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Selected aspects of transgenerational epigenetic inheritance and resetting in plants

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Transgenerational epigenetic inheritance (TEI), which is the inheritance of expression states and thus traits that are not determined by the DNA sequence, is often postulated but the molecular mechanisms involved are only rarely verified. This especially applies to the heritability of environmentally induced traits, which have gained interest over the last years. Here we will discuss selected examples of epigenetic inheritance in plants and artificially divide them according to the occurrence of inter-generational resetting. The decision which epigenetic marks are reset and which ones are not is crucial for the understanding of TEI. We will consider examples of epialleles found in natural populations and epialleles induced by genetic and/or environmental factors used in experimental setups.

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Introduction

Epigenetic mechanisms can stably alter transcriptional activities and those can be transmitted through mitoses and sometimes also meiosis. Therefore, they can be propagated across generations as alternative epigenetic states of genes, so-called epialleles. Although epigenetic states are stable, at certain frequencies they can revert, thus that transgenerational epigenetic inheritance (TEI) has a stochastic component that has to be considered. The transgenerational transmission of traits that are not determined by DNA sequence, i.e. meiotic epigenetic inheritance, is of interest to both researchers and the public [1–3]. Various mechanisms contributing to epigenetic gene regulation and to TEI are reviewed in other chapters of this volume.

In plants, the germ line differentiates late in development as gametes are formed by the haploid gametophytes, which develop through mitotic divisions from the meiotic products [4,5]. Thus, TEI requires the propagation of epigenetic states over many mitotic DNA replication cycles, meiosis, and the post-meiotic mitoses during gametophyte development. The mechanisms responsible for the continuity in the propagation of epialleles during these various developmental transitions are poorly understood, with the exception of cytosine methylation in a CG sequence context. Replication of CG methylation patterns is performed by the maintenance METHYLTRANSFERASE1 (MET1), which uses post-replicative, hemimethylated DNA to copy methylation to the newly synthesized strand. Although it was recently suggested that *MET1* is down-regulated in the female gametes [6], it is currently not clear whether this truly reflects MET1 activity or the turnover of the complex fusion protein used. Moreover, a loss of *MET1* activity in the gametes seems incompatible with genetic evidence [7], such that, in plants, there is currently no compelling evidence for transgenerational resetting of CG methylation as it has been documented in mammals. Therefore, the formation and transmission of heritable epialleles is expected to correlate with the different levels and/or distribution of CG methylation and such marks should be resistant to transgenerational resetting. Indeed, *MET1* seems to be essential for the precision of TEI not only during vegetative growth but also during post-meiotic development of the gametophytes. Its depletion during male or female gametogenesis results in an alteration of the epigenetic states at a genome-wide scale in progeny plants [7]. It has been proposed that heritable patterns of CG methylation provide the epigenetic information scaffold that coordinates the distribution of other more dynamic epigenetic marks, such as DNA methylation in a non-CG context and various histone modifications [8]. The faithful propagation of CG methylation by MET1 requires additional activities, most notably the chromatin remodeling factor DECREASE IN DNA METHYLATION1 (DDM1). Consequently, studies of epialleles induced experimentally by mutations in either the *MET1* or *DDM1* gene substantially contributed to our current understanding of TEI in *Arabidopsis thaliana*, the model organism in which these studies were performed.

Owing to their possible role in adaptation, there is an increasing interest in studying the potential transgenerational inheritance of environmentally induced, epigenetic traits. Here we will briefly discuss selected examples of naturally occurring epialleles and epialleles induced by the genetic alteration of CG methylation levels. We will also provide a brief overview of recent experiments

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suggesting that environmental factors are possible inducers of heritable epialleles. To provide a broader perspective of TEI we will also discuss selected examples, factors, and *cis*-acting elements that are involved in the transgenerational resetting of epigenetic states which prevent TEI, and thus allow the acquisition of particular epigenetic states at every generation.

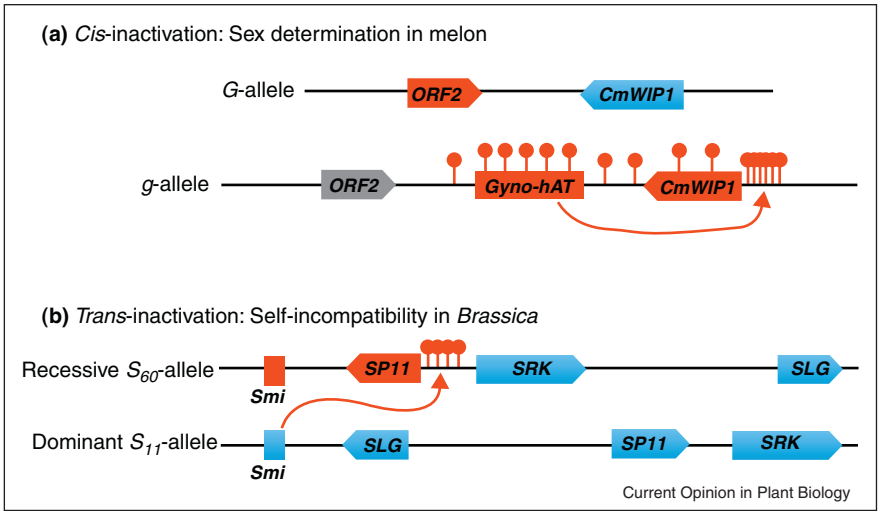
Naturally occurring epialleles

The first, classical example of an epiallelic gene variant causing the radially symmetric, peloric phenotype of *Linaria* flowers, which are usually bilaterally symmetric, has been attributed to hypermethylation of the *CYCLOIDEA* gene [9]. This study provided compelling evidence by linking the levels of DNA methylation of the *CYCLOIDEA* promoter to phenotypic alterations in flower symmetry. The various flower phenotypes and associated methylation levels were also documented for flowers on different branches of the same plant. Therefore, the epigenetic nature of *CYCLOIDEA* epialleles and the involvement of DNA methylation in their formation and maintenance for hundreds of years in nature is well documented and provides a prominent example of naturally occurring TEI in plants.

More recently, the map-based cloning of the *Colorless non-ripening* (*Cnr*) locus in tomato, responsible for fruit ripening, revealed that a hypermethylated epiallele determined the fruit phenotype [10]. *Cnr* hypermethylation affected cytosines in CG sequences, thus that the hypermethylated state may have been propagated by the tomato MET1 homolog. Another interesting feature of the *Cnr* locus is the presence of a COPIA-like retrotransposon in the vicinity the *CNR* gene, which mapped to the middle between two recombination breakpoints encompassing the *CNR* region. Actually, one of the recombination break points was in the promoter of the *CNR* gene, such that the protein-coding part of the *CNR* gene was outside the recombinationally defined region responsible for the *Cnr* phenotype. It would have been desirable to examine the methylation status of the retrotransposon, which is likely influencing methylation levels of neighboring genes including *CNR* (see below).

The epigenetically silenced *CNR* gene in the vicinity of a transposon resembles the structure of another naturally occurring epiallele that is responsible for sex determination in melon. It has been shown that the presence of a *hAT* transposon at the locus is essential for the initiation and spreading of DNA hypermethylation to the promoter of the neighboring *CmWIP1* gene encoding a transcription factor controlling the development of the floral sexual organs (Figure 1a) [11••]. Therefore, a genetic element triggers epigenetic modifications in this case, resulting in the transcriptional repression of a regulatory gene that has a decisive influence on flower development. Interestingly, this example resembles the well characterized epigenetic regulation of the *Arabidopsis FLOWERING WAGENINGEN* (*FWA*) locus. Although the *fwa* alleles were recovered in a mutagenesis screen, epigenetic regulation of this locus is very well documented and thus will be briefly discussed here. *FWA* is a maternally expressed gene, likely regulated by genomic imprinting, which is transcriptionally silenced during sporophytic development and expressed only in the female gametophyte and endosperm. Sporophytic

Figure 1



Examples of epialleles that are controlled by DNA sequence elements in *cis* and *trans*. **(a)** Sex determination in melon is controlled by two loci (*a*, *andromonoecious*; *g*, *gynoecious*). The recessive *g* allele carries a transposon (*Gyno-hAT*), a *cis*-acting genetic element, from which methylation spreads into the sex determining *CmWIP1* gene leading to its silencing (after [11••]). **(b)** Self-incompatibility in *Brassica* is determined by allele-specific interactions of the stigma-expressed *S*-Receptor-Kinase (SRK) and the secreted pollen factor SP11 (also known as SCR [66]). In the recessive S_{60}-allele, the *SP11* promoter gets methylated in *trans* by RdDM guided by siRNAs produced from the *Smi* gene on the dominant S_{11}-allele (after [15••]). Expressed genes are in blue, silenced loci in red, and grey indicates that the expression level was not reported, red lollypops symbolize DNA methylation.

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silencing of *FWA* promotes early transition to flowering and correlates with hypermethylation of its promoter. In *Arabidopsis* mutants affected in the maintenance of CG methylation, *FWA* becomes transcriptionally active and flowering is delayed. The *FWA* promoter includes a transposon-derived DNA sequence. It has been demonstrated that DNA methylation is targeted to this particular sequence and maintained during the entire vegetative development allowing for early flowering [12]. This is yet another example where a transposon or transposon-related sequence provides genetic information in *cis* that directs epigenetic modifications. Remarkably, in both melon and *Arabidopsis*, these transposon-related sequences influence developmental programs involved in reproduction via the formation of heritable epialleles.

Another recent and intriguing example for epigenetic regulation modulating plant reproduction was identified by studying the dominance relationships of *S*-alleles determining self-incompatibility in Brassicaceae. Determinants of self-incompatibility can behave as dominant or recessive alleles and this is achieved by monoallelic transcription in certain heterozygous combinations [13]. Subsequently, it has been shown that transcriptional silencing was accompanied by hypermethylation of the recessive alleles [14]. However, the specificity of methylation targeted to the recessive alleles remained a mystery. Recently, it has been demonstrated that a small RNA produced from a precursor RNA transcript of the *Smi* gene residing at the *S*-locus has perfect homology to the methylated region of the recessive allele (Figure 1b) [15••]. Therefore, also in this system, a genetic element directs developmentally programmed epigenetic modifications that influence plant reproduction. However, here there is no direct link to transposon-related sequences nor is the silencing of recessive alleles meiotically heritable. Rather, the system seems to involve RNA-directed DNA methylation (RdDM), which is known to affect DNA methylation in any sequence context but mostly in non-CG sequences. It is well documented that RdDM, being directed by small RNAs, provides DNA methylation patterns that are much more flexible than methylation at CGs, which is maintained by MET1. Therefore, in this case the epigenetic regulation of self-incompatibility is based on the dynamic interaction of two chromosomal loci in *trans* mediated by small RNAs. Such transcriptional regulation resembles paramutation [16]; however, in contrast to paramutation where the paramutated epiallele becomes stable and is inherited in an autonomous fashion (i.e. in the absence of the paramutagenic allele), the *trans*-interaction of S-alleles requires the supply of small RNAs.

In summary, where investigated in detail, it appears that naturally occurring epialleles can be linked to the presence of a genetic element acting in *cis*, for example a transposon or transposon-related sequence, or in *trans*, such as the siRNAs derived from the *Smi* gene at the *Brassica rapa S*-locus.

Genetically induced epialleles

The above-mentioned examples of epialleles, controlled through a genetic element in *cis* or *trans*, provide the most convincing examples of TEI. However, these specific examples do not address the molecular mechanisms and factors involved in the creation, maintenance, and stability of epialleles in general. It is also not clear whether there is a broader spectrum of loci that are subjected to similar mechanisms of epiallelic regulation within the genome. Experiments addressing these issues exploit mutations in genes encoding various epigenetic regulators. Such studies showed that the chief components involved in the formation of epialleles, which can be propagated over many generations in the absence of the mutation initially triggering the epigenetic changes, are linked to the maintenance of CG methylation. The two best studied factors required for the maintenance of CG methylation are DDM1 and MET1, such that *ddm1* and *met1* mutants were used to establish plant strains with genome-wide alterations of epigenetic marks [17–19,20•,21].

Since MET1 maintains CG methylation patterns following DNA replication, its depletion results in the rapid, uniform and genome-wide depletion of this epigenetic mark due to its passive loss at each replication cycle. Therefore, in loss-of-function *met1* mutants, CG methylation is completely erased in the first homozygous mutant generation. In contrast, the loss of DNA methylation in *ddm1* mutants progresses gradually over several generations and occurs at different rates at distinct chromosomal regions. Hypermethylated centromeric and pericentromeric repeats lose methylation marks rapidly whereas the euchromatic chromosomal arms are demethylated at a slower rate. It was observed that after self-pollination of *ddm1* over several generations, when centromeric and pericentromeric repeats lost most or all DNA methylation, the loss in DNA methylation cannot be restored even when demethylated regions are introduced back to the wild type [22]. Thus, their acquired hypomethylated state (new epiallelic state) can be inherited for many generations in the presence of all epigenetic components. This observation suggests that a certain level of CG methylation depletion results in the loss of information guiding remethylation. A parallel situation was observed when demethylation was triggered by a *met1* null mutation [7]. This is in contrast to epigenetic changes triggered by the depletion of various other epigenetic regulators, such as those affecting non-CG methylation or histone modifications; such epigenetic states that can rapidly revert when the wild-type functions are restored. Therefore, it became apparent that in order to create and study hidden epiallelic diversity, *ddm1* and *met1* mutations are most suitable for the generation of stable

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epiallelic variants. Following this rationale two isogenic recombinant inbred populations with chimeric epigenomes derived from meiotic recombination between wild-type and *ddm1*-derived or *met1*-derived chromosomes were constructed [17,18].

In general, the data obtained from studies of the two epigenetic recombinant inbred populations (epiRILS) led to similar conclusions. For instance, both populations demonstrated that mutant-derived, hypomethylated epialleles were stably inherited over eight generations of inbreeding. Furthermore, a similar range of novel phenotypes was observed; however, these were so far not mapped and, thus, could not be attributed unequivocally to particular epiallelic gene variants. However, some distinct characteristics for each population were also unraveled (discussed in [21]). These differences were most likely due to the properties of the initial mutations used for the construction of the epiRIL populations. Moreover, mapping turned out to be more difficult than anticipated due to firstly, the observed remethylation of chromosomes derived from the mutants [17–19] and secondly, to methylation losses on chromosomal segments derived from the wild type [21], which were observed during inbreeding. In addition, for certain traits, significant phenotypic instability has been observed that could also obstruct mapping approaches (Jon Reinders and JP, unpublished). Surprisingly, in both populations the most apparent and the most stable phenotypic variants were late flowering epiRILs. Their phenotypes can be traced to the *FWA* gene and the demethylation of the transposon sequences in its promoter. Since in both populations dynamic changes in DNA methylation were observed during inbreeding, it is still not clear whether any loci other than *FWA* can be classified as stable epialleles responsible for a given phenotype. Obviously, discovery and characterization of additional epialleles in these populations are ongoing and, if successful, this could also shed light onto DNA sequence elements that are possibly responsible for epiallelic diversity. So far, although intriguing, it remains unanswered in how far transposon-related sequences and/or particular chromosomal repeats and their specific arrangements are able to provide the critical information for directing alternative, heritable epigenetic states.

Environmentally induced epialleles

It has been a long-lasting desire to induce superior heritable traits through subjecting plants to various environmental influences. In recent years a considerable number of studies reported the environmentally triggered acquisition of new, heritable plant properties or persistent changes in DNA methylation [23–29]. In some cases these traits could be inherited for several generations [23–25,28,29]. Inheritance over a number of generations is an indispensable criterion in claiming transgenerational inheritance, since only then the direct parental influence, that is that of the treated individual, can be neglected. Since the studies were directed toward the establishment of new, stable epialleles, most of them used clones (e.g. apomicts [24]) or well-defined starting material securing minimal genetic diversity. Furthermore, a relatively big number of plants grown under well-controlled conditions were analyzed to increase statistical power [23–29]. Reported results suggested the acquisition of various heritable traits after diverse treatments, including biotic and abiotic stresses. These outcomes were interpreted predominantly as being consistent with epigenetic inheritance. Intriguingly, it has been suggested that an acquired hyper-recombination phenotype is transmitted through male and female gametes and acts on newly crossed-in recombination substrates in *trans*, suggesting the induction of a heritable mechanism with abilities to alter naïve loci [23]. Moreover, in experiments with tobacco where heritable traits were initially induced by virus infection, subsequent grafting experiments suggested that not the infection itself but a systemic signal is responsible for triggering new stable traits [28]. These results, suggesting environmental induction of heritable phenotypes or changes in DNA methylation levels and/or distribution, are very exciting and meet increasing attention. They also stimulated follow-up experiments. Unfortunately, the results described in the first report aiming to reproduce the experimental setup, which triggered the acquisition of heritable hyper-recombination [23], did not confirm the generality of the previous observation [30•]. Obviously, many uncontrolled factors that may differ between the two experimental setups performed in two different laboratories may have contributed to the conflicting outcomes. Nevertheless, in order to experimentally reveal the molecular mechanisms responsible for the acquisition and transgenerational maintenance of the environmentally induced heritable traits, the robustness of the initial findings is of critical importance.

So far, although epigenetic mechanisms were often postulated as being involved in trait acquisition and transgenerational inheritance, the experimental evidence for this is scarce. In this respect it is essential to consider the recent reports suggesting that environmentally induced destabilization of suppressive epigenetic marks leading to the alleviation of transcriptional gene silencing occurs only transiently. To date, a release of silencing has only been observed after particular environmental stress regimes, even if well-defined loci known to be silenced by epigenetic mechanisms were studied [26,31,32]. Moreover, although epigenetic regulation at these loci could be altered under environmental stress, there are no indications for an induction of stable epialleles [31,32]. Therefore, mechanisms of repeatedly reported environmental inductions of heritable traits may use novel, unanticipated mechanisms, which may not be directly related to epigenetic inheritance.

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Resetting of environmentally induced, epigenetic states

Although a series of studies suggest that TEI of environmentally induced traits may exist (see above), it is clear that most of the plastic responses a plant shows to environmental challenges are not transmitted to the progeny and are, thus, reset between generations. At present, it is unclear what determines whether an epigenetic state is transmitted or reset/erased. It is possible that this decision is linked to GC DNA methylation, but this proposition is based on the study of only a small number of transgenerationally inherited epialleles (see above). In order to better understand TEI and, thus, to define which loci are excluded from erasure, it will be important to identify the factors and *cis*-acting elements that are involved in resetting.

One of the best-studied examples of an environmentally induced state is caused by vernalization, the acceleration of flowering by the exposure of a plant to a long period of cold. This response has long been known to be epigenetically inherited over many cell divisions, as the memory of an exposure to cold is kept throughout the life span of winter annuals — long after the initial stimulus is gone — even through clonal propagation and cell culture (reviewed in [33]). The progeny of a vernalized plant, however, loses this memory and the epigenetic state is thus reset. A key mediator of the vernalization response is *Flowering Locus C* (*FLC*), a repressor of flowering encoded by a MADS-box gene. Exposure to cold triggers the progressive enrichment of trimethylation at Lys 27 of histone H3 (H3K27me3), resulting in the silencing of the *FLC* locus, whose expression decreases quantitatively with an increasing period of cold treatment. The H3K27me3 modification is mediated by a specific variant of the evolutionary conserved *Polycomb* Repressive Complex 2 (PRC2), which maintains the repressed state of *FLC* over many cell divisions (reviewed in [34,35]).

In contrast to the establishment and maintenance of *FLC* silencing, resetting from one generation to the next is less well understood at the molecular level. Recently, it was shown that independent of its expression state in adult plants, *FLC* expression cannot be detected in male and female gametophytes [36,37•]. After fertilization, both maternal and paternal *FLC* alleles are expressed in the embryo but not the endosperm, the second product of double fertilization in flowering plants. Thus, it can be inferred that the repressive H3K27me3 marks are removed sometime during reproductive development and that the epigenetic state of *FLC* is reset. When precisely this resetting occurs is currently not clear. Although *FLC* expression is only re-established post-fertilization in the embryo, the mark may already have been removed earlier during sporogenesis or gametogenesis. Removal of the repressive marks may not result in *FLC* expression if *trans*-activating factors are not present at this stage of development. Interestingly, key factors that are required for *FLC* activation in adult plants, such as FRIGIDA and SUPPRESSOR OF FRIGIDA4, are not required for *FLC* reactivation in the early embryo, such that the regulation of *FLC* during gametogenesis and embryogenesis is not well understood. A complex containing PHOTOPERIOD-INDEPENDENT EARLY FLOWERING1 (PIE1) appears to be involved in *FLC* activation because in *pie1* mutants, *FLC* is not fully reactivated [37•]. As the PIE1-containing complex mediates the exchange of histones H2A with H2A.Z at the *FLC* locus in adult plants [38], such an exchange of histone variants may be involved in the epigenetic resetting of *FLC*. A similar exchange of histone variants occurs before the production of germ cells in the mouse, where it may also be involved in the erasure of epigenetic marks [39]. The recent finding that specific H3 variants present in plant gametes are removed from the chromatin of the zygote [40•] indicates that the removal and exchange of histone variants may be an integral part of the resetting mechanism. However, its effects on epigenetically regulated gene expression and, thus, transgenerational inheritance or resetting remain to be elucidated.

Resetting of imprinted alleles in the germ line?

While certain epialleles are stably inherited over many generations, other epigenetic marks have to be erased and reset in each generation. This is, for instance, the case with imprinted loci in mammals, where the expression state of an allele depends on its parental origin. The corresponding imprints have to be reset in the germ line of the individual to ensure that the alleles are transmitted to the progeny in the appropriate imprinting state according to the sex of that parent. Until recently, many considered genomic imprinting in plants as endosperm-specific and, because the endosperm is a terminal tissue, the resetting of imprints was not widely considered; a notion that should be reassessed.

Epialleles of *FWA*, recovered after mutagenesis or through removal of DNA methylation in *ddm1* or *met1* mutant backgrounds, are stably inherited over many generations [41–43]. Thus, the epigenetic state of the *FWA* gene, controlled by the methylation of transposon-derived sequences that contribute the first two exons of *FWA*, is not reset before or during embryogenesis. This is in contrast to the situation in the wild type, where the *FWA* locus is silenced in all tissues and gets specifically reactivated in the female gametophyte's central cell, which gives rise to the endosperm after fertilization. As a result, only maternally derived *FWA* transcripts are detected during seed development. In principle, these transcripts could be stored in the central cell and used up after fertilization and/or they could be derived from post-fertilization monoallelic transcription in the endosperm, that is imprinted expression of *FWA* [44]. Although active transcription of the *FWA* locus after fertilization awaits

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demonstration, its specific demethylation in the central by the DNA glycosylase DEMETER (DME) and the stability at which a demethylated state can be propagated, makes *FWA* very likely regulated by genomic imprinting [45]. *DME*-dependent demethylation of maternal alleles in the central cell has also been proposed for several other imprinted genes (recently reviewed in [46–48]), although the situation may be more complex for some imprinted loci because DNA methylation and demethylation does not always correlate with gene expression (discussed in [49]).

Importantly, recent investigations of genome-wide methylation patterns in the embryo and endosperm of *Arabidopsis* revealed the extensive demethylation of repetitive elements in the endosperm, which depends to a large extent on DME [50•,51••]. It has been proposed that DME targets transposable elements or transposon-derived sequences. This can bring neighboring genes under their control leading to imprinted expression [51••], as it was previously as demonstrated for *FWA* [47,52]. Future molecular investigations of the newly identified candidate genes proposed to be regulated by genomic imprinting will shed light onto the exact role transposon-derived sequences play in imprinting regulation. But it is conceivable that this epigenetic regulation is largely mediated by genetic elements derived from transposons and repeats, although this may not be true for every imprinted locus [53] and alternative mechanisms likely exist, too. Furthermore, a large variety of 24 nucleotide siRNAs, which play a central role in RdDM, was detected in developing endosperm [54•]. These RNA polymerase IV-dependent siRNAs are derived from the maternal genome only and, thus, their production dependent on imprinted maternal expression. How their parent-of-origin-specific production is regulated at the molecular level and how these siRNAs may be interrelated with the demethylation of transposons and repeats in the endosperm is currently not known.

As imprinted expression appears to largely depend on demethylation by DME, which was reported to be preferentially expressed in the central cell [55], many researchers consider genomic imprinting as specific to the endosperm that develops from the fertilized central cell. In this situation, the imprinting mark(s) reflecting parental origin will not have to be erased and reset because the endosperm does not contribute to the next generation. However, if imprinted expression also occurred in plant embryos, erasure and resetting mechanisms would also have to exist in plants as they do in mammals. To date, two plant genes have been proposed to show imprinted expression not only in the endosperm but also in the embryo. For the *MEDEA* (*MEA*) locus, reports on expression and imprinting in the embryo are controversial (for a detailed discussion see [49]). While selected transgenic lines may show reporter gene expression under the control of the *MEA* promoter that is specific to central cell and endosperm (e.g. in [56]), there are several reports showing *MEA* mRNA and reporter gene expression in both embryo and endosperm, strongly suggesting expression in both fertilization products [57–61]. While expression in isolated embryos at seven to eight days after pollination was found to be biallelic [58,63], paternal *MEA* expression could not be detected in other experiments [57], even using highly sensitive quantitative RT-PCR [62]. Such differences may depend on the genetic background used, as many accessions carry genetic modifiers of *mea* [53,57,63], but imprinting in the embryo appears to occur at least in some accessions. The situation is clearer for a recently identified maize gene, *Maternally expressed in embryo1* (*Mee1*), which is expressed both in the embryo and endosperm. During embryogenesis, *Mee1* shows transient expression and is activated only at three days after pollination, excluding maternally deposited RNAs [64•]. Maternal alleles get demethylated after fertilization and remethylated in the embryo but not the endosperm later during development [64•]. As *Mee1* is methylated in both egg and sperm cells, this suggests allele-specific demethylation, the specificity of which thus must rely on germ line marks other than DNA methylation. Although DNA methylation gets re-established during embryogenesis, the resetting of the unknown germ line marks must occur later during development. Only a resetting mechanism that occurs after the lineages for male and female reproductive organs have separated can explain parent-of-origin-specific germ line marks. At present, neither the nature nor the timing of such a resetting mechanism is known.

Conclusions and outlook

Studies over the last few years have clearly shown that epigenetic variation exists and can be stably transmitted over several generations. As outlined above, stable transmission is often related to altered DNA methylation in a GC context. On the other hand, other epigenetic marks are reset between generations, as has been shown for some histone modifications and histone variants that are erased or exchanged in the embryo, respectively. However, this is not true for all histone modifications. It was recently shown that some epigenetic marks are differentially established during gametogenesis, leading to epigenetically differentiated egg and central cells [65••]. These specific epigenetic states, including differential patterns of H3K9me2, H2B turnover, and LIKE HETEROCHROMATIN1 localization, is transmitted to embryo and endosperm, respectively. This raises a set of interesting questions. Which epigenetic information is reset and which is transmitted to the next generation? Which parts of the genome are reprogrammed and which are subjected to TEI? Is the relevant information contained in the genome sequence?

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An important additional aspect of TEI is its contribution to traits induced by external stress factors. Such acquired TEI, when understood, will provide novel aspects of long-term environmental adaptation. However, various discussions link current research results and experimental approaches in this area to those of Trofim D. Lysenko, who questioned Mendelian laws and — based on not rigorously performed experiments — postulated the environmental acquisition of heritable traits. Therefore, studies in this area have also an unfortunate history. Consequently, it is crucial that progress in this field is now based on strong experimental evidence including the characterization of the molecular basis of the observed phenomena. Recent reports show that investigations along this challenging path begin.

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