic series in methylation-deficient backgrounds.

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On the issue of chromatin structure, the trail does not end with DDM1. Both MEA and FIE, a gene in which an independently isolated mutation has similar maternal effects on zygotic development [12], encode Polycomb-group proteins. The best understood role of Polycomb-group proteins in animals is to maintain the repressed state of homeotic genes in cell lineages. Studies with Drosophila have shown that repression is initiated by transcription factors which are transiently expressed at a specific stage of development, but is later taken over by Polycomb-group proteins, which lock mitotically stable states of repression [13]. In a sense, these proteins are also responsible for ‘imprinting’, as they act as reminders of past transcriptional states. No targets for MEA/FIE are known yet, but it is tempting to hypothesise, according to the parental conflict theory, that they might include growth-promoting genes, which would be repressed in the maternal complement. Many surprises are to come, as this Arabidopsis chromatin thread unwinds.

References