

Chapter 4

Epigenetics and Heterosis in Crop Plants



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Abstract Heterosis refers to improved or altered performance observed in F1 hybrid organisms when compared to their parents. Heterosis has revolutionized agriculture by improving key agronomic traits in crop plants. However, even after decades of research in this area a unifying molecular theory of heterosis remains somewhat elusive. For many years the *dominant*, *overdominant*, and *epistasis* models have prevailed for explaining multigenic heterosis. The use of whole transcriptome, proteome, metabolome, and epigenome profiling approaches can further generate and inform hypotheses regarding heterosis. This chapter reviews the models that have been used to explain heterosis. We also review the mechanistic basis of epigenetic pathways in plants and describe how they may also be considered in relation to understanding heterosis. There are number of findings that support potential links between epigenetic regulation and heterosis in model and crop plants, including the potential for DNA methylation, histone modification, and small RNAs to influence heterotic effects in F1 hybrids. Overall, we assess some opportunities and challenges for epigenetic research to advance the molecular understanding of heterosis.

4.1 Importance of Heterosis for Crop Improvement

Heterosis is the phenomenon observed when the F1 progeny of a cross exhibit improved or transgressive values for growth or other traits when compared to their parents. The discovery of heterosis was recorded as early as the 1700s when the

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botanist Joseph Koelreuter observed that F1 hybrid tobacco plants exceeded the height of their parents (Reed 1942). The first characterization of heterosis was performed in a pioneering study performed by Darwin in 1876. By comparing the self-fertilized and cross-fertilized progeny of pairs of inbred parents of 60 plant species he observed that the F1 hybrid plants from crossed plants were typically taller and more vigorous than self-fertilized crosses (Darwin 1876). This phenomenon was later verified independently by George Shull (Shull 1908) and Edward East (East and Jones 1919) in breeding programs of maize (*Zea mays* L.), with Shull being the first to coin the term “heterosis” in a lecture given in 1914.

The exploitation of heterosis has had revolutionary effects on global agriculture and has led to increased yields in a range of crop species (Mendoza and Haynes 1974; Duvick 2001; Schnable and Springer 2013). Heterosis has been applied with particular success in maize (Crow 1998; Duvick 2001), but has also been deployed in other crops such as wheat (Wang et al. 2006; Qi et al. 2012), tomatoes (Williams and Gilbert 1960; Krieger et al. 2010), and rice (Yu et al. 1997). Heterosis has also been harnessed in livestock including cattle (Neufeld Arce 2006) and observed in other mammals such as mice (Leamy and Thorpe 1984; Han et al. 2008). The phenomenon of heterosis is assumed to be widespread among eukaryotes (Goff 2011; Baranwal et al. 2012).

In plants, heterosis is often considered to be a complex and multigenic trait, involving alterations to numerous quantitative traits such as vegetative growth rate and plant stature, accumulation of metabolites, flowering time, biomass, seed size, and tolerance to biotic and abiotic stresses (Baranwal et al. 2012). Such changes can lead to heterotic phenotypes leading to increased yield of a crop. Notably, heterosis can occur in either “direction,” either increasing the trait value of interest relative to the parents or decreasing it. Depending on the trait in question, either may be of potential interest in crop breeding programs (for example, the so-called negative heterosis for seed size may be of value for fruit crops). Heterosis can be classified in two ways: (1) heterosis that exceeds the mean of the parental values (termed mid-parent heterosis) or (2) heterosis which exceeds the values of both parents (termed best-parent heterosis).

Adoption of hybrid maize became more widespread in the USA in the 1930s. Maize yields increased by approximately 2% year-on-year through the use of heterotic F1 hybrids in the period 1930–1940. Heterosis research improvements occurred in parallel to agronomy improvements, including advances in farm machinery and fertilizers. Heterosis breeding systems have also been subject to ongoing improvements (e.g., through the establishment of double haploid approaches to create inbred lines more rapidly than conventional methods like single seed descent). The success of hybrid crops relies upon the willingness of farmers to purchase F1 hybrids each year from breeding companies, because heterosis is largely restricted to the F1 generation (Hufford and Mazer 2003).

A range of genetic models have been advanced to explain the occurrence of heterosis in the offspring of certain crosses, whether in plants or other organisms. However, it is recognized that these models may not be able to wholly explain all aspects of heterosis (Groszmann et al. 2013). These models are described below.

4.2 Genetic Models for Explaining Heterosis: Successes and Limitations

Although the underlying mechanisms of heterosis are still not fully understood, increased heterozygosity is often positively correlated with increased fitness in many species (Darwin 1876). When genetically distinct genomes hybridize for the first time they may encounter genetic shock and asynchrony effects (Gernand et al. 2005). If the genomes are genetically incompatible, post-fertilization aberrations and seed abortion may occur, preventing the production of viable F1 progeny. This is termed hybrid incompatibility (Burke and Arnold 2001), which is observed in some inter-specific hybridizations (Burkart-Waco et al. 2013). However if two genetically distinct genomes hybridize and overcome the post-fertilization barriers and produce viable offspring, heterosis may be observed in some instances (Birchler et al. 2010; Chen 2010).

Inbreeding depression is commonly considered the conceptual opposite of heterosis. In maize it has been predicted that heterosis can occur by reversing inbreeding depression on self-fertilized lines (Good and Hallauer 1977). Inbreeding depression is defined as “the reduced survival and fertility of offspring of related individuals” (Charlesworth and Willis 2009). Outcrossing organisms including plants and animals which undergo multiple rounds of inbreeding generally display slower growth, lower fertility, and increased disease susceptibility (Charlesworth and Charlesworth 1987). Most genetic models for explaining heterosis rely upon considerations of the impact of heterozygosity and homozygosity at particular loci in inbred and outbred individuals. The most widely considered genetic models for explaining heterosis are the *dominance*, *overdominance*, and *epistasis* models (Lewontin 1964).

These three models have been developed to allow better scientific understanding of the biological phenomenon of heterosis. The development of accurate models is a prerequisite for rational exploitation of the potential value of heterosis in agriculture and other applied biology areas. However, despite consistent research in this field for over 70 years, a clear unifying molecular or genetic model remains elusive. It is likely that no one model can fully explain either hybrid vigor or heterosis. It is important to note that these theories are not mutually exclusive, and that it is likely that different mechanisms can explain heterosis observed under different combinations of crosses in different species, or affecting different phenotypes (Chen 2013; Schnable and Springer 2013).

4.2.1 Dominance Model of Heterosis

The *dominance model* of heterosis proposes that following hybridization between genetically distant genomes, the F1 generation displays heterotic characteristics as a result of the complementation of multiple slightly deleterious alleles from the

genome of one parent line by superior, dominant alleles from the other (Birchler et al. 2003). This can lead to F1 offspring that exceed the trait values observed in either parent. In Fig. 4.1a, slightly deleterious alleles (“a” and “b”) are present in the genomes of parental lines P1 and P2, which have genotypes aa, BB and AA, bb, respectively. Although alleles significantly reducing the fitness of the organism are expected to be purged by natural selection (Schnable and Springer 2013), mildly deleterious alleles may persist in a population due to linkage with beneficial or essential alleles. Upon hybridization, the F1 offspring will be heterozygous at both loci, i.e., genotype Aa, Bb. The deleterious alleles at both loci can thus be complemented, leading to increased fitness or enhanced values of other traits observed. The heterosis effect observed in the F1 progeny is not stably inherited in subsequent generations due to independent segregation. The dominance model is also applicable in the case of crosses in which one parent contains advantageous genes which are entirely missing or non-functional in another (Fu and Dooner 2002; Birchler et al. 2010). In both cases, the dominance model (masking of deleterious recessive alleles) presents heterosis as a simple reversal of inbreeding depression (unmasking of deleterious recessive alleles).

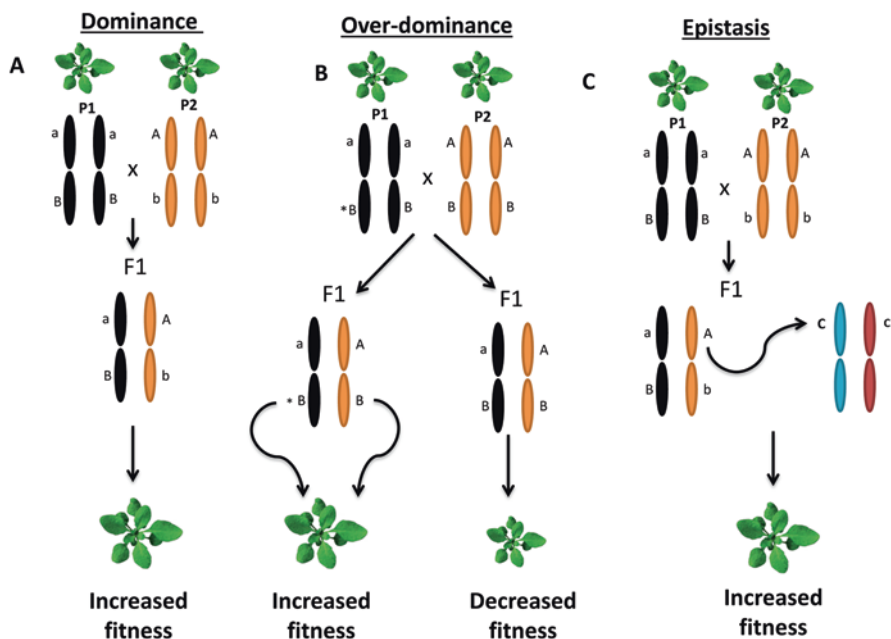


Fig. 4.1 Schematic of genetic models for explaining heterosis. (a) Dominance model; (b) Overdominance model; (c) Epistasis. For full descriptions, see text

4.2.2 Overdominance Model of Heterosis

Since its development in the early part of the twentieth century, the dominance model has explained significant aspects of heterosis (Davenport 1908; Jones 1917; Troyer 2006). However, the dominance model also suffers from certain limitations which suggest that it is only a partial explanation for the phenomenon of heterosis. A key criticism of this model is that if complementation of deleterious alleles is causal for heterosis, then the potential to generate heterosis by crossing commercially available inbred lines should decrease over time (Springer and Stupar 2007). Elite maize germplasm has been exploited in breeding programs for nearly 90 years, and during this period the majority of slightly deleterious alleles would be expected to have been purged (Duvick 2001). Models of heterosis relying entirely on the concept of dominance would predict that the potential for heterosis should also have decreased over the same time period (Birchler et al. 2003). However, the extent of heterosis generated in breeding programs has not reduced over time, and may even have increased somewhat (Duvick 1999), suggesting that heterosis is more than a simple complementation of deleterious alleles by dominant ones.

The extent of heterosis and inbreeding depression in polyploid plants when compared with their diploid counterparts also suggests that dominance models of heterosis are incomplete. Since polyploids have the potential to possess higher allelic diversity than their diploid counterparts, the onset of inbreeding depression in polyploids should occur more slowly during the self-fertilization of polyploids than in diploid progenitors, as homozygous offspring are produced less frequently. However, it has been shown that inbreeding depression rates are similar in diploids (2 \times) and tetraploids (4 \times) of various plant species (Rice and Dudley 1974; Birchler et al. 2005). Furthermore, the levels of heterosis observed when inbreeding depression is reversed continue to increase with increasing heterozygosity (Birchler et al. 2005), which would not be the case if heterosis depended upon the masking of slightly deleterious alleles. In the case of polyploid plants, it is likely that complementation of deleterious alleles by dominance therefore plays only a limited role in heterosis.

Limitations in genetic models of heterosis based on dominance led to the development of alternative models based on transgressive (or overdominant) interactions between alleles rather than simple complementation, or based on allelic dosage effects (the onset and reversal of inbreeding depression in polyploids has been explained with reference to allelic dosage effects, Birchler et al. 2005). The *overdominance model* proposes that synergistic allelic interaction at particular heterozygous loci leads to superior performance in the F1 progeny. In Fig. 4.1b, *B is an allele variant of B (irrespective of dominance in this case). F1 hybrids inherit both alleles and act synergistically to cause a heterotic effect. If *B is not inherited the F1 progeny exhibit no heterotic effect.

One of the most exciting developments in our understanding of overdominant heterosis is the identification of cases of “single locus overdominance” (Mckeown et al. 2013a) such as that involving *SINGLE FLOWER TRUSS (SFT)* locus in tomato.

SFT is a *FLOWERING TIME (FT)* related gene that when present in a heterozygous state increases tomato yields by up to 60% (Krieger et al. 2010). Other cases of single locus heterosis have been observed in the model plant organism *Arabidopsis thaliana* (Meyer et al. 2010; Smith et al. 2011), as well as in other agronomic crops including wheat (Li et al. 2013), rice (Hua et al. 2003; Goff and Zhang 2013), and maize (Schnable and Springer 2013).

The identification of overdominant loci could potentially lead to easier and faster deployment of heterosis. The conventional method of generating hybrids (crossing inbred lines in different combinations to identify non-additive traits in F1 progeny, Duvick 2001) is time consuming, laborious, and expensive. With the aid of denser genetic maps for agronomic crops, quantitative trait loci (QTL) maps relevant for the study of heterosis are being generated (Basunanda et al. 2010; Schön et al. 2010; Mckeown et al. 2013b; Wallace et al. 2014). Such methods still face potential pitfalls such as false positives arising as a result of pseudo-overdominance, where pseudo-overdominance is defined as a phenomenon where two or more tightly linked dominant alleles in a repulsion phase can induce heterosis in F1 offspring which mimics overdominance effects (Crow 1952; Schnable and Springer 2013). Heterosis due to epistatic interactions can also mimic overdominance (see below). Accurate identification of individual loci that can induce heterosis when in a heterozygotic state could be extremely useful for crop breeding programs as it would allow better prediction of heterotic crosses, and, potentially, direct manipulation of the loci concerned. The advent of genome editing techniques using transcription activator-like effector nucleases (TALENs) or clustered regularly interspaced short palindromic repeats (CRISPRs) could potentially be used to efficiently generate overdominant alleles to induce artificial overdominant heterosis as previously proposed (Mckeown et al. 2013a).

4.2.3 Heterosis, Epistasis, and Complexity

Whereas the overdominance model proposes that interactions at individual loci can induce heterosis (for example, by producing heterodimeric protein complexes with greater activity than a homodimeric complex), the *epistasis* model posits that heterosis can arise from epistatic interactions between alleles at different loci. Many heterotic epistatic relationships could in principle occur in F1 hybrids when one allele is complemented and its gene product affects the function of one or more products of other genes. For example, in Fig. 4.1c the gene product of dominant allele “A” has an epistatic interaction with the gene product of “C,” an unlinked locus. In some instances, this interaction can cause heterotic effects in the F1 progeny. An allele having an epistatic relationships with the allele of another locus *in trans* can mimic an overdominant heterotic QTL.

QTLs associated with heterosis suggest that in most crosses the molecular basis of heterosis is likely to be complex, and likely multigenic (Meyer et al. 2010; Riedelsheimer et al. 2012). It is quite likely that heterosis cannot be entirely

explained by any single unifying mechanism. Instead, heterosis is likely to be a complex, multifactorial trait that can involve allelic interactions at one or several loci. Microarray-based transcriptome profiling of maize inbred lines B73 and Mo17 and their resulting F1 hybrids has identified many different types of effects on gene expression levels including additive, high- and low-parent dominance, overdominance, and underdominance (Swanson-Wagner et al. 2006). Some researchers have proposed that terms such as dominance, overdominance, and epistasis should be abandoned in the context of heterosis models as they may be imposing artificial distinctions which do not easily correspond with the biological effects (Birchler et al. 2010).

4.3 Is There an Epigenetic Component to Heterosis?

Despite the successes of the *dominant*, *overdominant*, and *epistatic* models, a comprehensive framework for understanding heterosis still remains elusive. This has led to the suggestion that even the sum-total of all genetic interactions in a hybrid F1 genome cannot fully explain every aspect of heterosis (Baranwal et al. 2012; Groszmann et al. 2013; Schnable and Springer 2013). Indeed, consideration can be given as to whether non-genetic mechanisms underlying heterosis might exist. Such cases of heterosis could fall into the category of “epigenetic” effects, of the kind which have been shown to regulate gene expression, cell fate, and non-Mendelian inheritance (Mckeown and Spillane 2014). Here we review the evidence that suggests that there may be epigenetic components to heterosis in at least some cases, beginning with a summary of what epigenetic effects are, and how they could be contributing to heterosis effects.

Epigenetics is broadly defined as the study of heritable changes in gene activity that cannot be attributed to DNA sequence changes (Mckeown and Spillane 2014). It has been said that “epigenetics emphasizes heritable changes in gene expression that cannot be tied to genetic variation” (Richards 2006). A critical consequence of epigenetic effects is that the same genotype can display diverse phenotypes due to differential modification of the epigenetic state. For example, epialleles are alleles of a locus which have identical DNA sequences but display different epigenetic states, and which have been proposed to influence a variety of phenotypes in plants and animals (Richards 2006). The inheritance of epigenetic marks can deviate from the rules of Mendelian inheritance. The transmission of epigenetic marks through generations (as opposed to cell lineages) is a hotly investigated arena of biology due to its implications for the inheritance of acquired characteristics.

Some of the most studied epigenetic mechanisms are DNA methylation, histone modifications and chromatin remodeling, and the RNAi pathway (including RNA directed DNA methylation, RdDM). Such epigenetic regulatory mechanisms can target and epigenetically modify DNA sequences (Kooter et al. 1999). Epigenetic variation at the level of DNA and chromatin can cause gene expression to spatio-temporally change throughout development of an organism, and during

gametogenesis and sexual reproduction in mammals and plants (Hsieh et al. 2009; Slotkin et al. 2009; Feng et al. 2010; Calarco et al. 2012). The following section of this chapter describes three well-known epigenetic pathways, and presents some studies that suggest that epigenetic mechanisms may contribute to heterosis effects.

4.3.1 DNA Methylation and Heterosis

DNA methylation refers to the covalent addition of methyl groups to the bases of a DNA molecule, usually at the 5' positions of cytosine residues as catalyzed by DNA methyltransferases (He et al. 2013). DNA methylation occurs in many taxa. The function and control of DNA methylation has been deeply investigated in the model plant *Arabidopsis thaliana*. Whereas cytosine methylation (^mC) in animal genomes is often restricted to CpG contexts, in plant genomes it occurs more widely (Fig. 4.2a). In all sequence contexts the DOMAINS REARRANGED METHYLATION 2 (DRM2) gene product plays a major role in establishment of ^mC (Cao and Jacobsen 2002). Symmetric methylation in CpG contexts is maintained by the methyltransferase METHYLTRANSFERASE 1 (MET1). Cytosine methylation in CpHpG contexts (where H = A, C, or T) is maintained by a feedback loop involving CHROMOMETHYLASE 3 (CMT3) and the H3K9me2 methyltransferase, KRYPTONITE (KYP) (Cao and Jacobsen 2002). In contrast, asymmetric cytosine methylation (in a CpHpH context) is maintained by *de novo* methylation through a pathway known as RNA directed DNA methylation (RdDM) in which the methyltransferase DRM2 methylates CpHpH motifs. Active demethylation can also occur through the action of DNA glycosylase-ligases such as DEMETER (DME) (Penterman et al. 2007; Zhu 2009). DNA methylation is known to be important for the silencing of active transposons, genetic repeat elements found in pericentromeric regions of chromosomes, and promoter regions of genes (Lippman et al. 2004).

A number of correlative studies have suggested that epigenetic effects, including cytosine methylation (^mC) of DNA, may be involved in pathways contributing to heterosis. Several studies have identified differences in ^mC patterns in heterotic F1 hybrids when compared to their respective parents (in maize, for example, Zhao et al. 2007). Similarly, in rice, differences in ^mC patterns are observed between inbred lines and are correlated with transcript level changes at some of the differentially methylated regions (DMRs) in the F1 hybrids (He et al. 2010).

Two studies analyzed crosses between *A. thaliana* accessions in which the F1 offspring display heterosis for biomass. Shen et al. (2012) performed genome-wide methylation profiling by constructing methyl-seq libraries of *A. thaliana* accessions Landsberg *erecta* (*Ler-0*) and C24 parental inbred lines and their reciprocal hybrid lines, *Ler-0* × C24 and C24 × *Ler-0*. Through this approach it was possible to analyze global methylation patterns in the parental and F1 genotypes. It was found that the overall level of DNA methylation was higher in the F1 hybrids compared to the

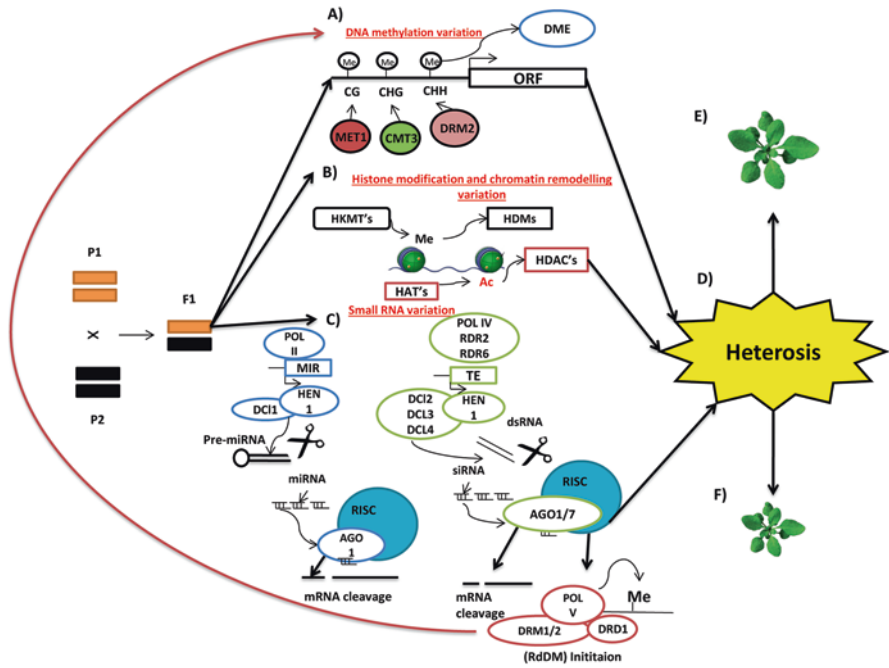


Fig. 4.2 A possible model linking epigenetics to the alteration of biological networks. Two distinct genomes hybridize to create a heterotic F1 hybrid: (a) Differential methylation patterns can occur in F1 hybrids where there is allelic variation at particular loci. Such methylation patterns are established and maintained symmetrically (CpG, CpHpG) by METHYLTRANSFERASE 1 (MET1) and CHROMOMETHYLASE 3 (CMT3), respectively, and asymmetrically (CpHpH) by the de novo methyltransferase DOMAINS REARRANGED METHYLATION 2 (DRM2). De novo methylation can be established by RdDM (Red arrow). (b) Histone lysine methyltransferases (HKMTs), demethylases (HDMs), histone acetylases (HATs), and deacetylases (HDACs) can produce unique histone modification patterns in F1 hybrids to activate (H3K4me) or repress transcription (H3K27me3). (c) sRNAs can accumulate at different levels in hybrids. miRNAs are established by POL II mediated *MIR* transcription to create precursor miRNA (Pre-miRNA) which is diced by DICER LIKE 1 (DCL1) in collaboration with HUA ENHANCER 1 (HEN1). Mature miRNA are loaded into the RNA ASSOCIATED SILENCING COMPLEX (RISC) associated with ARGONAUATE 1 (AGO1) and mediate post-transcriptional gene silencing (PTGS). sRNAs are derived primarily from transposons in heterochromatic regions or by endogenous *MIR* genes. They are diced by DCL2, 3, or 4 and loaded into RISC accompanied by AGO and either mediate PTGS or initiate *de novo* methylation by RdDM (red arrow). Such epigenetic pathways have the potential to either independently or synergistically establish heterosis (d), and either improve (e) or deteriorate (f) vigor in F1 hybrids

parents. In a similar approach Greaves et al. (2012) performed whole methylome profiling on *Ler-0* and C24 parental lines and their reciprocal F1 hybrids. By using a methylation clustering approach the differences in total ¹³C between the parents were determined to be 23% (Greaves et al. 2012). Of this, CpHpH methylation showed the greatest variation. In addition, regions with differential methylation in a CpHpH context were enriched in gene bodies and their flanking regions. When

assessing the methylome of F1 hybrids, both additive and non-additive methylation differences were observed, with CpHpH methylation being predominantly lower than the mid-parent value in hybrids. Non-additive methylation clusters were enriched in genic regions, in a similar pattern to their parental lines. This could suggest a possible link between differential ³CpHpH in parental plants and the occurrence of non-additive methylation in this context in their F1 hybrid offspring, at least in *A. thaliana* (Greaves et al. 2012).

4.3.2 Heterosis and Histone Modifications

DNA methylation frequently interacts with covalent modifications of the histone octamers which “package” the DNA into nucleosomes and into chromatin. Histone modification refers to the covalent modification of histone proteins, usually on their N-terminal tails, which causes nucleosome rearrangement, chromatin remodeling, and altered transcriptional potential. A multitude of histone modification marks have been documented in plants and other eukaryotes (Berger 2007). Key histone modifications include methylation and acetylation, especially of lysine (Lys, K) residues (which are abundant on histone N-terminal tails). Such modifications are orchestrated by complexes of histone lysine methyltransferases and demethylases (HKMTs and HDMs), and acetylases and deacetylases (HATs and HDACs) (Fig. 4.2b) (Cao and Jacobsen 2002; Chandler and Stam 2004; Gendrel et al. 2005; Fuchs et al. 2006; Pfluger and Wagner 2007). Histone modification marks can act as binding sites for different chromatin remodeling enzyme complexes, as in the case of KYP mentioned above, and can lead to the formation of stable epigenetic loops involving feedback between DNA methylation and histone modification.

A possible link between histone modifications and heterosis has been suggested (Ni et al. 2008). This study demonstrated that genes involved in the circadian clock of *A. thaliana* underwent transcriptional changes in both diploid and allotetraploid F1 hybrids which were associated with altered histone modifications. The circadian clock, which is an intracellular biochemical mechanism that synchronizes biological events between day and night cycles, operates by matching daily changes in gene or protein activity (defined by their periods and amplitudes) to aspects of the external environment, such as daylight (Dodd et al. 2005). In plants, the circadian clock is known to control many biological processes, which include starch biosynthesis and growth rate. Plants that are exposed to environments that match its internal circadian rhythm are more vigorous than plants that are not. By using antibodies against the H3-Lys-9 acetylation (H3K9ac) and H3-Lys4 dimethylation (H3K4me2) marks which commonly correlate with gene activation in *A. thaliana* (Jenuwein and Allis 2001), Ni and colleagues found both modifications to occur at key clock regulatory genes in F1 hybrids. Functional alterations of the internal clock by histone-mediated control of the *CCA1* and *LHY* genes may lead to the differential biomass accumulation observed in hybrids and polyploids (Miller et al. 2012; Shen et al. 2012; Chen 2013).

Studies in rice have shown that overexpressing or knocking out histone deacetylase genes can lead to non-additive gene expression in hybrids at some loci, which could in principle lead to overdominance for a trait controlled by the locus. By using high-throughput ChIP-Seq with three histone marks (H3K4me3, H3K9ac, and H3K27me3) global histone mark patterns could be compared between two rice subspecies and their resulting F1 hybrid (He et al. 2010). Correlations were found between the transcriptional activation mark, H3K4me3, and the transcriptional repression mark, H3K27me3, linked to dynamic expression patterns between hybrids and parents. Independent studies on 6 days after pollination (DAP) F1 hybrid maize endosperm transcriptomes identified significant expression variations in the key histone variant HTA112, when compared to parental inbred lines (Jahnke et al. 2010). These studies raise the possibility that features of heterosis could be associated with alterations of epigenetic histone modifications.

4.3.3 sRNAs: Roles in Epigenetic Regulation and Heterosis

In plants, epigenetic regulatory loops may also involve small RNA molecules, i.e., short (20–27 nucleotide, nt) non-coding RNAs (Simon and Meyers 2011). Such sRNA can regulate gene expression and also act as an RNA-based immune system to counteract against foreign viral RNA or transposons which are deleterious to genome integrity (Vaucheret 2006). These sRNA-mediated processes include transcriptional gene silencing (TGS) and post-transcriptional gene silencing (PTGS) (Vance and Vaucheret 2001; Waterhouse et al. 2001; Boutet et al. 2003; Lippman et al. 2004).

Plant sRNAs include two major classes, the microRNAs (miRNAs) and small interfering RNAs (siRNA) (Fig. 4.2c). miRNA precursors are endogenously transcribed from endogenous *MIR* genes by RNA POLYMERASE II (RNA Pol II) and are then cleaved (“diced”) to a length of 20–27-nt by DICER LIKE 1 (DCL1). The mature miRNAs are then loaded into the RNA Induced Silencing Complex (RISC) complex, accompanied by the ARGONAUTE 1 (AGO1) endonuclease (Bartel 2004). The loaded complex is then guided to messenger RNAs with sequence similarity to the mature miRNAs in order to cleave the mRNA transcripts and/or inhibit translation. Small interfering RNA (siRNA) biogenesis pathways are mostly stimulated by the presence of aberrant double stranded RNAs produced from transposons in heterochromatic regions or by invading viral RNA. They act to maintain genome stability by silencing transposons and help to protect against viral RNA invasion (Baulcombe 2004; Slotkin and Martienssen 2007). Although there is some uncertainty regarding how the biogenesis of plant siRNAs is regulated, it is considered that RNA is transcribed by RNA POLYMERASE IV (Pol IV) and reverse transcribed into double stranded RNA (dsRNA) by RNA DEPENDANT RNA POLYMERASE 6 (RDR6) or RNA DEPENDANT RNA POLYMERASE 2 (RDR2). dsRNAs are subsequently diced by either DCL2, 3, or 4 to generate mature 20–24-nt siRNAs which are loaded into RISC (accompanied by AGO proteins) to

catalyze either mRNA cleavage or stimulation of the RdDM pathway for *de novo* DNA methylation and/or histone modifications (Vaucheret 2006; Castel and Martienssen 2013). It should be noted that this model is based upon *Arabidopsis thaliana* and could vary between species.

As RdDM can direct DNA methylation and heterochromatin formation (Feng et al. 2010), it has been speculated that sRNAs could also regulate epigenetic changes associated with heterosis. Indeed, sRNA levels show substantial variation between parental inbred lines and their F1 hybrid or allopolyploid offspring in several taxa, e.g., the *Arabidopsis* genus (Ha et al. 2009; Groszmann et al. 2011; Li et al. 2012; Shen et al. 2012), and the monocot cereals such as wheat (Kenan-Eichler et al. 2011), maize (Barber et al. 2012; Ding et al. 2012), and rice (Chen et al. 2010; He et al. 2010; Chodavarapu et al. 2012).

A number of studies have provided evidence to support the hypothesis that such non-additive changes might be involved in heterosis. For example, crosses between the *A. thaliana* accessions Col-0 and *Ler-0* demonstrated a decrease in the accumulation of 24-nt siRNA in the hybrids compared to the parents, concomitant with altered patterns of CpHpH methylation (Groszmann et al. 2011). Potentially, heterosis could be induced by the hybridization of epigenetically divergent parents as a result of increased epiallelic variation within the offspring (Chen 2013). When differences in DNA methylation between parental and heterotic F1 hybrid *A. thaliana* lines were mapped at single base-pair resolution across the genome, the hybrids displayed elevated methylation levels, especially in transposable elements (Shen et al. 2012). A parallel genome-wide sRNA-seq experiment demonstrated that production of sRNA differed between the parental lines and hybrids. In addition, sites of sRNA synthesis were significantly associated with loci undergoing increased DNA methylation (Shen et al. 2012). This study suggests a link between sRNA and ¹³C accumulation with altered expression in F1 hybrids at selective loci.

To date, most studies of the possible links between sRNAs, DNA methylation, and heterosis have been based upon inference and correlation. However, similar to the use of histone modification mutants in rice, some studies have functionally tested the possibility that sRNA-mediated pathways might be necessary for heterosis. HUA ENHANCER 1 (HEN1) is an *A. thaliana* methyltransferase that methylates mature sRNAs of both siRNA and miRNA classes to increase their stability (Vilkaitis et al. 2010). When a *hen1* mutant was crossed to the *Ler-0* background to generate F1 hybrids (*hen1* x *Ler-0*) it was found that the resulting F1 hybrids showed reduced size, and that plant vigor was compromised. These results indicate that the association between sRNAs and some heterotic traits might indeed be causal. However, contrasting results were presented by studies using mutants for the maize MODIFIER OF PARAMUTATION 1 (MOP1) gene, which is considered to be the homologue of *A. thaliana* RDR2 and is essential for the biogenesis of heterochromatic 24-nt siRNAs in maize (Lisch et al. 2002; Barber et al. 2012). The maize functional study found that heterosis was not disturbed in *mop1* hybrids (Barber et al. 2012). Such differences may be because HEN1 is important not only for the stability of 24-nt siRNAs but also other classes of sRNAs including miRNAs, while the role of MOP1 is restricted to the generation of 24-nt siRNA.

4.3.4 Genome-Wide Epigenetic Networks as a Component of Heterosis?

Allelic methylation differences in F1 hybrids have been shown to occur through *trans*-acting phenomena termed trans-chromosomal methylation (TCM) (Fig. 4.3) and trans-chromosomal demethylation (TCDM) (Greaves et al. 2012). Such methylation events predominantly occur in F1 hybrids at allelic sites where differentially methylated regions exist between the genomes of the parents. In such cases, it is sometimes found that the methylation of one allele will be increased or decreased such that it matches the methylation status of the homologous allele derived from the other parent. Between them, TCM and TCDM events accounted for 86% of the total non-additive methylation differences observed in F1 hybrids (Greaves et al. 2012). Comparative analysis of methylation and siRNA distribution in parental

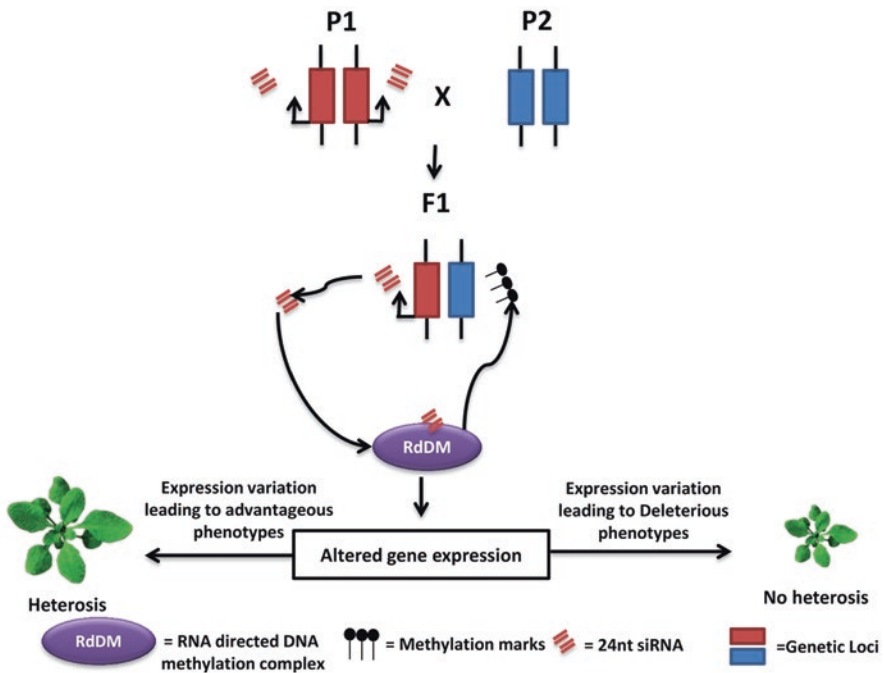


Fig. 4.3 Possible roles for methylation, siRNA, and RdDM in heterosis. (a) Two distinct genomes (P1, P2) with various levels of siRNA accumulation hybridize to create a heterotic F1 hybrid (F1). (b) Upon hybridization siRNAs can interact in cis or trans with genetic elements containing their complementary sequence. siRNAs can interact with RdDM pathways to silence genes via trans-chromosomal methylation. (c) Methylation marks may be removed allowing expression of both alleles in the F1 hybrids. (d) siRNA may be generated via the allele inherited by P1 but does not methylate its homologous allele leading to allele specific expression. Such types of epigenetic amendments may lead to altered expression levels in F1 hybrids which could potentially lead to heterotic effects which either improve (g) or deteriorate (f) vigor in F1 hybrids

(C24, *Ler-0*) and F1 hybrid lines (C24×*Ler-0*, *Ler-0*×C24) indicated that there was also a positive correlation between siRNA abundance and such non-additive methylation. These changes were in some cases also found to correlate with gene expression changes that departed from the mid-parent value at these loci. These studies suggest that RdDM may play a role in modulating DNA methylation levels between the alleles at hybrid loci, leading to non-additive methylation and heterotic gene expression in hybrid plants.

A recent study investigated the inheritance pattern of TCM and TCDM at specific loci in the *A. thaliana* genome (Greaves et al. 2014). By assessing total methylation levels at loci previously shown to undergo TCM and TCDM in reciprocal *Ler-0* × C24 F1 hybrids, it was determined that altered methylation patterns were stably inherited into the F2 generation. Interestingly, however, ¹³C patterns were transmitted to the F1 offspring outcrosses or backcrosses by the C24 genomic segment only. When *Ler-0* segments that were newly methylated were backcrossed to unmethylated *Ler-0* segments, a paramutation-like phenomenon occurred and this phenomenon appeared to direct *de novo* methylation via TCM.

4.4 Parent-of-Origin Genome Dosage Effects and Their Links to Heterosis

To test for evidence of parent-of-origin effects on heterosis in phenotypic traits, our lab investigated the effects of polyploidization and hybridization on the phenotypes of triploid plants produced from inter-ploidy crosses. The phenotypes measured were the reproductive traits of ovule number and fertility (Duszynska et al. 2013). These were determined in *A. thaliana* F1 hybrid triploids generated by crossing 89 diploid accessions using tetraploid *Ler-0* plants, again using a reciprocal design to allow parent-of-origin effects to be identified. All traits showed dramatic alterations in certain F1 hybrid lines, which were in many cases found to be heterotic. Strikingly, a strong parent-of-origin-effect was displayed between maternal excess 3× (M) and paternal excess 3× (P) F1 hybrid triploids with respect to both total ovule number per silique, and their fertility (Duszynska et al. 2013). Our study suggests that parent-of-origin effects (argued to be *sensu lato* epigenetic in nature) can determine whether the F1 progeny display heterosis for certain traits. Regardless of its mechanistic basis, some of the modulation of parental effects on heterosis by natural variation are manifested in diploid–diploid crosses, while other elements can be “cryptic,” and are only manifested in inter-ploidy crosses.

Are such effects a peculiarity of *A. thaliana*, or other plants consisting of highly inbred homozygous populations, or are they of broader relevance? The effect of genome dosage on heterosis in *Z. mays* has been investigated using inbred diploid lines (B73, Mo17) and their reciprocal F1 hybrids, when compared to matched triploid derivatives (Yao et al. 2013). It was observed that reciprocal F1 triploid hybrids varied in the extent of heterosis. Such studies contradict the predictions of a strict

dominance model of heterosis as it is predicted that complementation of recessive mutations would occur equally in both triploid hybrids. Such studies demonstrate that parent-of-origin effects can influence heterosis in both monocots and dicots.

4.5 Future Directions

The search for a unifying biological mechanism for heterosis still remains elusive even after over 100 years of research in this area. The key models of *dominance*, *overdominance*, and *epistasis* are still in use for describing multigenic heterosis. However, investigations of epigenetic processes including DNA methylation, histone modification, and sRNA expression and accumulation provide some new perspectives in relation to heterosis. Early studies suggesting links between non-additive DNA methylation with heterosis in F1 hybrids (Zhao et al. 2007) have been complemented with additional studies correlating sRNA, DNA methylation, and histone modification with heterosis (Ni et al. 2008; He et al. 2013). Global siRNA differences have been observed between F1 hybrids and parents in *Arabidopsis thaliana* (Groszmann et al. 2011). An increased understanding and prediction of TCM and TCDM events in plant epigenomes in both *Arabidopsis thaliana* and crops has the potential to contribute to further unraveling of the molecular basis of heterosis. To date, the bulk of epigenetic heterosis research has been conducted in the model crop *Arabidopsis thaliana* and *Zea mays*. Expanding epigenetic research into other crops that display heterosis effects will contribute to advancing of understanding regarding the molecular basis of heterosis. Clearly, while there is evidence that epigenetic variation may be linked to heterosis, the functional studies to test whether epigenetic regulation is causally central to heterosis are currently lacking. The ongoing rapid advances in functional genomics and epigenomics now pave the way for a deeper mechanistic understanding of both the genetic and epigenetic contributions to heterosis effects.

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