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Polyploidization and epigenetics

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The occurrence of polyploidy, or whole genome duplication, can result in instantaneous speciation. Because new polyploids are immediately reproductively isolated from their non-polyploid congeners, polyploidization has played an important role in the diversification of flowering plants and some vertebrates. Newly formed polyploids must respond to this instantaneous genomic change, which resembles "genome shock" syndrome to survive and reproduce successfully. Epigenetic changes, which do not cause changes to the sequence of DNA, can significantly contribute to the survival of and ultimately to the evolutionary success of new polyploids. Epigenetic regulation, both transcriptional and post-transcriptional, entails changes in DNA methylation, gene status and/or nucleolus dominance. These changes provide effective and flexible ways for a new polyploid to respond quickly to the enormous change in genetic material, to survive and potentially reproduce. We examine and assess certain epigenetic phenomena and possible pathways that may facilitate the evolutionary success of polyploid organisms.

polyploidization, epigenetics, DNA methylation, gene status, nucleolus dominance

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1 Occurrence of polyploidy and its evolutionary significance

Polyploidy is an important evolutionary feature of flowering plants and vertebrates [1,2]. Most angiosperms and many animals have undergone this process many times [3]. There are indications that most vertebrates are paleopolyploids, derived from an ancestor in which two rounds of genome doubling or whole genome duplication (WGD) occurred. Teleosts, which are the most species-rich group of vertebrates, are derived from a lineage that experienced a third round of WGD [4–8]. Study of the production, survival and subsequent success of polyploids may provide insight into some of the more dramatic evolutionary events, and epigenetic changes may be crucial to this understanding.

2 Polyploidization and phenotypic expression

Two types of polyploids can be recognized, allopolyploids and autopolyploids. Allopolyploids are often derived from a two-step process: hybridization between two species and then doubling of all the chromosomes by the union of unreduced gametes (or doubling may occur by other mechanisms) [9]. In contrast, autopolyploids are usually formed by chromosome doubling within a species [9].

Contemporary work on polyploidization mainly focuses on the duplicated genome and evolution post-polyploidization, e.g., the occurrence of polysomic inheritance after genome

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doubling, changes in genome structure, gene structure, gene expression, and subsequent phenotypic changes. Current studies suggest that once polyploidy occurs, whatever the mechanism involved, the polyploid genome evolves rapidly [10].

Epigenetic changes promote the survival and reproduction of neopolyploids and subsequently facilitate genomic changes. Subsequent to polyploidization, genomic shock syndrome and dramatic changes are likely to produce severe stress on the survival and reproduction of polyploids. Chen [10] argued that "Epigenetic changes, which are potentially reversible, provide an effective and flexible means for a polyploid cell to respond to polyploidy or genomic shock. Moreover, gene silencing or activation that is initially epigenetic and reversible could be one step toward a genetically fixed and irreversible state". Observed phenomena provide clues to the potential effects of polyploidization-dependent epigenetic changes. For example, the single genome of an autopolyploid is the same as its parents' except in the number of chromosomes, yet the phenotype of an autopolyploid may be distinguishably different from that of its parents. Compared to chromosome doubling, inter-specific hybridization exerts greater effects on gene expression [9]. Gene expression changes affect the phenotypes of organisms. For example, allopolyploids (or interspecific hybrids) can present various phenotypes, some of which may be inherited from their parents, some of which are newly formed and some of which are lost in polyploid offspring. In newly formed *Tragopogon* allopolyploids, transcriptional investigation indicated that 5% of genes have been silenced and that 4% exhibited novel patterns of expression [11]. These results suggest that such expression changes may be epigenetically controlled.

3 Epigenetics versus genetics

Epigenetics generally refers to heritable changes in gene expression that do not involve DNA mutations or chromosome changes (genetics) [12 and therein]. Epigenetic regulation of gene expression occurs by DNA and/or histone modification. In polyploids, epigenetically produced phenomena include gene silencing, gene activation, genomic imprinting, maternal effects, nucleolus dominance and chromatin conformation changes.

4 Phenotypic variation and hybrid vigor in polyploids

Many studies have demonstrated that genetic and epigenetic changes, triggered by hybridization and whole genome duplication, play major roles in phenotypic expression [13–16], which in turn determines the fitness of newly formed polyploids [17]. Several important crops such as wheat, cotton and canola are allopolyploid and contain two or more different genomes [10,18]. Some animals, including several commercially raised fish are polyploid, such as *Carassius auratus gibelio* [19], allotetraploid progenies of *Carassius auratus* crossed with *Cyprinus carpio* and allotriploids produced by crossing allotetraploid progenies of *Carassius auratus* × *Cyprinus carpio* with *C. auratus cuvieri* [20,21]. These crops and fish breeds show more phenotypic variation and hybrid vigor than their parents or progenitors [18–21]. For example, segregating hybrids and stable allopolyploids of *Arabidopsis thaliana* and *Arabidopsis arenosa* are larger than their parents [18]. In vertebrates, the growth rates of triploid *C. a. gibelio*, allotriploids produced by crossing allotetraploid *Carassius auratus* × *Cyprinus carpio* progeny with *C. auratus cuvieri* and allotetraploid progeny of *Carassius auratus* × *Cyprinus carpio* were 13.7%–34.4% [19], about 100% [20] and 21.8% [21] faster than their parents, respectively. The latest evidence indicates that newly formed genomic interactions in allopolyploids induce phenotypic variation and increased growth [18]. Furthermore, the over-dominance model suggests that heterosis should reach its peak at the maximum levels of heterozygosity and dissipate when approaching homozygosity [22].

Once successful polyploidization has occurred, genome complexity and fitness can be enhanced to produce greater genomic flexibility. This can result in higher growth rates, larger size and increased survival fitness in harsher environments.

5 Epigenetic phenomena and relevant mechanisms in polyploidy

5.1 Polyploidization and DNA methylation

(i) DNA methylation in polyploids. The production of polyploids is often accompanied by alterations in DNA methylation. Some studies [16,23] found that changes in DNA methylation patterns occurred more frequently in synthetic allotetraploids than in their parents. Cytosine methylation is a universal epigenetic phenomenon [24,25], and is the most important epigenetic change associated with plant polyploidy [26–28]. For example, tobacco possesses a highly repeat-rich allotetraploid genome in which about 28% of cytosines appeares to be methylated [29]. In addition, DNA methylation changes are observed in newly formed allopolyploids of *Arabidopsis* [16] and wheat [30]. Many *A. thaliana* genes were transcriptionally suppressed in resynthesized allotetraploids. In natural allopolyploids, a subset of *A. thaliana* loci, including transposons and centromeric repeats, were heavily methylated and subjected to homologous genome-specific RNA-mediated DNA methylation; however, reduced methylation at some loci was also observed [22]. In a synthetic allotetraploid (*Aegilops sharonensis* × *Ae. umbellulata*), some genetic alterations were

associated with comparative hypomethylation of the promoter region within the *Ae. umbellulata*-derived rDNA units [31]. In another example, the endoreduplication of cells during tomato fruit ripening was not accompanied by material changes in global methylation levels; there was no change in the level of methylation, but the patterns of methylation had changed [32]. This suggests that methylation patterns may be rapidly established on dramatically multiplied chromosomes [23].

(ii) Mechanisms of DNA methylation. What causes alternations of methylation in a polyploid? Recent findings have indicated alternative possibilities. Firstly, methyltransferases are responsible for methylation, and disruption of *met1* expression affects gene regulation and development in allopolyploids [22]. Chen et al. [16,22] treated resynthesized and natural *Arabidopsis* allotetraploids with 5-aza-2′-deoxycytosine (5-aza-dC), a chemical inhibitor of DNA methyltransferases, and observed dramatic changes in phenotypes. Protein-coding genes [22,33] were also reactivated, suggesting that DNA methylation was responsible for reactivation of some genes in the allotetraploids [22]. Hence, the expression level of methyltransferases plays a crucial role in gene expression, gene regulation, growth and development. Methyltransferase 1 (MET1) mainly affects CG methylation in allotetraploids, and an increase in DNA methylation is likely to be caused by an indirect effect of a *met1* defect [22]. The increase in DNA methylation in the promoter region is probably associated with the down-regulation of this gene in a genetic background that is predominantly demethylated. This is reminiscent of the dense methylation that is observed around the transcription start site and within the coding region of the *SUPERMAN* (*SUP*) gene in *A. thaliana*, which over-expresses antisense MET1 [22,34]. In allopolyploids, down-regulation of MET1 led to an overall reduction of CG methylation and to increased levels of transcription of the SCP repetitive gene family and of En/Spm transposons and centromeric repeats, suggesting a causal link between CG methylation maintained by MET1 and transposon suppression [10]. Secondly, Chen [10] suggested that allopolyploidy and DNA methylation might have distinct and overlapping effects on gene regulation in allopolyploid genomes. They also observed that both the density and the specific sites of DNA methylation could affect gene regulation [10]. Reduced DNA methylation can cause severe growth and developmental abnormalities, and the severity increases in self-fertilized progeny. This is probably because hypomethylation induces other changes in the genome [22], as observed in the selfed progeny of ddm1 mutants [35,36]. Thirdly, changes in DNA methylation patterns occur more frequently in synthetic allotetraploids than in their parents [16,23]. This is perhaps related to the silencing of "redundant" genes in a doubled genome, or maybe because DNA methylation systems are perturbed by the effects of interspecies hybridization and polyploidization [23]. Fulnecek et al. [23] speculated that in tobacco, which has a repeat-rich heterochromatic genome, natural selection might favor additive expression of parental DNA methyltransferase genes that maintain high levels of DNA methylation. Methylation of repeat sequences could also be important for their stability in the cell nucleus [37], decreasing their recombination frequency [38] and preventing intergenomic homogenization [39]. Recent studies suggest that DNA methylation is likely to be involved in harmonizing genome structure and expression after genome merging in allopolyploid plants [23]. Therefore, maintaining high levels of DNA methylation may be important for an organism. Polyploidization can induce changes of DNA methylation, and DNA methylation plays a pivotal role in genome regulation and gene expression, thereby affecting growth, development and phenotype. Subsequent to polyploidization, organisms possess novel characteristics, different from those of their parents.

(iii) Levels of DNA methylation related to gene status. There may be close relationships between levels of DNA methylation and gene status [40,41]. The studies of Chen et al. [22] suggested that allopolyploidization induced DNA methylation changes in genes that were suppressed in synthetic allopolyploids, and reduced DNA methylation induced activation of suppressed genes in natural allopolyploids. Both polyploidization (interspecific hybridization) and DNA methylation affect the status of certain genes in *A. thaliana*. These genes are, however, generally suppressed in natural allotetraploids [10] and are sensitive to changes in DNA methylation [22]. Suppression or activation of these genes in a polyploid is likely to result in different levels of expression. Chen et al. [22] studied the relationship between gene status (gene silencing or gene activation), and the pathways involved. First, for homologous-specific centromeric DNA methylation, methylation of some homologous loci (e.g., locus At2g23810) was correlated with gene suppression in resynthesized and natural *Arabidopsis* allotetraploids [22]. Second, to test if loss of DNA methylation was associated with gene activation in allopolyploids of *Arabidopsis*, Chen et al. [22] analyzed DNA methylation variations in reactivated genes and transposons using DNA blot analysis. Their results indicated that activation of At5g36180 (SCP), At4g08010 and At1g44070 was related to reduced CG methylation [22]. Third, At2g23810 encoded a putative senescence-associated protein (SAP1). SAP1 was expressed at high levels in *A. thaliana* and was suppressed in resynthesized (F5) allotetraploids [22,42], while SAP1 was silenced in the natural allotetraploid *A. suecica*. Silencing of this gene correlated with hypermethylation of both CG and non-CG sites; however, when the overall methylation levels of both CG and non-CG sites were dramatically reduced, SAP1 was reactivated [22]. Fourth, when DNA methylation was reduced in the natural allotetraploid *A. suecica*, the expression of about 200 genes was altered [22]. These results indicate that DNA methylation or hypermethylation can result in gene suppression or silencing, and reduced DNA

methylation or hypomethylation can lead to gene activation. Other investigations with different organisms, using alternative methods support the above conclusion [22,33,43–46].

5.2 Polyploidization and gene silencing

(i) Gene silencing in polyploids. Numerous cases of gene silencing have been reported in polyploids. For example, diploid *A. thaliana* line C outcrosses to tetraploid plants can result in reduced hygromycin phosphotransferase (HPT) transgene activity [47]. Silencing of orthologous genes from one progenitor was estimated to occur at a frequency of ~0.4% in synthetic allotetraploids and ~2.5% in natural allotetraploids, with genes of *A. thaliana* and *A. arenosa* being equally likely to be silenced [43]. Sometimes, parallel expression and silencing patterns are observed in natural and neopolyploids that are of similar genomic constitution [44,48,49]; wheat allohexaploids also show organ-specific silencing of homologs [9]. Adams et al. [48] documented considerable variation in the expression and silencing patterns of homologous genes in different organs of allopolyploid *Gossypium hirsutum*. Indeed, unequal expression of homologs and silencing of one copy have been observed in neopolyploids, as shown by several studies of allotetraploid *Gossypium*, *Triticum* and *Arabidopsis* [13,45,49–51]. These studies indicate that gene silencing is widespread following direct or indirect polyploidization.

Subfunctionalization may result in genes that are reciprocally silenced in polyploids. Adams et al. [48,52] showed reciprocal, organ-specific silencing of homologs of the alcohol dehydrogenase gene *AdhA* in various floral organs of *G. hirsutum*, such that one homolog had been silenced in some organs and the other homolog had been silenced in other organs. This was then followed by subfunctionalization [9]. Subfunctionalization may lead to changes in gene expression levels and gene expression patterns, ultimately resulting in phenotypic differentiation [2,53]. Moreover, in allotetraploids, after the formation of epialleles, genes will be reciprocally silenced [45]. In summary, reciprocal silencing of genes may be a new epigenetic event, and it affects gene expression levels and thus potentially may change phenotypes.

(ii) Silencing of redundant genes due to balance dosage. The underlying molecular mechanism of gene silencing may result from the requirement to shut down redundant gene copies. Pikaard [43] noted that when chromosomes pair, there would be some 'chatter' in new allopolyploids, and that "evidence emerged that new allopolyploids might deal with these challenges by silencing some of the redundant 'chatter' and by finding ways to reduce the incidence of chromosomal infidelity", meaning that changes of ploidy seem to result in epigenetic silencing [47]. In addition, he noted that "transposable elements, the troublesome hounds of the genome, are often unleashed in new hybrids and can roam their new environment causing damage, which ultimately leads to gene silencing" [43]. Simultaneously, to reduce damage, cells may also limit changes in gene expression by silencing some gene regions [43].

5.3 Polyploidization and nucleolus dominance

(i) Nucleolus dominance in polyploids. Nucleolus dominance in hybrids and allopolyploids of both plants and animals mainly describes rRNA gene silencing [54], where rRNA genes are highly methylated [55–57] and silenced over one or several loci [58,59]. Nucleolus dominance is a widespread epigenetic phenomenon [58,59], which is exhibited by diploids and allopolyploids [31,59,60]. Nucleolus dominance has been observed in both plants and animals, including *Drosophila* interspecific hybrids and *Xenopus*, *Arabidopsis*, *Brassica* and wheat allopolyploids [61,62]. Nucleolar dominance does not, however, appear to be part of a broader genome silencing phenomenon [62]. Dadejova et al. [39] reported the establishment of epigenetic patterns of rRNA gene expression in synthetic hybrids of *Nicotiana* species, and Pikaard [43] found that synthetic allotetraploids that recreated four naturally occurring *Brassica* or *Arabidopsis* allotetraploid species displayed the same patterns of nucleolar dominance observed in natural allotetraploids, beginning as early as the F_1 generation and becoming completely established by F_2.

(ii) Possible mechanisms of altering nucleolus dominance. Nucleolus dominance is reversible and developmentally regulated and is controlled by chromatin modifications involving DNA methylation and histone acetylation [10,58,63]. Blocking histone acetylation or DNA methylation suppresses the silenced rRNA genes [10]. In *Arabidopsis* and *Brassica*, the nucleolar dominance phenomenon was found to be related to allopolyploid formation and subsequent DNA methylation and histone modifications [46,58,63,64]. The role of chromatin modifications in silencing or activating protein-coding genes in allopolyploids has been reported in several recent studies [16,33,44,45,65,66]. Silenced rRNA genes subjected to nucleolar dominance are suppressed by 5-aza-dC (a chemical inhibitor of cytosine methylation) or by chemical inhibitors of histone deacetylation, indicating a role for chromatin modifications in the silencing process [58,64].

As early as 1982, Martini et al. [67] suggested that the dominant effect of U-genome nucleolus organizer regions (NORs) was determined by the large number of repeats in the IGS (intergenic spacer) compared with those in other *Triticum* species. Reeder [62] proposed the hypothesis of an "enhancer imbalance mechanism" to explain nucleolar dominance, in which there was competition between IGS repeats for enhancer elements in limiting supply. Shcherban et al. [31] suspected that the repeats in the insertion served as a transcription enhancer that promoted higher activity of the *Ae. umbellulata*-derived NORs in different hybrid combinations. Another appealing hypothesis to explain nucleolar dominance is that dominant rRNA genes have the

most transcriptional enhancers [62,63,68]. In plants, the evidence supporting this hypothesis, however, is indirect and still under survey [63].

Chen and Pikaard [63] suggested that reactivation of suppressed rRNA genes in *B. napus* was associated with the developmental transition from the inflorescence to the floral meristem. Likewise, Wilson et al. [69] showed that nucleolar dominance was complete in early embryos of *Xenopus* hybrids, but transcripts from under-dominant genes could be detected late in embryonic development and in organs of adult frogs. Therefore, in both animals and plants there is evidence that nucleolar dominance is developmentally regulated independent of gamete formation.

Although nucleolar dominance phenomena are often found in polyploids, there seems to be no relationship between nucleolar dominance and polyploidization. For instance, in *B. carinata*, whose tetraploid genome is derived from *B. oleracea* and *B. nigra*, 6 clones had 100% identity to the promoter of *B. oleracea* and 4 clones had 100% identity to the *B. nigra* promoter [63]. No sequence polymorphisms were observed in the promoter region sequenced [63]. These data suggest that rRNA gene promoter sequences are unchanged since the formation of the polyploid, presumably thousands of years ago [63] and are in agreement with the view of Prakash and Hinata [70]. Thus, nucleolar dominance is independent of ploidy [63]. Furthermore, Chen and Pikaard [63] pointed out that nucleolar dominance was also independent of maternal effect, rRNA gene dosage, size of repetitive region or number of repetitive elements.

The mechanisms for the establishment and maintenance of nucleolar dominance are still poorly understood [31,71]; there is no single hypothesis that can explain the range of expression patterns found in different organisms [31,72]. Chen and Pikaard [63] also indicated that we could not explain nucleolar dominance according to expression levels of rRNA genes in plants. Other work indicates that under-dominant rRNA genes are subjected to suppression, suggesting that enhancer dosage and transcription factor availability are also unlikely to explain all aspects of nucleolar dominance [63]. Chen and Pikaard [63] stated that experiments were needed to determine whether changes in DNA methylation were a cause or an effect of nucleolar dominance and to explain how dominant and under-dominant genes were first discriminated within the nucleus. Future tasks will be to determine whether nucleolar dominance is a hybrid-specific dosage compensation mechanism or a product of the same mechanisms controlling the number of active rRNA genes during normal development [63].

6 Other epigenetic events and causes of epigenetic alterations

Ongoing studies indicate that RNA interference (RNAi) and microRNAs (miRNAs) may interfere with epigenetics in newly formed polyploids. Ha et al. [73] argued that because interspecific hybrids and allopolyploids are mergers of two sets of microRNA sequences and divergent genomes, accumulation of miRNA and target sites might be influenced. RNAi affects gene status via gene silencing and gene suppression, and this feature has been used to study epigenetics [18,22].

miRNAs are associated with non-additive expression of target genes in allotetraploids. Genome merging in allotetraploids induces genetic and epigenetic changes [10], leading to non-additive expression of miRNA targets and miRNA primary transcripts [73]. Bartel [74] proposed that non-additive accumulation of miRNAs might be caused by non-additive expression of miRNA biogenesis genes. At the post-transcriptional level, non-additive expression of miRNA biogenesis genes resulted in non-additive accumulation of miRNAs [75]. This non-additivity is partly associated with transcriptional regulation of miRNA loci and their targets [10,73,75]. Many microRNA (miRNA) targets are non-additively expressed in the allotetraploids [42,73], suggesting a role for miRNAs in buffering genetic clashes between species [73,75].

Ha et al. [73] studied the natural allotetraploid *Arabidopsis*, and found that miRNA sequences were conserved between species, but that their expression patterns were highly variable between the allotetraploids and their progenitors. There can be rapid and dramatic changes of miRNA expression levels in allopolyploids, for the following reasons: miRNAs play an important role in maintaining flower morphology and development in stable allotetraploids; miRNAs mediate expression diversity between closely related species and in allotetraploids [73]; many miRNA targets encode transcription factors or proteins that are important for growth and development in plants and animals [73,74]; non-additive regulation of some miRNAs and their targets may lead to novel phenotypes in allopolyploids [73]. Interestingly, many miRNAs accumulate differently between diploid *Arabidopsis* and its allotetraploid (*A. suecica*), indicating a role for miRNAs in allopolyploid evolution. Expression variation of miRNAs can lead to changes of gene expression, growth vigor, and adaptation [73].

To validate the effects of RNAi on gene status, Chen et al. [22] carried out RNAi on methyltransferase 1 (*met1*) using the allotetraploid *A. suecica*. RNAi of *met1* reduced DNA methylation and altered the expression of some genes, many of which encoded transposons, predicted proteins, and centromeric and heterochromatic RNAs. Some transposons were also activated and even gene At2g23810 was demethylated [22]. When At2g23810 and genes that encode transposons were activated, gene expression levels were subsequently changed. Developmental pathways of *A. suecica* must be quite different from those of its parents *A. thaliana* and *A. arenosa*.

Kovarik et al. [76] suggested that there might be a potential link between rDNA homogenization and epigenetics

based on a study of rDNA evolution in *Nicotiana* allopolyploids. Following allopolyploidy, rDNA may homogenize, leading to reduced complexity and a decreased number of rDNA variants [31,77,78]. Rapid evolution and/or amplification of a new rDNA family and its homogenization between rDNA loci have previously been observed in a synthetic tobacco allopolyploid [79]. Although the causes of DNA sequence homogenization need to be determined, it undoubtedly influences growth and development of organisms.

7 Conclusions and perspectives

After polyploidization, one epigenetic event or mechanism is not independent of another; phenotypes of organisms are determined by converging effects (Figure 1). DNA methylation is widespread in polyploids and can lead to gene silencing. Nucleolar dominance is an epigenetic phenomenon that describes rRNA gene silencing, which is controlled by chromatin modifications involving DNA methylation and histone acetylation. Furthermore, the role of chromatin modifications in silencing or activating protein-coding genes in allopolyploids is important. Therefore, investigations into epigenetic events and relevant polyploidization pathways are in demand.

Epigenetic phenomena are involved in the early events of neopolyploid formation and provide flexibility for polyploid progeny to respond to the substantial changes of a doubled genome. The study of how epigenetic phenomena facilitate polyploidization will elucidate vital events in evolutionary history and the early stages of speciation, and of how polyploidization can lead "to an increase in biological complexity and the origin of evolutionary novelties" [2].

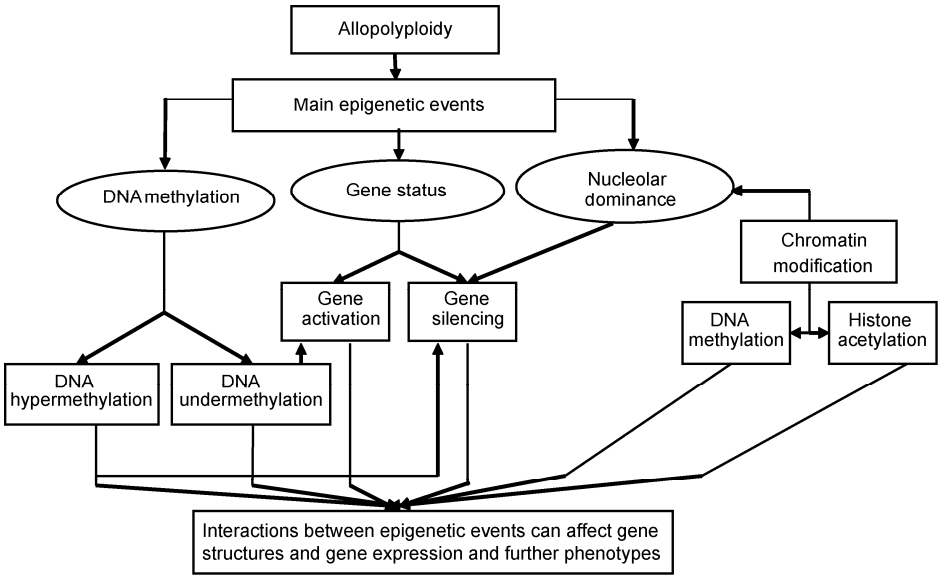

Figure 1 Main epigenetic events and mechanisms and their potential relationships after allopolyploidization.

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