Genomic Imprinting

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What You Will Learn in This Chapter

A typical cell contains two sets of chromosomes: one that was inherited from the mother, the other from the father. Usually, autosomal alleles are expressed at similar levels from the maternally and paternally inherited chromosomes. This chapter is dedicated to an exception of this rule: the expression of genes that are regulated by genomic imprinting depends on the parental origin of the allele, leading to the non-equivalence of maternal and paternal genomes. Genomic imprinting is a paradigm of epigenetic gene regulation as genetically identical alleles can exist in two expression states within the same nucleus. The imprints marking the parental alleles are established in the parental germline, maintained during the development of the offspring, but reset before they are passed on to the next generation. In mammals, the primary imprint is usually a differentially methylated region at the locus but there are also examples where histone modifications mark the parental alleles. Many imprinted genes play important roles for development and are associated with human disease. Interestingly, genomic imprinting evolved independently in mammals and seed plants and similar mechanisms have been recruited to regulate imprinted expression in the two kingdoms. We will discuss evolutionary constraints that could have led to the evolution of genomic imprinting in these seemingly disparate lineages.

5.1 Discovery of the Non-equivalence of Maternal and Paternal Genomes

In general, the genetic contributions from father and mother are equivalent (except for the sex chromosomes; see book ► Chap. 4 of Wutz), each contributing a chromosome set to the progeny. Genetically, this manifests itself by the recessive nature of the vast majority of loss-of-function mutations, i.e., if a mutant allele is only inherited from one of the parents, the offspring is phenotypically wild-type. Studies on traits that did show surprising parent-of-origin-specific effects suggested the non-equivalence of the parental genomes and led to the discovery of genomic imprinting.

5.1.1 Genome-Wide Imprinting in Insects

The first evidence that parental contributions may not be equivalent came from cytogenetic observations that Charles Metz made nearly 100 years ago. He found that, during the first meiotic division in the male germline of the fungus gnat *Sciara coprophila*, an entire chromosome set was eliminated. Genetic studies using the autosomal recessive *truncate* mutant, which affects wing shape, showed that only the maternal allele was transmitted by the sperm while the paternal chromosomes were lost. Metz concluded (Metz 1938): "… that the difference in behavior [of the chromosomes] is due to a general qualitative difference between maternal and paternal chromosomes …, and that this [difference] is impressed on the chromosomes in the preceding generation by the sex of the parent." And he continued: "It should be emphasized that this qualitative difference or modification persists for only one generation and is reversible." Although the term "imprint" was only introduced by Helen Crouse when studying the genetic determinants of paternal X-chromosome elimination in *S. coprophila*, Metz' conclusions set the foundations of the imprinting field, namely:

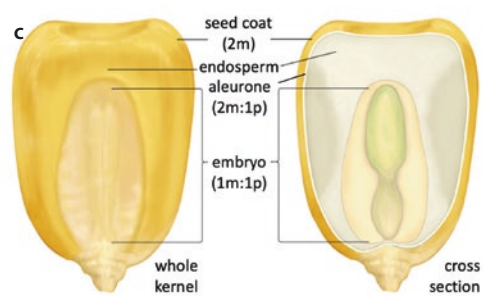
1. The differential behavior of the chromosomes implies the existence of differential modifications, i.e., imprints, on the chromosomes.
2. Imprints may be on the maternal or paternal chromosomes, or both; the imprint represents a difference between them, not an absolute state.
3. Imprints are established in the parents but persist during development of the progeny. Thus, they must be maintained during cell division as the genetic material is replicated.
4. Imprints are reversible and have to be reset in every generation: both maternal and paternal chromosome sets of a male are passed on to the progeny with paternal imprints, those of a female with maternal imprints.

Parental imprints were later also postulated in coccids, scale insects where the paternal genome is heterochromatinized and silenced or even eliminated early during embryogenesis. Although maternal and paternal chromosome sets in these embryos are distinctly marked (e.g., by differences in DNA methylation, H3K9me3, and H4ac), the mechanisms underlying the establishment, maintenance, and resetting of the imprints are not well understood (Sánchez 2014). While studies in insects provided a conceptual framework for imprints that distinguish maternal and paternal chromosomes, the focus of this chapter is not on genome-wide imprinting but rather on the imprints that distinguish individual genes or gene clusters and ultimately lead to the parent-of-origin-dependent expression of the corresponding alleles.

5.1.2 Discovery of Genomic Imprinting at an Individual Locus in Maize

Genomic imprinting regulating an individual gene rather than an entire chromosome set was first discovered when studying seed coloration in maize (*Zea mays*). In flowering plants, seeds develop from ovules after double fertilization, involving the fusion of two pairs of gametes: one haploid sperm fuses with the haploid egg cell to form the diploid embryo whereas the second haploid sperm unites with the homo-diploid central cell and develops into the triploid endosperm. The endosperm is a placenta-like tissue supporting the development of the embryo by providing nutrients. Both fertilization products are surrounded by the protective seed coat, a maternal tissue derived from the integuments of the ovule. In the 1960s, Jerry Kermicle studied various alleles of the *colored1* (*r1*) locus in maize. The *R-r:standard* allele (hereafter referred to as *R*) controls the production of anthocyanin pigments in the aleurone, the outermost layer of the endosperm in maize seeds (kernels). If crossed to the recessive null allele *r-g* (hereafter referred to as *r*), Kermicle obtained fully colored kernels if *R* was inherited from the mother and mottled kernels with purple-brownish spots when *R* was inherited from the father (◘ Fig. 5.1).

Such a difference in phenotype depending on the direction of the cross indicates a parental effect. In this case, there is a maternal effect on *R* expression as most aleurone cells do not express *R* if it is inherited from the father. However, there are several fundamentally different mechanisms that could lead to such a maternal effect and Kermicle used an elegant series of genetic experiments to distinguish between them (Kermicle 1970).



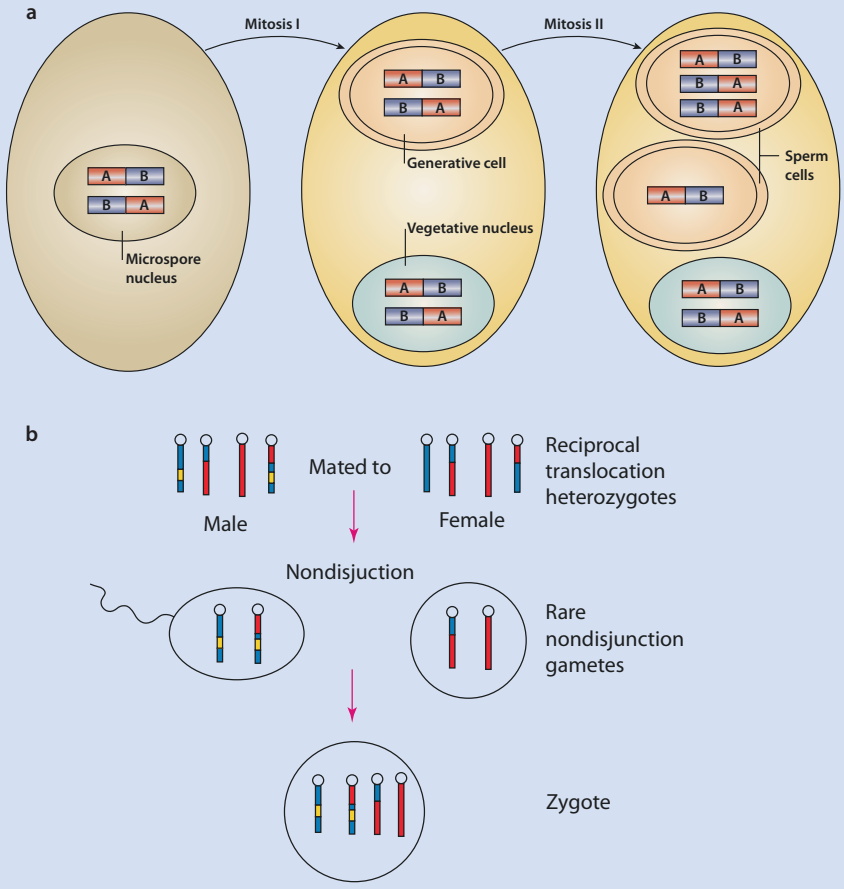
d

Female	Male	Endosperm genotype (m:p)	Kernel phenotype
R R	*r r*	*R R:r*	
r r	*R R*	*r r:R*	
r r	*R* TR	*r r*:TR TR	
		r r:R	
R TR	*r r*	TR TR:*r*	
		R R:r	
R R, mdr1 mdr1	*r r, mdr1 mdr1*	*R R, mdr1 mdr1:r, mdr1*	
R R, mdr1 mdr1	*r r*, MDR 1 MDR1	*R R, mdr1 mdr1:r*, MDRr1	

Fig. 5.1 Imprinting at the *r1* locus. **a** Kernel with maternal (full pigmentation) and **b** with paternal inheritance of the *R* allele (mottled pigmentation) after a cross with the recessive, colorless *r* allele. (From Evans and Grossniklaus 2008). **c** Schematic representation of a maize kernel showing the composite structure of the seed with the purely maternal (2m) seed coat, the triploid endosperm including the pigmented aleurone with 2 maternal and 1 paternal genomes (2m:1p), and the diploid embryo (1m:1p). (From Encyclopedia Brittanica). **d** Results of crosses involving *R* and *r* alleles as well as the T*R* translocation where the long arm of A-chromosome 10 containing the *R* locus is translocated onto a non-essential B-chromosome. Such B-A translocation stocks can produce sperm cells with either two T*R*s or none, allowing the generation of kernels with different *R* dosage. The *maternal derepression of r1* (*mdr1*) mutation disrupts a *trans*-acting factor of imprinting required for full expression of the maternally inherited *R* allele. (From Baroux et al. 2002)

The maternal effect could be due to a dosage effect in the triploid endosperm as it contains two chromosome sets from the mother and only one from the father. Therefore, the copy number of *R* differs in reciprocal crosses and it is possible that full pigmentation requires a threshold that is only reached if two *R* copies are present. To test this hypothesis, Kermicle used B-A translocations (▶ Box 5.1) to generate kernels with two paternal copies of *R*. As the phenotype remained mottled, he excluded that different dosage was the basis of the maternal effect (Fig. 5.1).

Methods Box 5.1: Genetic Analyses Using Translocation Stocks (Box Fig. 5.1)



Box Fig. 5.1 In both maize and mice, genetic studies that were crucial for the demonstration of genomic imprinting relied on translocation stocks. **a** Maize B-chromosomes do not carry any essential genes but can participate in translocations with the normal A-chromosomes. In the meiotic product (microspore) shown below, only the reciprocal A-B and B-A translocation chromosomes are shown. Microspores undergo two mitotic divisions to form the sperm. In mitosis I, the vegetative and generative lineage separate, while in mitosis II, the generative cell divides once more to form the two sperm cells that will participate in double fertilization. The B-chromosome can impart a peculiar behavior onto the translocated part of the A-chromosome that does not contain a centromere: during mitosis II, non-disjunction produces one sperm with two and one without a B-A translocation chromosome, allowing the manipulation of dosage for the genes present on the translocated part of the A-chromosome. (From Walbot and Evans 2003). **b** In mice, balanced reciprocal translocation heterozygotes were used to map chromosomal regions that contain imprinted loci. Crossing such translocation stocks carrying appropriate markers (yellow segment in the example below) generates some progeny that have a fully balanced genome, i.e., all chromosomal regions are present in two copies, but the translocated segments (distal parts of red and blue chromosomes) are of the same parental origin. If such progeny shows phenotypic abnormalities, the corresponding segments must contain imprinted genes required for normal development or behavior. (From Oakey and Beechy 2002)

Alternatively, the maternal effect could be of cytoplasmic nature, i.e., it depends on organelles, proteins, or RNAs produced prior to fertilization and deposited in the female gametes. If full pigmentation depended on a product that is maternally stored in the central cell but required only later in endosperm development, the phenotype would be independent of the presence of the maternal *R* allele after fertilization. To test this, Kermicle used a genetic trick to induce the loss of the chromosome fragment carrying the *R* locus shortly after fertilization and found that the sectors in which the maternal *R* copies had been lost were mottled. As *R* was present when the female gametes were formed, this experiment ruled out that a cytoplasmically stored product was responsible for the maternal effect.

Given that the maternal effect on *R* expression was neither due to dosage nor of a cytoplasmic nature, Kermicle concluded that it was of chromosomal nature, i.e., that the *R* locus itself was somehow modified during gamete formation and carried an imprint that was maintained during development and ultimately affected the expression of *R* later during aleurone formation. These findings were confirmed by the isolation of the *maternal derepression of r1* (*mdr1*) mutant, disrupting a factor required for the establishment of the active state of the maternal *R* allele. Even if a wild-type *MDR1* copy was present after fertilization, only mottled kernels were produced by *mdr1* mutant females. This indicates that the active state of *R* is set during female gametogenesis, even though the maternal *R* allele is only expressed much later in development.

In summary, through sophisticated genetic experiments, Kermicle clearly demonstrated that the expression of the very same *R* allele differs depending on its parental origin. He thus identified the first case of genomic imprinting affecting an individual locus rather than an entire chromosome set, demonstrating that this type of epigenetic gene regulation can act on single genes. This is clearly different from the genome-wide effects first described in insects and also from chromosome-wide repression, such as imprinted or random X chromosome inactivation (see book ▶ Chap. 4 of Wutz).

5.1.3 Demonstrating the Non-equivalence of Parental Genomes in Mammals

In mammals, chromosome-wide imprinting was also described in the 1970s when X-chromosome inactivation (see book ▶ Chap. 4 of Wutz) was found to be paternal-specific in marsupials and in the extraembryonic tissues of the mouse (Cooper et al. 1971). Furthermore, using translocation stocks (▶ Box 5.1), mouse geneticists identified regions with parental-specific phenotypes and described a maternal effect in the "hairpin-tail" mouse. However, they did not conclude that this was due to genomic imprinting but favored the interpretation of a cytoplasmic maternal effect. This view only changed with the seminal nuclear transfer experiments performed in Davor Solter's and Azim Surani's groups, which unequivocally showed that maternal and paternal genomes of the mouse are not equivalent (McGrath and Solter 1984; Surani et al. 1984).

After fertilization, the nucleus contributed by the sperm decondenses and forms a pronucleus that is larger than the female pronucleus, making them easily distinguishable. As these pronuclei remain observable for more than 12 hours, the parental genomes in the zygote can be manipulated. Using micropipettes to remove and add pronuclei, Surani and Solter generated zygotes that had either two maternal, two paternal, or one maternal and paternal pronucleus each (◘ Fig. 5.2).

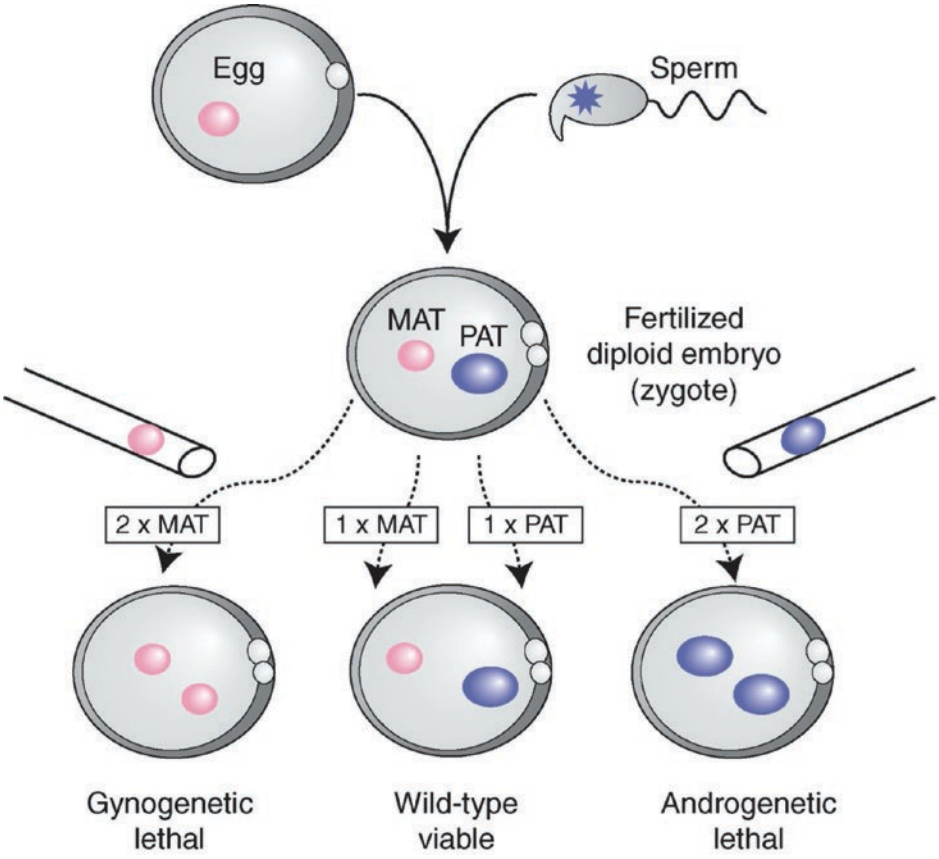


Fig. 5.2 Both maternal and paternal genomes are required for normal embryogenesis in the mouse. The zygote contains a maternal (pink) and paternal (purple) pronucleus that can be manipulated using a micropipette. By removing and adding different pronuclei, the following combinations were generated: gynogenetic and androgenetic embryos containing two maternal or two paternal pronuclei, respectively, aborted during embryogenesis, while embryos, in which the wild-type situation with one paternal and maternal pronucleus was recreated, developed normally. (From Barlow and Bartolomei 2014)

Only embryos with a biparental constitution survived and produced viable pubs, while gynogenotic (two maternal genomes) and androgenotic (two paternal genomes) embryos died early during embryogenesis. These results clearly demonstrate that both, maternal and paternal genomes are required for normal embryogenesis and suggest that the two parental genomes are not equivalent. Unlike in genetic crosses, neither genome dosage nor the cytoplasm of the zygote differed in these experiments, pointing to the existence of imprinted genes with only the paternally or maternally inherited allele being expressed. Nevertheless, it was only with the cloning of three imprinted genes and the demonstration of their parental-specific expression in the early 1990s that the idea of genomic imprinting in mammals gained widespread acceptance.

Conceptuses that develop from zygotes with only paternal or maternal genomes also spontaneously occur in humans and lead to abnormal pregnancies. Hydatidiform moles are derived from androgenetic zygotes that were generated by fertilization of an anuclear egg cell either by one sperm followed by chromosome doubling or by two sperm cells.

Hydatidiform moles do not contain differentiated embryonic tissues and are derived from trophoblast cells that would normally give rise to the embryonic parts of the placenta. In contrast, benign ovarian teratomas are tumors that contain various differentiated tissues, often including hair, muscle, and bone. Most mature ovarian teratomas contain two maternal chromosome sets and are thus gynogenotes. They are derived from egg cells containing two maternal genomes because of an aberrant meiosis, spontaneous genome doubling, or the fusion of two egg cells, and develop without fertilization (parthenogenesis).

5.2 Characteristics of Imprinted Genes in Mammals

After the demonstration of the non-equivalence of the parental genomes by Solter and Surani, earlier genetic experiments using translocation stocks (▶ Box 5.1) were revisited and extended, leading to the identification of imprinted regions on 8 of the 19 mouse autosomes (Oakey and Beechy 2002). Most of these regions were found to be required from both the father and the mother, indicating that they contain paternally as well as maternally expressed imprinted genes. The molecular isolation of imprinted genes confirmed that they typically occur in clusters with at least one maternally and one paternally expressed gene.

5.2.1 Molecular Characteristics of Imprinted Gene Clusters

The molecular isolation of imprinted genes allowed more detailed studies on the mechanisms that lead to their unique regulation. The first three imprinted loci were described in the early 1990s and have since been studied intensely: the genes encoding the insulin-like growth factor type-2 receptor (*Igf2r*, Barlow et al. 1991), the *H19* non-coding RNA (Bartolomei et al. 1991), and the insulin-like growth factor 2 (*Igf2*; DeChiara et al. 1991). To date, about 150 genes are known to be imprinted in the mouse. Further characterization has shown that not all imprinted genes are exclusively expressed from only one parental allele; rather, many show a consistent, biased expression of one parental allele. Thus, the definition of genomic imprinting should be broadened to include all genes that show non-equivalent expression from the parental alleles. Although many imprinted genes are expressed in the placenta, some show rather specific expression patterns, often restricted to adult tissues such as the brain. This emphasizes a key point initially made by Metz: while the imprints are established during gametogenesis, their readout, i.e., the biased or specific expression of one of the parental alleles, can manifest itself much later in development. Thus, the imprint itself and its effect on gene expression should not be confounded. Some genes show imprinted expression in some tissues but are biallelically expressed in others. Imprinted expression can also be stage-specific, whereby even the parental association of the active and silenced alleles can be reversed in different tissues. In other words, the effect of the imprint that has been established in the germline differs depending on developmental stage and tissue. These observations suggest complex mechanisms that translate the imprinting mark into a discernable effect on gene expression and ultimately the phenotype.

A typical imprinted gene cluster contains both maternally and paternally expressed genes and usually also biparentally expressed ones (◘ Fig. 5.3). The expression of the imprinted genes is controlled by an imprinting control element, also known as ICE. In most imprinted gene clusters, the ICE shows differential DNA methylation,

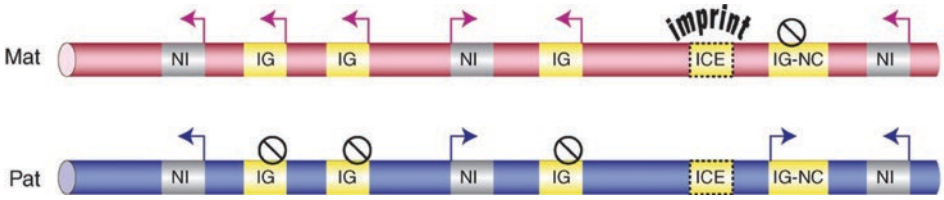


Fig. 5.3 A typical imprinted gene cluster with non-imprinted genes (NI) as well as paternally and maternally expressed imprinted genes (IG). Maternal (Mat) and paternal (Pat) chromosomes of a typical imprinted gene cluster are shown with silenced (slashed circles) and expressed (arrows) alleles. Most clusters contain at least one imprinted, non-coding RNA gene (IG-NC). Imprinted expression is controlled by an imprinting control element (ICE) that carries an epigenetic imprinting mark that was established in and inherited from one parental gamete. The ICE often controls expression of the imprinted IG-NC, which plays an important role in regulating the cluster. (From Barlow and Bartolomei 2014)

which was established in only one of the parents during gametogenesis (gametic or primary imprint) and then maintained exclusively on this allele throughout post-fertilization development. Thus, the gametic imprint marks the maternal or paternal alleles differently in the respective zygote. Initially, all studied imprinted gene clusters in the mouse carried a gametic imprint based on 5-methyl-cytosine (see book ▶ Chap. 1 of Wutz), but this is not true in macaques where several ICEs do not show differential DNA methylation in the gametes. More recently, it was shown that primary imprints can also be based on the histone modification H3K27me3 (Inoue et al. 2017). Deletion of the ICE leads to parent-of-origin-specific effects. Such a deletion has usually no effect on the expression of the genes on the allele carrying the imprinting mark. In contrast, deletion of the ICE on the allele without the gametic imprinting mark leads to a switch, such that the expression of the imprinted genes in the cluster now resembles that of the allele that is normally carrying the imprinting mark.

5.2.2 Molecular Mechanisms Leading to Imprinted Expression

The majority of imprinted gene clusters contains at least one imprinted gene for a non-coding RNA (IG-NC). While several imprinted non-coding RNA genes are very long, ranging from about 100 kb for *Airn* to over 1000 kb for *LNCAT*, the *H19* non-coding RNA is only 2.3 kb. Two observations suggest that the IG-NC plays a role in regulating imprinted expression. First, the IG-NC usually shows the opposite parental-specific expression of the protein-coding genes in the cluster, and second, the ICE of the cluster carrying the gametic imprint typically overlaps with the IG-NC promoter, controlling its expression (Fig. 5.4). The role of the long non-coding RNA in regulating imprinted expression was tested in several clusters by truncating it through the insertion of a polyadenylation signal at the endogenous locus. Truncation of *Airn*, *Kcqnt1ot1*, and *Nespas* showed that expression of these long non-coding RNAs was necessary to silence the paternal alleles of the protein-coding genes in the cluster. How exactly they silence other genes in *cis* is currently not known, although models involving RNA-interference (see book ▶ Chap. 6 of Grossniklaus), transcriptional interference, or coating of the local chromatin and recruitment of repressive marks similar to X-chromosome inactivation (see book ▶ Chap. 4 of Wutz) have been proposed. In all three clusters, the maternal allele carries the gametic DNA methylation imprint that prevents transcription of the IG-NC (Fig. 5.4a).

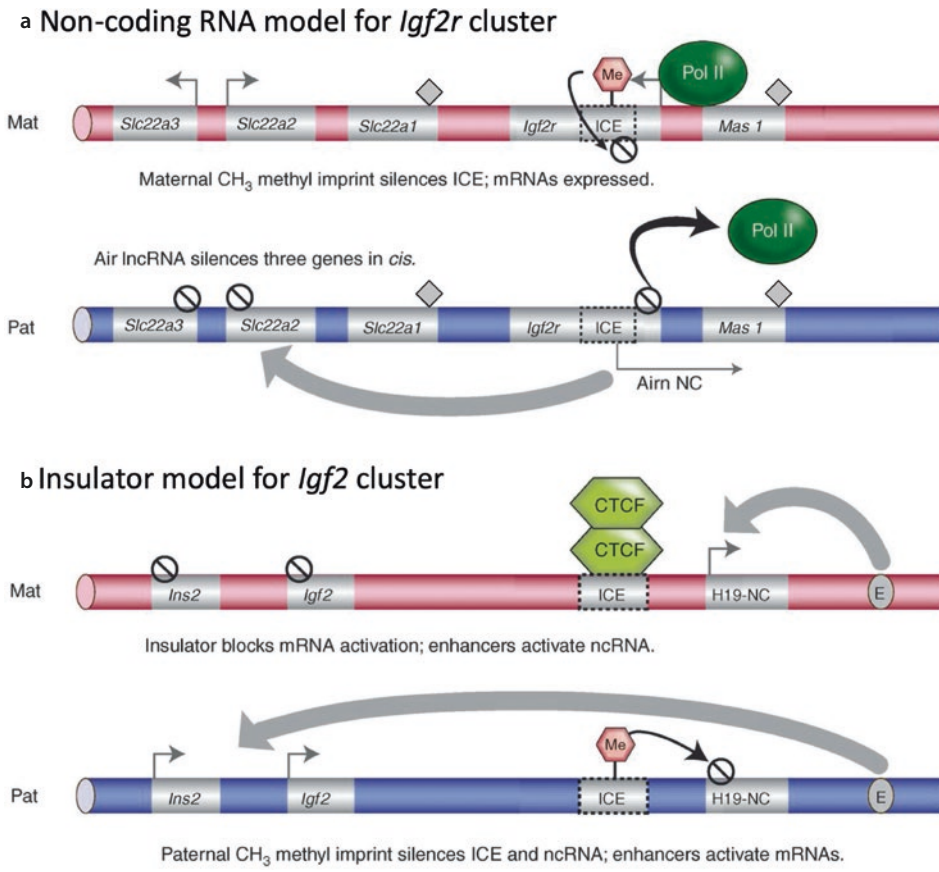


Fig. 5.4 Molecular mechanisms underlying parental-specific expression in imprinted gene clusters. **a** In the *Igf2r* cluster, the ICE carrying a DNA methylation imprint overlaps with the promoter of the *Airn* non-coding RNA (Airn NC). Expression in the placenta is shown where *Slc22a1* and *Mas1* are not expressed (diamonds). On the maternal (Mat) chromosome, the *Airn* promoter is methylated and silenced (slashed circle), while *Igf2r*, *Slc22a2*, and *Slc22a3* are expressed (arrows). On the paternal (Pat) chromosome, lacking the gametic methylation imprint, *Airn* is expressed and silences *Igf2r*, *Slc22a2*, and *Slc22a3* in *cis*. **b** The genes of the *Igf2* cluster are regulated by the same enhancers (E) distal to the *H19* non-coding RNA gene (H19-NC). On the maternal chromosome, the ICE is unmethylated, allowing CTCF proteins to bind and form an insulator that blocks the enhancers from interacting with the promoters of *Igf2* and *Ins2*, while they can interact and activate *H19*. On the paternal chromosome, the ICE carries a gametic methylation imprint which prevents the binding of CTCF and, thus, the formation of an insulator. The enhancers now preferentially interact with and activate the *Igf2* and *Ins2* promoters, while *H19* is not expressed. (From Barlow and Bartolomei 2014)

In contrast, at the *Igf2* cluster the gametic methylation imprint is on the paternal allele and deletion of the *H19* non-coding RNA gene had no effect on silencing the maternal alleles of *Igf2* and *Ins2*. The *Igf2* cluster uses an insulator-based mechanism that results in parent-of-origin-dependent expression (Fig. 5.4b). All genes in the cluster are regulated by the same enhancers located downstream of the *H19* gene. As described in ▶ Chap. 1, chromatin structure and topology contribute to the regulation of gene expression (see book ▶ Chap. 1 of Wutz). In particular, CTCF was implicated in the formation of insulators and chromatin loops. The unmethylated

ICE, located upstream the *H19* gene, serves as a binding site for CTCF proteins, which form an insulator that blocks the interaction of these enhancers with the *Igf2* and *Ins2* promoters, while activating the *H19* promoter. If the ICE is methylated, CTCF proteins cannot bind and the insulator does not form, thus allowing the interaction of the enhancers with the more distant promoters of the protein-coding genes *Igf2* and *Ins2*. CTCF binding sites were also identified at other imprinting clusters, where a similar mechanism may regulate imprinted expression.

5.2.3 The Life Cycle of a Genomic Imprint

As described above, essentially all imprinted gene clusters carry differentially methylated regions (DMRs) as well as regions that differ with respect to H3K27me3 on the maternal and paternal chromosomes. However, not all of these are necessarily the primary or gametic imprints that mark the parental chromosomes and are typically located at ICEs. As DNA methylation is associated with repression, DMRs can also simply coincide with the silenced alleles in a cluster, as can other repressive marks such as H3K27me3. To identify the primary imprint, whether it is differential DNA methylation or a chromatin modification, it is therefore important to determine when the imprinting mark is established during development. If it forms during gametogenesis and is maintained throughout development, it is likely the primary imprinting mark that confers a memory of its parent-of-origin. On the other hand, if the mark is established after fertilization, when the two parental genomes are in the same nucleus, it might be considered a consequence of the silenced state of imprinted genes.

As imprints have to be reset in each generation, they undergo a life cycle of erasure and reestablishment in the germline. This occurs in primordial germ cells where DNA methylation is erased genome-wide and then reestablished, with DMRs getting methylated according to the sex of the individual (◘ Fig. 5.5a). There are many more DMRs that are methylated on the maternal allele (22 of 25 well-studied DMRs) than on the paternal one, and the timing of methylation differs between males and females. Maternal methylation imprints are established during oocyte maturation in meiotic prophase, which occurs after birth. Paternal methylation imprints are established in the developing testes of the fetus in prospermatogonia long before the germ cells enter into meiosis. Despite this difference in timing, the acquisition of gametic methylation imprints depends on the *de novo* DNA methyltransferase 3A (Dnmt3A, see book ▶ Chap. 1 of Wutz). In oocytes, Dnmt3A is aided by the non-catalytic Dnmt3L that directs DNA methylation to transcriptionally active regions, which are enriched in H3K36me3 but devoid of H3K4me2/3 (Stewart et al. 2016). In the male germline, *de novo* DNA methylation is linked to Piwi-interacting RNAS (piRNAs) that direct it to repeats and transposable elements (see book ▶ Chap. 6 of Grossniklaus). This targeting mechanism includes the paternal gametic imprint in the *Rasgrf1* cluster, which contains a retrotransposon. In addition to Dnmt3A/L, Dnmt3B is required for methylation at the *Rasgrf1* DMR but it plays no role for the maternal methylome. In sperm, DNA methylation is more evenly distributed in both genic and intergenic regions. Why Dnmt3A/L appears to act rather unspecifically in sperm but is precisely targeted to transcribed genes in oocytes is currently not understood.

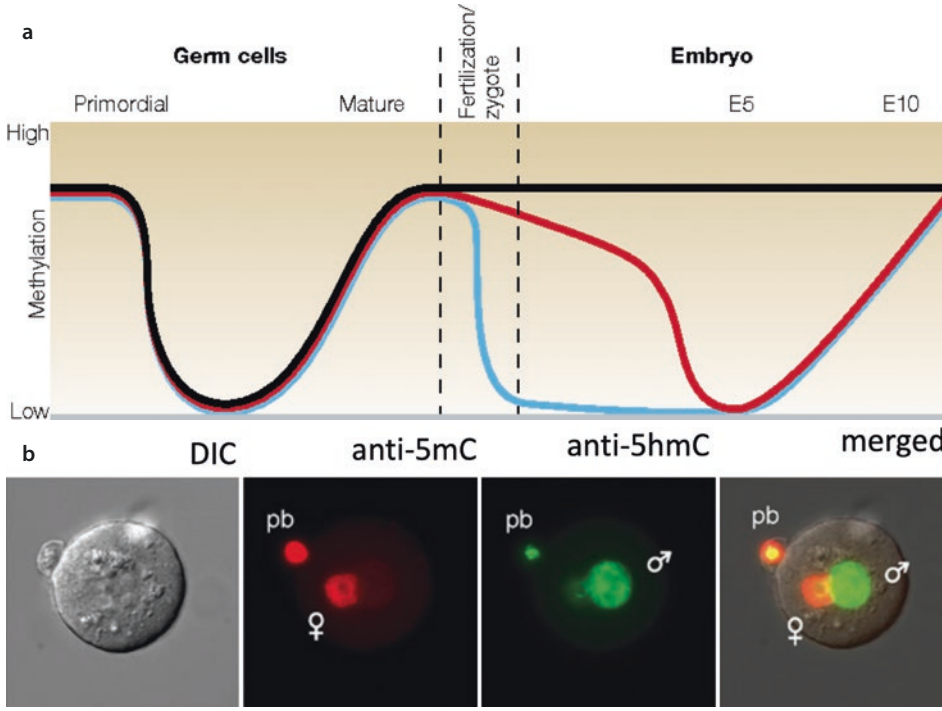


Fig. 5.5 Reprogramming of DNA methylation in the primordial germ cells and the embryo. **a** The schematic shows how the level of methylation in non-methylated (grey) and methylated (black) DMRs of imprinted genes and in non-imprinted sequences (red: maternal, blue: paternal genome) changes during development. The x-axis indicating developmental timing and the y-axis showing relative methylation levels are not to scale. In germ cells, the methylation imprints are erased and reset according the sex of the individual. In the zygote and early embryo, there is global demethylation that, however, does not affect the gametic imprints, which are maintained. The paternal genome loses DNA methylation in the zygote very rapidly, even before the first division. In contrast, the maternal genome loses DNA methylation gradually, possibly through passive demethylation by DNA replication. (From Reik and Walter 2001). **b** The rapid loss of 5-methyl-cytosine (5mC) in the male pronucleus occurs concomitantly with oxidation, resulting in 5-hydroxymethyl-cytosine (5hmC). As revealed by antibody staining, the female pronucleus und polar body (pb) still show high levels of 5mC when the male pronucleus has completely lost this antigen but gained 5hmC. (From Iqbal et al. 2011)

Once the gametic imprints are established, they are propagated over cell divisions by the maintenance DNA methyltransferase Dnmt1, which methylates hemimethylated CpG sites directly at the replication fork (see book ▶ Chap. 1 of Wutz). However, imprinted loci are challenged by a second genome-wide reprogramming event immediately following fertilization, involving demethylation during preimplantation development and *de novo* methylation in the postimplantation embryo (Fig. 5.5a). This wave of genome reprogramming is postulated to allow the resetting of the zygotic genome to a totipotent state. The parental genomes that contribute to the zygote are derived from highly differentiated gametes and, therefore, carry epigenetic markings that have to be removed in order to generate totipotent cells and, subsequently, all cell types of the organism (see also book ▶ Chap. 7 of Paro).

While the paternal genome undergoes a rapid loss of DNA methylation even before the first division, the maternal genome is demethylated more slowly during

preimplantation development (◘ Fig. 5.5a). These kinetics indicate an active demethylation of the paternal genome whereas the maternal genome likely loses DNA methylation passively in a DNA replication-dependent manner (see book ▶ Chap. 1 of Wutz). Active demethylation was proposed to be mediated by ten-eleven translocation methylcytosine dioxygenases (TETs), which can oxidize 5-methyl-cytosine (5mC) to 5-hydroxymethyl-cytosine (5hmC) and further to 5-formyl- and 5-carboxyl-cytosine (see book ▶ Chap. 1 of Wutz). Indeed, TET3 expression coincides with loss of 5mC and the accumulation of 5hmC in the male pronucleus (◘ Fig. 5.5b), leading to the widely held view that demethylation occurs via oxidation by TET enzymes. However, recent studies have shed some doubt on this notion as demethylation seemed complete prior to the accumulation of 5hmC and still occurred in an oocyte-specific *Tet3* knock-out mouse (SanMiguel and Bartolomei 2018).

One of the most intriguing questions is how gametic imprints escape demethylation in the embryo and unmethylated DMRs are protected from becoming methylated when the rest of the genome regains DNA methylation. This is probably achieved by *trans*-acting factors that bind to specific *cis*-regulatory regions to protect gametic DMRs. One of these proteins is DPPA3, which is responsible for the maintenance of DNA methylation at some of the gametic DMRs. Another protein that protects methylation imprints is ZFP57, a zinc finger protein with binding preference for TGCCGC, which occurs at most gametic DMRs involved in imprinting. ZFP57 binds only to methylated sites and interacts with the KAP1 protein that recruits various repressive histone modifying enzymes and, importantly, Dnmt1, which maintains methylation at the DMR. In maternal-zygotic *Zfp57* mutant embryos, there is a loss of DNA methylation at many gametic DMRs accompanied by extensive changes in the expression of imprinted genes, thus illustrating its importance in protecting the DMRs during reprogramming (SanMiguel and Bartolomei 2018).

5.3 Genomic Imprinting and Human Disease

Imprinted genes are expressed in diverse tissues and their functions are manifold. Consistent with the expression of many imprinted genes in the embryo and placenta, mutations often affect fetal growth and development. Other imprinted genes show preferential expression in the brain and mutations in the mouse can lead to the development of behavioral phenotypes. Given that only one of the parental alleles of imprinted genes is active, it is not surprising that mutations in imprinted gene clusters are often linked to human disease (see book ▶ Chap. 8 of Santoro). While some of these diseases are clearly linked to a specific imprinting cluster, others show disruptions of imprinting at multiple loci across the genome. A selection of imprinting disorders is shown in ◘ Table 5.1 (based on Monk et al. 2019):

As becomes obvious from the selection in ◘ Table 5.1, many imprinting disorders affect the same genomic region but lead to distinct clinical features depending on which of the parental alleles is affected. For instance, in some patients with Silver-Russel Syndrome, the paternal copy of the *Igf2*/*H19* imprinting cluster is missing or affected, while in Beckwith-Wiedemann Syndrome, it is the maternal copy. Likewise, in Temple Syndrome, the paternal allele of the *Meg3-Dlk1* cluster is affected, while the maternal one is missing in most patients with Kagami-Ogata Syndrome.

Table 5.1 Selected human disorders related to defects in imprinting clusters

Imprinting disorder	Chromosome	Affected imprinting cluster
Silver-Russel Syndrome	11p15.5 7p11.2 Others	*Kcnq1, Igf2/H19* *Grb10* …
Beckwith-Wiedemann Syndrome	11p15.5	*Kcnq1, Igf2/H19*
Temple Syndrome	14q32	*Dlk1-Dlo3*
Kagami-Ogata Syndrome	14q32	*Dlk1-Dlo3*
Prader-Willi Syndrome	15q11-q13	*Snrpn/Ube3A*
Angelman Syndrome	15q11-q13	*Snrpn/Ube3A*

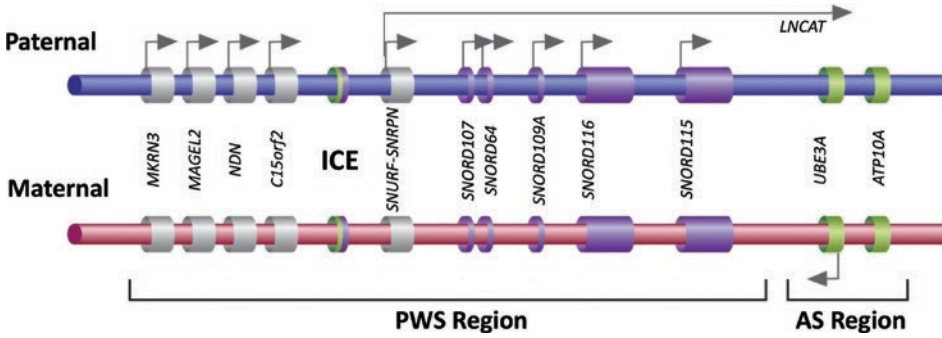


Fig. 5.6 The *Snrpn/Ube3A* imprinted gene cluster associated with Prader-Willi Syndrome (PWS) and Angelman Syndrome (AS). The ICE controls imprinted expression of the genes in the cluster. The *SNURF-SNRPN* gene encodes not only two protein products, Snurf and Snrpn, but also a paternally expressed long non-coding antisense transcript (*LNCAT*) that overlaps with the promoter of the maternally expressed *UBE3A* gene responsible for AS. (From Zoghbi and Beaudet 2016)

To illustrate the molecular basis of imprinting disorders, we focus on the Prader-Willi Syndrome (PWS) and Angelman Syndrome (AS), both affecting the *Snrpn/Ube3A* imprinted gene cluster. Among other clinical features, PWS is characterized by a lower birth weight, neonatal hypotonia, postnatal growth restriction followed by hyperphagia and obesity, short stature, hypogonadism, and mild intellectual disability. In contrast, AS patients can have an above-average birth weight (depending on the exact lesion), severe delay in postnatal development, ataxia, scoliosis, microcephaly, seizures, severe intellectual disability, minimal verbal skills, and a happy affect with frequent bouts of laughter.

The majority of patients with either disorder carry a deletion of about 5–6 Mb in 15q11. While the deletion is inherited paternally in PWS patients, it is derived from the mother in AS children. This difference explains the distinct clinical features in PWS and AS patients, as only the maternal or paternal alleles of the *Snrpn/Ube3A* cluster, respectively, are expressed. Apart from deletions, other genetic lesions leading to loss-of-function mutations of genes in the *Snrpn/Ube3A* cluster can cause the syndromes (Fig. 5.6). For AS, about 10–15% of the cases carry a mutation in the

maternally expressed *UBE3A* gene, identifying *UBE3A* as gene likely responsible for AS. It is not yet fully clear which of the genes in the PWS region of the imprinting cluster is responsible for PWS, although recent evidence points to the *SNORD116* locus encoding a set of small nucleolar RNAs.

Apart from genetic causes of these syndromes, there are also cases where the *Snrpn/Ube3A* cluster is intact but its genes are epigenetically misregulated. This is for instance the case if both homologs of chromosome 15 originate from the same parent, a condition called uniparental disomy (UPD). Maternal UPD is responsible for PWS, whereas AS is caused by paternal UPD. The origin of UPDs lies in non-disjunction during female meiosis that, after fertilization, leads to zygotes that either have only one paternal (monosomy) or two maternal and one paternal (trisomy) chromosome 15 in the case of PWS and AS. These aberrant zygotes can develop normally if they spontaneously loose or duplicate chromosome 15. Loss of the paternal copy from trisomic zygotes leads to maternal UPD, while duplication of the single paternal chromosome in monosomic zygotes to paternal UPD. As in individuals with UPD the two homologs stem from the same parent, they lack expression of imprinted genes specific to the other parent, resulting in imprinting disorders.

Importantly, PWS and AS can also stem from imprinting defects. Imprinted expression at the *Snrpn/Ube3A* cluster is controlled by an ICE upstream of the *SNURF-SNRPN* gene. If a mutation affects the function of the ICE, the genes in the cluster lose their parent-of-origin-specific expression. The same can result from a failure to reprogram the gametic imprint in primordial germ cells. For instance, if the methylation mark on the maternal ICE is not erased in prospermatogonia, a male will transmit it with a maternal rather than a paternal gametic mark. This will lead to the formation of a zygote with two "maternal" *Snrpn/Ube3A* clusters causing PWS. Although such imprinting defects are responsible for only a minority of the PWS and AS cases, they contributed important insights into the resetting of imprints and the regulation of imprinted expression at the *Snrpn/Ube3A* cluster.

5.4 Genomic Imprinting in Flowering Plants

Although imprinting of individual genes was originally discovered in plants and the parent-of-origin-dependent expression of a few genes was documented in the maize endosperm, the mechanisms underlying genomic imprinting were not well studied until the discovery of imprinted genes in the model plant *Arabidopsis thaliana* in the late 1990s. Plant imprinting studies were reinvigorated by the discovery of *MEDEA* (*MEA*) (Grossniklaus et al. 1998), a maternally expressed imprinted gene encoding a homolog of *Drosophila melanogaster Enhancer of zeste*, the histone methyltransferase in the conserved *Polycomb* Repressive Complex 2 (PRC2; see book ► Chap. 3 of Paro). The *mea* mutant was identified in a screen for maternal effect mutants, with the genotype of the female gametes determining the phenotype of the developing seeds. Because the gametes of plants are produced by haploid multicellular organisms (gametophytes) that form through mitotic divisions after meiosis, such mutants show a peculiar genetic behavior. In a heterozygous *mea/MEA* plant, half the meiotic products will carry the mutant *mea* allele and half the wild-type *MEA* allele. Correspondingly, also half of the gametophytes and the gametes they contain (egg and central cell in the female gametophyte; two sperm cells in pollen, the male game-

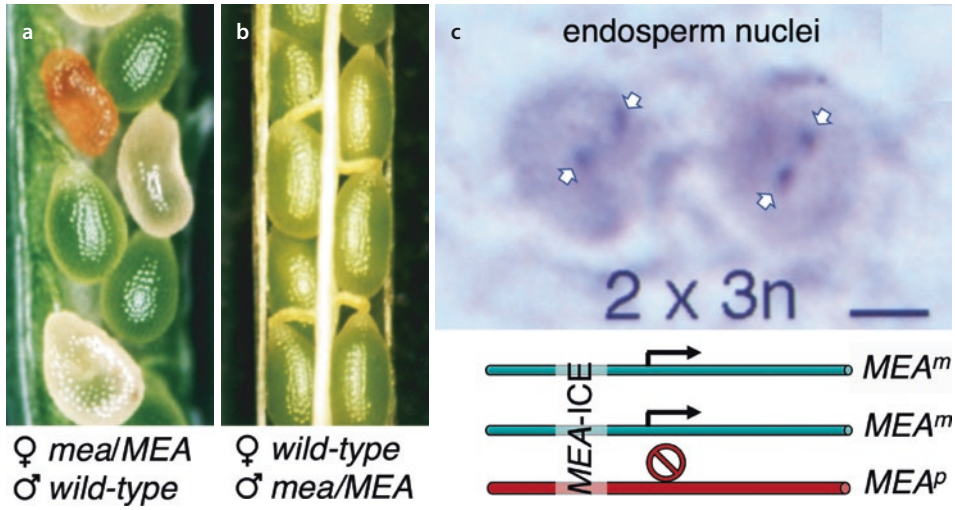


Fig. 5.7 Genomic imprinting is responsible for maternal effect seed abortion observed in the *mea* mutant. **a** and **b** show opened siliques with developing seeds from reciprocal crosses. If the mutant *mea* allele is inherited from the mother, seeds abort (red and brown seeds in **a** but if it is inherited from the father, it has no effect on seed development **b**. (From Grossniklaus et al. 1998). **c** Detection of nascent transcripts at the *MEA* locus (arrows) show that only two of the three alleles in the endosperm are actively transcribed, i.e., the two maternal copies as illustrated schematically. (From Vielle-Calzada et al. 1999)

tophyte) will carry the mutant allele. Thus, if a *mea*/*MEA* heterozygous female plant is crossed with a wild-type male, half of the seeds abort while, in the reciprocal cross, all seeds develop normally (Fig. 5.7).

Of course, similar to kernel pigmentation regulated by the *R* allele in maize, maternal effect seed abortion observed in *mea* mutants could be caused by (i) a dosage effect in the endosperm, (ii) a missing cytoplasmically stored product, or (iii) genomic imprinting. As *A. thaliana* does not offer the sophisticated genetic tools available in maize, a dosage effect was excluded by generating tetraploid plants that produce endosperm with *mea*:*MEA* allelic ratios ranging from 6:0 to 0:6. Distinction of the remaining two possibilities required the cloning of *MEA* and analysis of its allelic expression using parental-specific polymorphisms. This showed that only maternal transcripts could be detected in developing seeds. However, *MEA* was already expressed in the female gametes, such that these maternal transcripts could have either been produced exclusively before fertilization and stored in the egg cell or, in addition, also be derived from the maternal allele after fertilization. RNA *in situ* hybridization to detect nascent transcripts in triploid endosperm nuclei showed that only two of the three *MEA* alleles were transcribed. Nascent transcripts temporarily remain at their chromosomal site of transcription forming "nuclear dots" of high transcript concentration (Fig. 5.7). Thus, while *MEA* is already expressed in the female gametes, it is also regulated by genomic imprinting leading to the transcription of only the maternal *MEA* alleles in the endosperm (Vielle-Calzada et al. 1999). The discovery of genomic imprinting in *A. thaliana* stimulated many studies both at the genome-wide level as well as on the molecular mechanisms underlying parental-specific expression at specific loci in plants.

5.4.1 Genomic Imprinting Occurs Predominantly in the Endosperm But Also Exists in the Embryo

As all imprinted genes initially studied in maize were expressed in the endosperm (Messing and Grossniklaus 1999) and the DNA of the maternal genome was found to be hypomethylated in this tissue (Hsieh et al. 2009), imprinting is often referred to as endosperm-specific. Despite imprinted genes also having been identified in the plant embryo, most studies to date focused on endosperm. Over the last years, many genome-wide studies identified candidate imprinted genes in several species, including *A. thaliana*, maize, and rice (*Oryza sativa*). These studies relied on sequencing RNA isolated from dissected endosperm tissue or sorted endosperm nuclei, and subsequent searching for transcripts with parentally biased expression based on nucleotide polymorphisms between the parents.

All studies looked at steady-state levels of mRNA transcripts, which does not distinguish between mRNAs that were exclusively produced prior to fertilization and stored in the gametes and those that were differentially transcribed from the parental alleles after fertilization. Although endosperm was isolated several days after fertilization, the half-life of mRNAs is unknown and they could potentially be very stable. Thus, to conclude that a gene is regulated by genomic imprinting requires the demonstration of its active transcription after fertilization. Only in the zygote or fertilized central cell, both parental alleles will be present in the same nucleus and transcribed based on their gametic imprint. This would be the case if no transcripts were detected in the gametes, although cases like *MEA*, which is expressed in the female gametes as well as imprinted, would be missed by this strategy. Alternatively, active transcription can be shown by looking at nascent transcripts (◘ Fig. 5.7). While, in plants, this analysis has so far only been done for *MEA*, fluorescent *in situ* hybridization to detect RNAs (RNA-FISH) is a standard technique in mammals. In summary, while RNA sequencing experiments have identified hundreds of candidate imprinted genes in diverse species, regulation by genomic imprinting has unequivocally been demonstrated for only a few of them.

Moreover, the overlap of presumptive imprinted genes between genome-wide studies performed in different laboratories is very small (Wyder et al. 2019). It is possible that the hypomethylation of the maternal genome in the endosperm leads to stochastic changes in expression, which differ between individuals and are influenced by the environment, explaining the poor overlap between studies. This would mean that the parent-of-origin-dependent expression of these genes would be accidental and not play a crucial role in development. Consistent with this idea, the function of only a handful of imprinted genes is known, including *A. thaliana MEA* and *FERTILIZATION INDEPENDENT SEED2* (*FIS2*), which encodes a homolog of *Drosophila Su(z)12*. MEA and FIS2 are subunits of FIS-PRC2, a PRC2 variant (see book ► Chap. 3 by Paro) that is required for normal seed development.

Although most imprinting studies in plants focused on the endosperm, genomic imprinting has also been reported in the embryo. In *A. thaliana*, about a dozen candidate genes were identified, ten of which were not expressed in the gametes, indicating active expression after fertilization and thus *bona fide* imprinting. A recent study in maize identified several dozen candidate imprinted genes. As in mammals, gametic imprints of genes expressed in plant embryos would have to be reset in every genera-

tion. This is not true for imprints in the endosperm, which is a terminal tissue that does not contribute to the next generation. As in mammals, the primary imprints have to be established during gametogenesis when the parental genomes are separated. In both maize and *A. thaliana*, genes with parental allele-specific expression in young embryos become biallelically expressed later during embryogenesis. Whether this represents erasure of the gametic imprint or an alternative transcriptional regulation is currently unknown.

5.4.2 Mechanisms Underlying Imprinting Show Similarities Between Mammals and Plants

Both, the dissection of imprinting regulation at specific loci as well as genome-wide profiling studies of DNA methylation and histone modifications have shed light onto the mechanisms underlying genomic imprinting in plants. Again, studies on the regulation of the *MEA* locus paved the way to show that, as in mammals, DNA methylation plays a prominent role in genomic imprinting in plants. Unlike in mammals, where only CGs are methylated (see book ▶ Chap. 1 of Wutz), in plants, DNA methylation can occur in the CG, CHG, and CHH context (with H ≠ G). While DOMAINS REARRANGED METHYLTRANSFERASE2 (DRM2) is the major *de novo* methyltransferase for all Cs, distinct DNA methyltransferases are responsible to maintain methylation in the different contexts: the Dnmt1 homolog MET1 for CG, the CHROMOMETHYLTRANSFERASE3 (CMT3) for CHG, and DRM2 together with CMT2 for CHH.

Mutants affecting *MET1* indicated an involvement of DNA methylation in silencing the paternal *MEA* allele. This was confirmed by the identification of DEMETER (DME), a DNA glycosylase that can excise methylated cytosines through base excision repair (Choi et al. 2002). DME is preferentially expressed in the central cell, where it leads to demethylation that then results in the hypomethylation of the maternal chromosomes in the endosperm after fertilization. In the *dme* mutant, the maternal *MEA* allele fails to be expressed, indicating that demethylation is required to set its active state. Regulation of imprinting by the maintenance methyltransferase MET1 and DME, which removes this epigenetic mark, has also been demonstrated for several other loci.

In addition to DNA methylation, H3K27me3 mediated by PRC2 was shown to regulate many imprinted genes. This was first shown for *PHERES1* (*PHE1*), a direct target of FIS-PRC2. The paternal allele of *PHE1* is expressed at a much higher level than the maternal one, which is specifically repressed by FIS-PRC2 during seed development (Köhler et al. 2005). The repression of the maternal *PHE1* allele also depends on a DMR at tandem repeats downstream of the gene. It has been proposed that the unmethylated DMR allows the formation of a repressive chromatin loop through interaction with FIS-PRC2 at the *PHE1* promoter (◘ Fig. 5.8). In its methylated form, the DMR prevents the binding of yet unknown factors involved in loop formation, resulting in an active paternal allele. While loops involved in imprinting await experimental confirmation in plants, the existence of such loops has been shown in mice. As discussed earlier, at the *Igf2*/*H19* imprinting cluster, CTCF binds to the unmethylated ICE and, together with PRC2, forms a chromatin

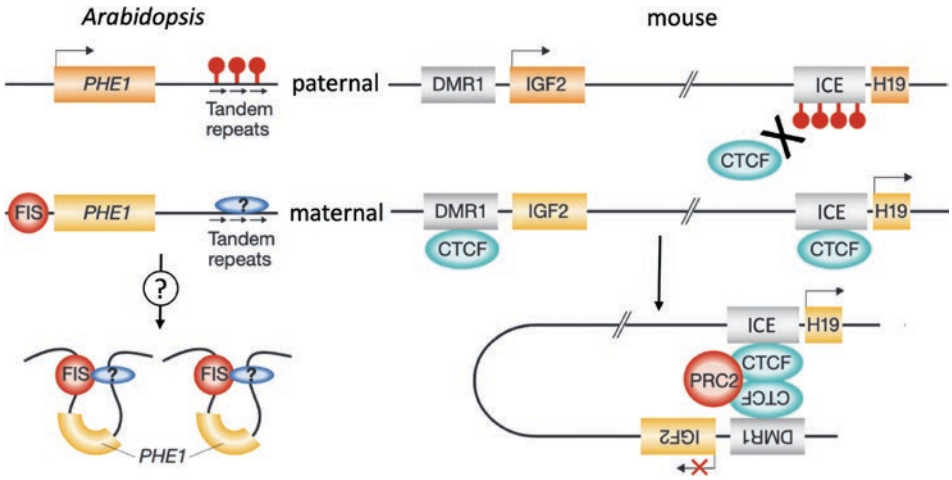


Fig. 5.8 Chromatin loops as part of the imprinting mechanism. On the left, the active paternal *PHE1* allele has methylated tandem repeats (DMR), while on the maternal allele, the DMR is unmethylated, which is proposed to allow the formation of a chromatin loop on the two maternal chromosomes in the endosperm. On the right, the *Igf2*/*H19* cluster is shown for which loop formation on the maternal chromosome, mediated by CTCF and PRC2, has been demonstrated. (From Loppin and Oakey 2009)

loop that represses *Igf2* (Fig. 5.8). PRC2 does not only play an essential role in seed development but also regulates some paternally repressed genes in the mouse placenta. Moreover, PRC2-mediated H3K27me3 can serve as gametic imprint, but relative to DNA methylation, the role of PRC2 is relatively minor (Barlow and Bartolomei 2014). Nevertheless, DNA methylation and PRC2 are involved in genomic imprinting in both mammals and plants, highlighting the independent recruitment of these two repressive modules in both lineages in the evolution of parental-specific expression.

Genome-wide profiling studies of DNA methylation, repressive chromatin marks, and gene expression in the endosperm have shown that a major determinant for maternally expressed imprinted plant genes is DNA methylation (Batista and Köhler 2020). In many cases, these genes are silenced by DNA methylation in most tissues and their demethylation by DME in the central cell leads to the expression of the maternal alleles in the endosperm. In other cases, *de novo* methylation of the paternal allele in sperm may lead to their imprinted maternal-specific expression (Fig. 5.9). For paternally expressed genes, histone modifications mediated by PRC2 (H3K27me3) or CHROMOMETHYLASE3 (CMT3) and plant SU(VAR)3-9 homologs (H3K9me2) play a major role in regulating imprinted expression. This can occur through demethylation of the maternal allele by DME followed by the deposition of repressive histone marks in combination with DNA methylation of the paternal allele, preventing the binding of PRC2 as it was shown for *PHE1* (Fig. 5.8). Alternatively, the maternal allele may be specifically targeted by recruiting PRC2 via selected transcription factors, or by removal of repressive histone marks of broadly silenced genes specifically in the sperm cell (Fig. 5.9).

While these mechanisms may explain the regulation of up to 30% and 90% of maternally and paternally expressed genes in the endosperm of *A. thaliana* (Batista and Köhler 2020), respectively, the regulation of many other imprinted genes does

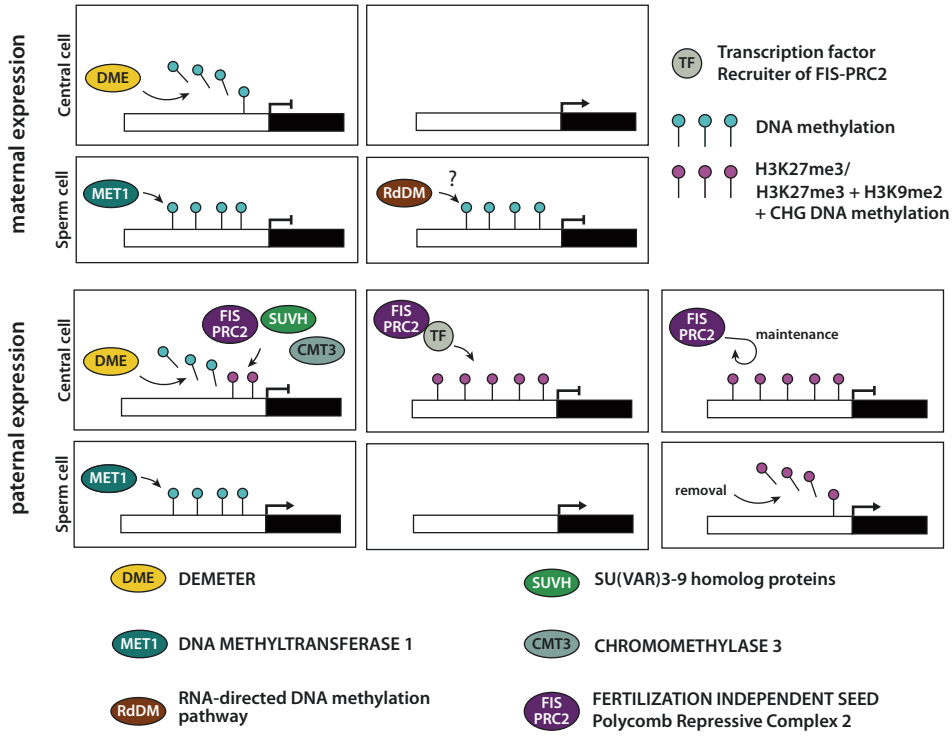


Fig. 5.9 Different models for the regulation of genomic imprinting in the endosperm. The events proposed to set gametic imprints in central cell and sperm are shown. For maternally expressed genes, the establishment of differential DNA methylation imprints, through demethylation or specific *de novo* methylation, is important. For paternally expressed genes, three scenarios involving PRC2 are shown. See text for details. (From Batista and Köhler 2020)

neither involve DNA nor histone methylation. This is particularly true for imprinted genes in the embryo, where none of 12 tested genes appears to be regulated by DNA methylation and only three were affected by mutants affecting PRC2 (Raissig et al. 2013).

There are also several cases of imprinted genes expressed in maize or *A. thaliana* endosperm carrying a DMR that, however, is not inherited from the parents but only established after fertilization. Differential DNA methylation does, therefore, not act as a gametic imprint in these cases.

That imprinting can be very complex was also shown through more detailed studies of the *MEA* locus. The expression of the maternal *MEA* allele depends on demethylation by DME and, after fertilization, the paternal allele is repressed by the maternally produced FIS-PRC2. The latter was concluded from several studies in which a paternal reporter gene driven by the *MEA* promoter became active in crosses to *mea* mothers. However, the analysis of the endogenous gene subsequently showed that the derepression of the paternal allele was minimal and *MEA* still showed a strongly biased allelic expression. Furthermore, imprinting could be conferred to a reporter gene using only 200 bp of the *MEA* promoter, which is completely free of any DNA methylation in the gametes. This was the first report of a DNA methylation-independent ICE, which were subsequently also found in mammals. As neither PRC2

nor DNA methylation is required for imprinted *MEA* expression, the primary imprints remain elusive (Wöhrmann et al. 2012). It was proposed that, similar to *PHE1*, chromatin loops may form depending on the DNA methylation status of flanking regions, thereby preventing access of imprinting factors that would establish the yet unknown gametic imprint.

Finally, the identification of *PHE1* as a target of FIS-PRC2 revealed the existence of an extensive regulatory cascade controlling seed development that is based on genomic imprinting. The maternally expressed imprinted genes *MEA* and *FIS2* control the paternal expression of *PHE1*, a transcription factor that, in turn, regulates a large number of paternally expressed imprinted genes in the endosperm. Thus, epigenetic gene regulation plays a major role in seed development.

5.5 Evolution of Genomic Imprinting

Several hypotheses have been proposed to explain the evolution of genomic imprinting. Among these, David Haig's parental conflict theory by far attracted the most attention from biologists working on imprinting. Haig tried to find an explanation why imprinting evolved in such different organisms as mammals and seed plants (Haig and Westoby 1989). He realized that both share a "placental habit", i.e., the development of seeds and fetuses depends solely on resources provided by the mother. Indeed, both the mammalian placenta and the endosperm of seed plants are major tissues with imprinted gene expression and play a central role in providing nutrients to the next generation. In contrast, the father contributes little to support the developing offspring. In polygamous species, this leads to a parental conflict between the paternal and maternal genomes in the progeny because of different kinship relationships. The mother is equally related to all her offspring and (epi)genotypes are favored that lead to a uniform distribution of resources to all her offspring over her lifetime. In contrast, the paternal genome is not the same in all the progeny, such that (epi)genotypes are favored that lead to an increased acquisition of nutrients at the expense of non-related siblings. This parental conflict will lead to the biased expression of genes involved in the acquisition of nutrients from the mother, and thus the growth of the seed or fetus. This theory makes clear evolutionary predictions: First, paternally expressed genes should promote growth of the offspring and second, maternally expressed genes should reduce it.

There is substantial evidence in support of the parental conflict theory (Pires and Grossniklaus 2014). For instance, the endosperm overproliferates in crosses of parents with different ploidy that increase the number of paternal genomes, while it proliferates less and differentiates earlier in seeds with an excess of maternal genomes (Haig and Westoby 1989). Similarly, androgenetic mouse embryos form a large trophoblast, from which the embryonic part of the placenta forms, while gynogenetic embryos produce an underdeveloped trophoblast. The parental conflict theory is also supported by its phylogenetic distribution in vertebrates. Genes that are imprinted in mammals are biallelically expressed in fish and birds, in which the amount of resources deposited in the egg is determined by the mother prior to fertilization, such that the paternal genome in zygotic tissues cannot influence nutrient acquisition from the mother. This is also true for egg-laying mammals like the platypus and echidna but not for marsupials. In the latter, much of the off-

spring's development occurs in the pouch, which from the point-of-view of the parental conflict theory is equivalent to the uterus in eutherian mammals or the seed in plants.

Intriguingly, the predictions of the parental conflict theory also play out at the level of individual genes. For instance, *MEA* is a maternally expressed gene and is predicted to restrict growth. Indeed, seeds developing from *mea* mutant gametes show overproliferation in both the embryo and endosperm before they abort (Grossniklaus et al. 1998). Similarly, the paternally expressed *Igf2* gene is expected to promote fetal growth in the mouse and, consistent with the theory, mutant pubs have a 40% reduction in birth weight compared to their wild-type siblings. In contrast, embryos lacking the maternally expressed *Igf2r* gene, encoding the receptor for Igf2, show overgrowth before they die. Most interestingly, double mutants are normal in size and are viable. In fact, Igf2r is a receptor that binds the growth factor Igf2 but targets it to the lysosome, preventing its growth-promoting action. Thus, Igf2r is a kind of decoy receptor expressed from the maternal genome to remove the paternally produced Igf2 growth factor: a molecular reflection of the tug-of-war between maternal and paternal genomes.

The parental conflict theory is very attractive as it explains the evolution of imprinting in both mammals and plants. To my knowledge, the phenotypes of all imprinted genes that have a function related to growth, either during embryogenesis or, in mice, also postnatally, e.g., by controlling feeding behavior, are consistent with the parental conflict theory. This includes some of the clinical features of patients with syndromes associated with imprinted gene clusters, which often affect birth weight or postnatal feeding behavior. However, not all imprinted genes regulate growth and for these, the parent conflict theory does not apply in an obvious manner. More than a dozen other theories for the evolution of imprinting have been proposed (Spencer and Clark 2014). Depending on the organism and the function of the imprinted gene, distinct selective forces may have driven the evolution of genomic imprinting. However, most alternative theories do not explain the peculiar phylogenetic distribution of genomic imprinting, i.e., its evolution in mammals and seed plants.

Take-Home Messages

- Genomic imprinting is a paradigm of epigenetic gene regulation that evolved independently in seed plants and mammals.
- Genomic imprinting leads to parent-of-origin dependent gene expression, rendering parental genomes non-equivalent, and is not related to the sex of the individual.
- Gametic or primary imprints are differential epigenetic marks that are acquired during gametogenesis and lead to allelically biased gene expression after fertilization.
- Gametic imprints have to be reset every generation in the germline according to the sex of the individual. In mammals, they withstand genome-wide reprogramming in the zygote, associated with a wave of DNA demethylation and remethylation.

- In mammals, imprinted genes typically occur in clusters that contain at least one maternally and one paternally expressed gene. Most clusters also contain a long non-coding RNA that is involved in imprinting regulation.
- Parental-specific expression in a cluster is regulated by imprinting control elements (ICEs) that are differentially marked, most often by DNA methylation imprints or, more rarely, by histone modifications that serve as gametic imprints.
- ICEs are long-range regulatory elements that control the expression of multiple genes in the cluster. Deletion of ICEs affects expression on the chromosome carrying the imprint.
- In both mammals and seed plants, genomic imprinting is regulated by DNA methylation and PRC2, although the former has a more predominant role in mammals. Differential DNA methylation can lead to topological changes, such as the formation of chromatin loops, that control imprinted expression.
- Although non-coding RNAs seem to play a role in the regulation of many imprinted gene clusters in mammals, the clusters are diverse and regulated in distinct ways, e.g., by modifying insulator elements.
- In plants, differential DNA methylation is associated with about 30% of the maternally expressed imprinted genes, the mechanisms regulating the rest are unknown. The majority of paternally expressed genes are controlled by PRC2, DNA methylation, or a combination thereof.
- Imprinted genes play an important role in development and behavior and, in agreement with the parental conflict theory for the evolution of imprinting, many of them regulate growth both in mammals and seed plants.

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