Mechanisms of genomic rearrangements and gene expression changes in plant polyploids

Z. Jeffrey Chen[1]* and Zhongfu Ni[1,2]

Summary

Polyploidy is produced by multiplication of a single genome (autopolyploid) or combination of two or more divergent genomes (allopolyploid). The available data obtained from the study of synthetic (newly created or human-made) plant allopolyploids have documented dynamic and stochastic changes in genomic organization and gene expression, including sequence elimination, inter-chromosomal exchanges, cytosine methylation, gene repression, novel activation, genetic dominance, subfunctionalization and transposon activation. The underlying mechanisms for these alterations are poorly understood. To promote a better understanding of genomic and gene expression changes in polyploidy, we briefly review origins and forms of polyploidy and summarize what has been learned from genome-wide gene expression analyses in newly synthesized auto- and allopolyploids. We show transcriptome divergence between the progenitors and in the newly formed allopolyploids. We propose models for transcriptional regulation, chromatin modification and RNA-mediated pathways in establishing locus-specific expression of orthologous and homoeologous genes during allopolyploid formation and evolution. *BioEssays* 28:240–252, 2006. © 2006 Wiley Periodicals, Inc.

Polyploidy and its forms

Polyploidy occurs throughout the evolutionary history of all eukaryotes,[1,2] predominately in flowering plants[3,4] including many important agricultural crops.[5] Compared to plants, polyploidy occurs rarely in animals, but clearly exists in some invertebrates (e.g. insects) and vertebrates (e.g. fish, amphibians and reptiles).[4,6] The relative paucity of polyploidy in animals is attributed to the delicate schemes of sex determination and animal development, which are disrupted by polyploidization.[7,8] Therefore, polyploid animals rarely exist.[9] Moreover, aneuploid and polyploid cells in animals and human are often associated with malignant cell proliferation or carcinogenesis.[10] However, endopolyploidy (somatic polyploid cells within a diploid individual) appears to be a physiological response to developmental changes in plants and some animals,[11,12] indicating plasticity of plant and animal genomes. In the post-sequencing era, polyploidy is proving to be a fascinating and challenging field of plant biology, stimulating many research advances and insightful reviews.[2,13–24] Here we attempt to update the views using genomic-scale results and provide new mechanistic insights.

Historically, Winkler (1916) introduced the term polyploidy,[25] and Winge (1917) called attention to the general importance of polyploidy in the evolution of angiosperms.[26] At that time, research in polyploidy was somewhat limited by the plant and animal materials that were available in nature. In 1937, Blakeslee and Avery induced polyploidy in plants using colchicine, a chemical inhibitor of mitotic cell divisions.[27] The technique has been successfully used to induce chromosome doubling in meristemic cells of diploids and interspecific hybrids. Doubling a single 'diploid' genome results in an autotetraploid, while doubling chromosomes in interspecific hybrids leads to the production of allotetraploids. Using this method, many synthetic (newly created or human-made) allopolyploids, including *Brassica* (combination of any two genomes among *Brassica oleracea*, *B. rapa* and *B. nigra*),[28,29] cotton (*Gossypium aboreum* x *G. thurberi* or *G. bickii*),[30,31] wheat (*T. monococcum* x *Aegilops sharonensis*),[32,33] and Triticale (*Triticum* x *Secale*),[34] have been generated de novo in the laboratory. Synthetic polyploids are excellent genetic materials for comparative analysis of gene expression and genomic changes in the early stages of polyploid formation because the exact progenitors are known, whereas the progenitors of many natural allopolyploids, except such recent polyploids as *Spartina*,[35] *Tragopogon*[36] and *Senecio*,[37] are unknown or unavailable.

[1]Molecular Cell and Developmental Biology, University of Texas, Austin, TX USA.
[2]Department of Plant Genetics and Breeding, China Agricultural University, Beijing, China.
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*Correspondence to: Z. Jeffrey Chen, Institute for Cellular and Molecular Biology, University of Texas at Austin, 1 University Station, A-4800, Austin, TX 78714-0159. E-mail: zjchen@mail.utexas.edu
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The concepts of autopolyploidy and allopolyploidy were introduced in 1926 by Kihara and Ono[38] and reinstated by Darlington.[39] In his *Plant Speciation* book, Grant (1971) divided polyploids into autopolyploids (AAAA), segmental polyploids (AAA_sA_s), disomic polyploids (AABB) or amphidiploids and auto-allopolyploids (AAAABB).[40] Here A and B designate the genomes originating in species A and B, respectively, and subscript "s" designates a subgenome within species A. Stebbins (1971), however, argued that "any attempt to maintain a division of natural polyploids into two discrete categories, autopolyploids and allopolyploids, is more likely to confuse than to clarify a very complex system of interrelationships."[41]

How are polyploids formed in nature? Two prevailing models (Fig. 1) may explain their origins.[40,41] The "two-step" model proposes that an allotetraploid is formed through hybridization between two diploid species followed by chromosome doubling of the F_1 hybrid (Fig. 1A).[38] Under natural conditions, spontaneous chromosome doubling, either in the zygote to produce a polyploid or in apical meristem to produce a polyploid chimera, is a rare event.[24] The "one-step" model suggests that an allotetraploid is formed by fusion of unreduced male and female gametes from two diploid species (Fig. 1B) or by direct hybridization between two autotetraploid species (Fig. 1C) as demonstrated in the production of new *Arabidopsis* allotetraploids.[42–44] Fusion of unreduced gametes may be the predominate mode because almost every plant species produces a variable but small amount of unreduced gametes via first or second division meiotic restitution,[45] and many plant species are autotetraploids.[24,41] It is notable that autopolyploids may be formed via either model, except that a single species is involved. In other words, fusion of unreduced gametes in the same plants results in an autopolyploid. Alternatively, chromosome doubling in a diploid species leads to the formation of autopolyploids. The models are oversimplified to explain initial

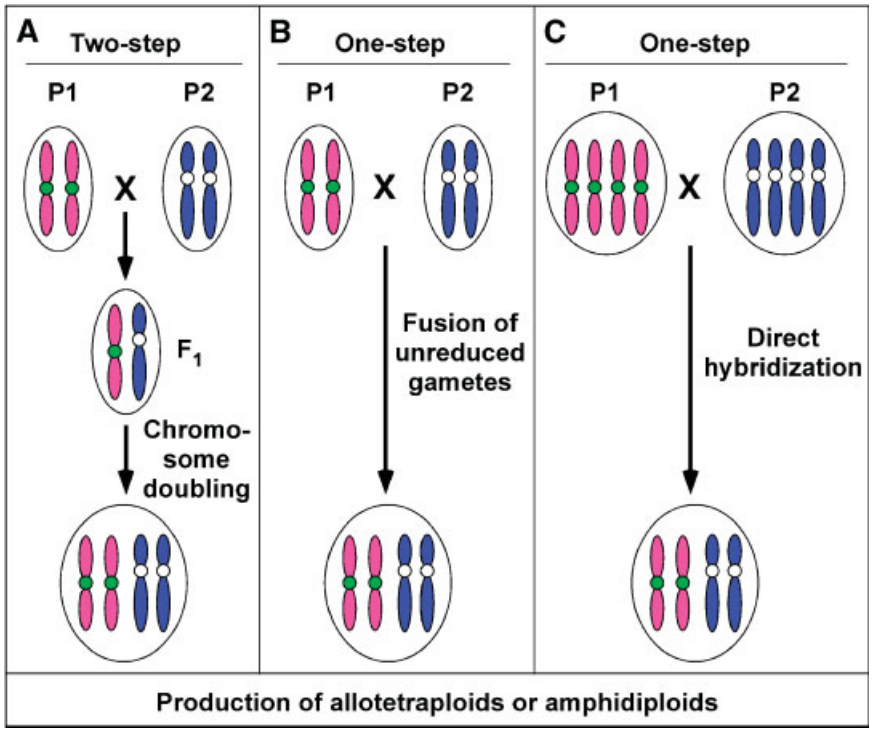

Figure 1. Two models ("one-step" and "two-step") for the formation of allopolyploids. **A:** An amphidiploid (synonymous with allotetraploid, combination of two divergent genomes) is formed by hybridization between two diploid progenitors followed by chromosome doubling (two-step model). For simplicity, each diploid species has one pair of chromosomes. P1 and P2 represent two progenitors. Here we considered allopolyploids and amphidiploids to be synonyms (R. C. King and W. D. Stansfield, A Dictionary of Genetics, 5th edition, Oxford University Press, 1997), although they may be distinguished by chromosome behaviors during meiosis. Strictly speaking, only bivalents are formed in amphidiploids, whereas multivalents may be formed in allopolyploids. **B:** Fusion of unreduced male and female gametes of two diploid progenitors leads to the production of an allotetraploid (one-step model). **C:** An allotetraploid is immediately formed by direct hybridization between two autotetraploid species (one-step model).

steps of auto- and allotetraploid formation. Readers should refer to excellent reviews[17,22] for additional steps of polyploidy formation involving triploid-bridge and variable ploidy levels.

Some allopolyploids are formed through multiple origins and by reciprocal crosses (with different combinations of maternal cytoplasm and paternal nucleus) such as in *Tragopogon*[46] and *Brassica*,[47] whereas others are formed by single or a few hybridization events, such as in cotton,[48] wheat[49] and *Arabidopsis*.[50] The new allopolyploids may cross-hybridize to diploid or autopolyploid progenitors and form hybrid zones in which the polyploids, progenitors and their intermediate forms coexist.[17,22,40,41]

During evolution, many polyploids undergo a diploidization process such that chromosomes pair and segregate in a "diploid" manner. This process of diploidization leads to massive gene loss and genome rearrangement.[51,52] As a result, ancestral polyploidy events can be clearly identified only by large-scale genome sequencing. *Arabidopsis* and maize are good examples of diploidized autotetraploids or paleopolyploids (ancient polyploids).[52,53] Mechanisms for the regulation of paralogous genes in ancient and segmental polyploids have been insightfully reviewed.[18]

Rapid and dynamic changes in genome structure in synthetic allopolyploids

Stebbins (1971) concluded "multiplication of chromosome sets either has little effect upon evolutionary progress at the gene level, or actually tends to retard it". However, molecular evidence suggests polyploid genomes display dynamic and pervasive changes in DNA sequence and gene expression probably as a response of "genomic shock" (release of genome-wide constraints on gene expression and sequence organization)[54] to intergenomic interactions (Fig. 2A).[15] Using RFLP analysis, Song et al (1995) first reported rapid changes in genomic organization in *Brassica* synthetic allotetraploids[28] and detected non-additive inheritance of genomic fragments in the synthetic allotetraploids. The changes include the absence of parental genomic fragments and the presence of novel fragments that were absent from both parents. Many of these changes in *Brassica* allotetraploids are likely caused by reciprocal translocations as well as non-reciprocal exchanges (or transposition) between homoeologous chromosomes (Fig. 2A).[55,56] Interestingly, the structural changes are not detected until the late generations of selfing (from S3 to S6 generations) and homoeologous genomes appear to be quiescent (few or no changes) in the early stages of allotetraploid formation. In later generations, massive genomic rearrangements may contribute to low levels of seed set[55] and changes in flowering time in *Brassica*.[57] Similar genomic changes in natural *Brassica* allotetraploids[55] correlate with flowering-time variation that is selected and preserved during evolution.

Unlike *Brassica* allotetraploids, wheat allotetraploids displayed 10–15% of genomic changes (mainly genome-specific sequence deletions) (Fig. 2A) immediately after hybrid (F_1) formation and the homoeologous genomes showed little changes by the third generation (S3) in selfing progeny.[32,33] Levy and Feldman (2002) proposed that allopolyploids undergo "revolutionary phase" or rapid genetic and epigenetic changes immediately after allopolyploid formation followed by "evolutionary phase" (slow changes in DNA sequence and rearrangement) in later generations.[14]

Contrary to the rapid genomic changes observed in *Brassica* and wheat allotetraploids, synthetic cotton allotetraploids display a negligible amount of changes in genomic sequences. Using AFLP analysis of over 22,000 fragments, Liu et al (2001) found additive patterns for nearly all AFLP fragments examined.[58] Molecular phylogenetic analysis indicated that the orthologous genes in the progenitors evolve independently at relatively similar rates to the homoeologous loci that are combined in the allotetraploids.[59] Similarly, genomic changes in *Spartina* polyploids occur at a very low frequency.[60] The data suggest that, compared to *Brassica* and wheat, cotton and *Spartina* have a high-level of tolerance for genome doubling and interspecific hybridization. Hence, they may represent just one of the diverse array of molecular evolutionary phenomena observed in polyploids in general.[16,58]

Genomic and chromosomal changes observed in *Arabidopsis* allopolyploids fall between those detected in cotton and wheat synthetic allopolyploids. A relatively low frequency (~1%) of genomic changes was detected in *Arabidopsis* allotetraploids.[42,44] However, meiotic abnormalities can be as high as 36%, which correlates with pollen sterility and low seed set in synthetic *Arabidopsis* allotetraploids[61] and rapid rearrangements in some specific genomic regions such as rDNA loci.[62] The various levels of genomic changes observed among different allotetraploids may be due to selection for the surviving allotetraploid individuals that can overcome meiotic abnormalities and reproductive barriers.

Inter-chromosomal exchanges may be mediated by loci that control pairing among homoeologous chromosomes such as homoeologous pairing locus 1 (*Ph1*) in hexaploid wheat[63] and *PrBn* in *Brassica napus*.[64] Alternatively, DNA recombination and repair pathways may be activated to correct a potentially high amount of non-homologous recombination in synthetic auto- and allotetraploids. AtRAD54 is a putative homolog of SNF2/RAD54 subfamily that is involved in both DNA repair and transcriptional regulation.[65] *AtRAD54* is activated in synthetic *Arabidopsis* auto- and allotetraploids but not in diploid parents and natural allotetraploids (*A. suecica*),[43] suggesting a role of DNA recombination and repair in the maintenance of genomic stability during early stages of polyploidy formation.

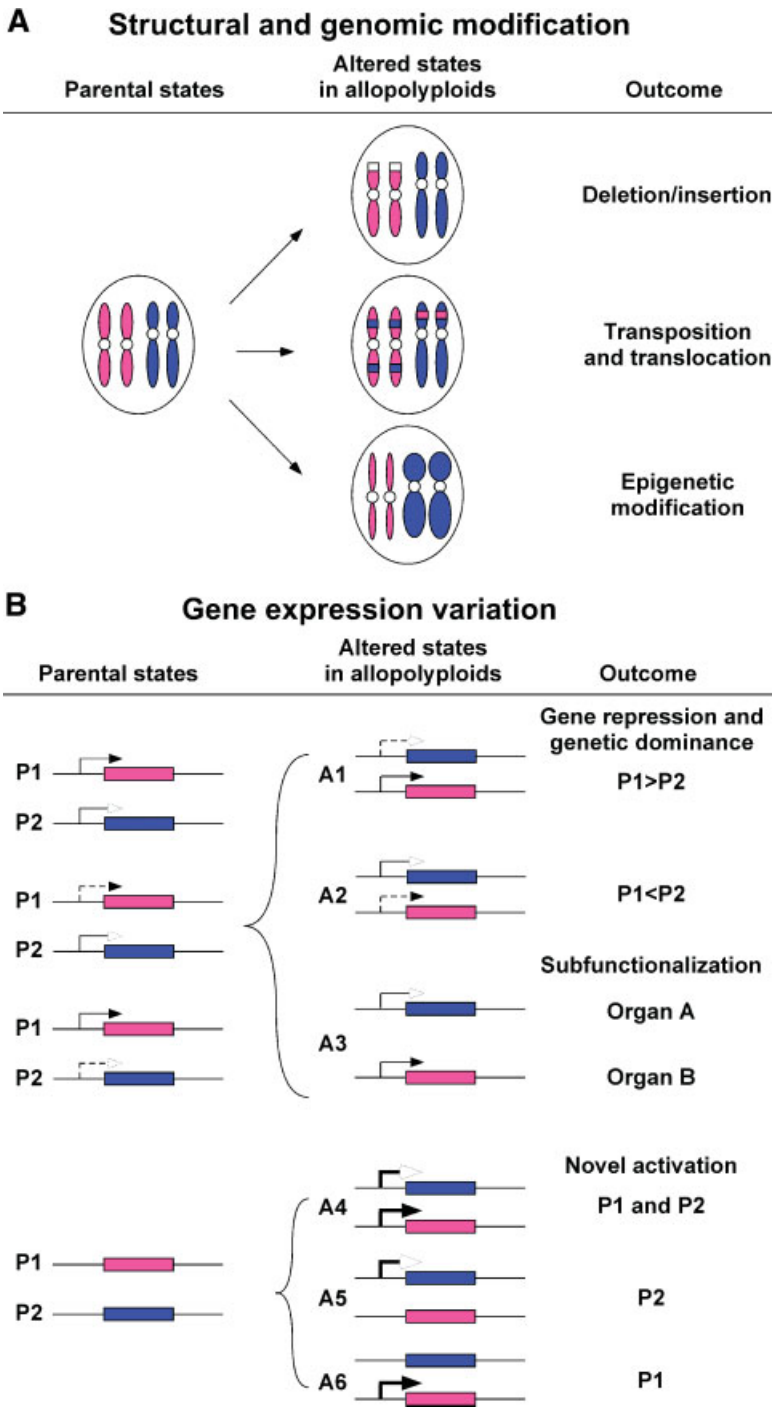

Figure 2. Types of genomic and gene expression changes documented in the polyploids. **A:** Genomic modification involves deletion, translocation and interstitial homoeologous exchanges (transposition),[55] and epigenetic modification (e.g. changes in DNA methylation). Blue and red colors represent two genomes or chromosomes from P1 and P2, respectively. **B:** Gene expression changes include genetic dominance, gene silencing, subfunctionalization and novel activation. In each case, the loci that display expression changes in allotetraploids (A1–A6) may be from either or both parents compared to the expression levels in the original parent (P1, red or P2, blue). For simplicity, only one of the two alleles in each parental locus was shown. The arrows on each locus indicate transcription (open head, P1, and filled head, P2, all solid lines), low levels of transcription (dashed lines) and no transcription (no arrows). Thick arrows indicate novel gene activation.

Activation of transposons and changes in dna methylation in allopolyploids

Transposable elements represent ~40% of the human genome and 50–80% of plant genomes.[66] Although most of them are quiescent in their respective genomes, they can be activated in response to certain stresses[67] and "genomic shock".[54] The combination of evolutionarily divergent genomes in allopolyploids resembles "genomic shock", leading to the activation of quiescent transposons in the allopolyploids (Fig. 2B, A4–A6).[15] Indeed, transposons including DNA transposons and retrotransposons are reactivated in wheat and *Arabidopsis* allotetraploids.[42,61,68] In wheat, activation of Wis 2-1A retrotransposons leads to the production of readout transcripts from neighboring genes in both sense and anti-sense orientations.[68] Using transposon display with template cDNA, Kashkush et al (2003) found that >7% of the Wis 2-1 A-containing transcripts are novel in the amphidiploids (synonymous with allotetraploids) but absent in both parents or absent in the amphidiploids but present in one or both parents. Moreover, neighboring genes are either "activated" due to overproduction of sense readout transcripts or silenced because of anti-sense readout transcripts that may serve as a negative regulator via RNA interference. The data indicate that activation of retrotransposons plays a role in *cis* and/or *trans* regulation[69] of neighboring genes in the allopolyploids.

Using genomic tiling microarray analysis, Madlung et al. (2005) found a *Ty-1 copia*-like retrotransposon and two *En/Spm*-like transposons that belong to the *Sunfish* (*Suf*) subfamily are reactivated in synthetic *Arabidopsis* allotetraploids.[61] In addition, *AtMu1*, a DNA transposon, displays differential expression in the *A. thaliana* L*er* autotetraploids and synthetic allotetraploids.

Reactivation of *Suf1-1* is correlated with reduction of CG and CNG methylation within the transposon coding sequences. *Sunfish* is methylated in the autotetraploid parent, but demethylated and reactivated in the allotetraploids, suggesting that allopolyploidization provokes perturbation of genomic structure and chromatin remodeling, giving rise to the reactivation and silencing of transposons[44] and protein-coding genes.[43,70] It is conceivable that changes in DNA methylation detected in recent *Spartina*[60] polyploids and synthetic *Arabidopsis*[44] allotetraploids are associated with gene expression changes and phenotypic variation. Notably, synthetic *Arabidopsis* allotetraploids are more sensitive than their parents to treatments of aza-dC,[44] a chemical inhibitor for DNA methylation, indicating that DNA methylation and other chromatin modifications become sensitized in the allotetraploids, probably due to remodeling activities during allopolyploid formation.

It is predicted that silencing transposons by DNA methylation is a defense mechanism against genome reorganization during allopolyploid evolution.[71] The high levels of genome-specific sequence deletions in wheat[32,33] and homoeologous chromosomal exchanges in *Brassica*[56,57] may suggest transposition events. However, available data in wheat synthetic amphidiploids do not support the movement of transposable elements.[68] Instead, illegitimate recombination (between homoeologous sequences) may induce sequence rearrangements in specific loci controlling grain hardiness (*Ha*)[72] and leaf rust resistance (*Lr10*)[73] in hexaploid wheat (*Triticum aestivum*) and its diploid and tetraploid relatives (*Triticum* and *Aegilops* species). Therefore, recombination between homoeologous chromosomes with or without transposon involvement may be a general mechanism for observed inter-chromosomal exchanges in allopolyploids.

Reprogramming of transcriptome divergence in allopolyploids

Alteration of parental gene regulation in interspecific hybrids was first implicated by Navashin[74] and Barbara McClintock[75] in a phenomenon known as nucleolar dominance. Nucleolar dominance results from selective silencing of one of the parental rRNA gene loci in an interspecific hybrid or allopolyploid.[21] Reeder and his colleagues have proposed the "enhancer-imbalance" model,[76] suggesting that active rRNA genes having more and stronger enhancers compete better than inactive genes with fewer and weaker enhancers for limiting transcriptional factors. Consistent with this model, dominant genes have longer spacers (putative enhancers) than inactive genes in hexaploid wheat.[77] However, in *Arabidopsis* and *Brassica* allotetraploids, differential rRNA gene expression is not associated with enhancers or availability of species-specific transcription factors.[78,79] Transient and in vitro transcription assays have shown that *Arabidopsis* and *Brassica* rRNA promoters can function with the RNA Polymerase I transcription machinery of the other species.[78,80] Silenced rRNA genes in *Arabidopsis* and *Brassica* allotetraploids were reactivated by chemical inhibitors for DNA methylation and/or histone deacetylation, suggesting that rRNA genes are silenced by DNA and histone modifications presumably associated with inactive chromatin structure.[81] Collectively, the data suggest that rRNA gene silencing acts on chromosomal loci that result in cooperative silencing of rRNA genes.[21]

How are orthologous protein-coding genes regulated in synthetic interspecific hybrids and allotetraploids? To address this question, several groups took AFLP-cDNA display approaches to uncover novel gene expression patterns in synthetic *Arabidopsis* allotetraploids and natural *A. suecica*,[42,70] cotton[31] and wheat[68,82] allotetraploids. Synthetic *Arabidopsis* allotetraploids were produced by direct hybridization between an *A. thaliana* L*er* autotetraploid and *A. arenosa*, a naturally outcrossing tetraploid.[20,42] *A. suecica* is a natural allotetraploid that was formed through interspecific hybridization between *A. thaliana* and *A. arenosa* ancestral species, from ~20,000 years[50] to ~1.5 million years.[83] The initial

survey of gene expression variation indicated ~2.5% of gene expression differences in *A. suecica* relative to *A. thaliana* and *A. arenosa*.[70] The levels of differential gene expression are higher in synthetic *Arabidopsis* allotetraploids[43] than in *A. suecica*. Approximately 11% of the cDNA fragments displayed changes that may be related to gene repression, activation and subfunctionalization (Fig. 2B, A1–A3). Among them, ~4% were from *A. thaliana* parent, ~5% from *A. arenosa* parent, ~1% from both parents, and ~1% from neither parents (or novel gene expression patterns).[43] Similar levels of changes were found in three independent allotetraploid lineages, suggesting that those genes are subjected to similar regulation during allopolyploid evolution.

Changes in gene expression can occur immediately after allopolyploid formation or stochastically in the selfing progeny.[43,79] For some genes, including rRNA genes, it takes 1–2 generations to establish a differential expression (silencing or activation) pattern. For others, it takes more than two and sometimes five generations to establish expression status. Although the expression patterns may be stochastically established, there is a trend toward silencing or expressing a particular locus during selfing compared to the expression pattern of homoeologous loci in natural *A. suecica*.[43] Moreover, the expression levels of some genes are highly variable between different individuals within a family. This may be advantageous for adaptation and establishment of a successful allopolyploid population.

If gene silencing or activation is established within a selfing progeny, does the expression status remain unchanged during developmental stages? The available data suggest that duplicate genes or homoeologous genes may change their expression in different organs or tissues as a response of functional diversification to changes in developmental programs. Silenced rRNA genes are reactivated in floral organs, which suggests a developmental role of activated or silenced homoeologous genes in allopolyploids,[84] which argues against the notion that reactivation of silenced RNA genes in microspores (pollen) is due to separation of repressors during meiosis.[75] In cotton many if not most homoeologous genes that display unequal expression in allotetraploids exhibit organ-specific expression patterns[30] (Fig. 2B, A3). Interestingly, for some homoeologous gene pairs, one locus (e.g. *AdhA*) is silenced in one organ, whereas the other locus is silenced in another organ. This silencing scheme is genotype-independent and occurs in both synthetic and natural cotton allotetraploids,[31] suggesting rapid subfunctionalization of duplicate genes and stable maintenance during evolution. Developmental regulation of orthologous genes immediately after allopolyploid formation is reminiscent of the theoretical predication about functional diversification of duplicates genes on an evolutionarily timescale.[85] Immediate divergence in the expression of orthologous genes in allopolyploids provides an inexhaustible reservoir for generating genetic variation and phenotypic diversification, which facilitates natural selection and polyploid evolution.

In a recent study, Wang et al. (2006) employed spotted oligo-gene microarrays to study transcriptome divergence in *Arabidopsis* synthetic allotetraploids and their parents.[86] Over 15% of the transcriptome was differentially expressed between the progenitors, of which 8% and 7% genes were highly expressed in *A. thaliana* and *A. arenosa*, respectively. This may represent "species-specific" regulatory changes as a consequence of 5.8 million years of evolution since these parental species diverged.[83] In synthetic *Arabidopsis* allotetraploids, >5% of the genes displayed expression divergence from the mid-parent value, suggesting non-additive gene regulation leading to gene activation, repression and novel expression relative to the expression states of parental loci (Fig. 2B). Significantly, ~68% of the non-additively expressed genes in the allotetraploids are "species-specific" genes, indicating that transcriptome divergence needs to be reprogrammed during allopolyploid formation. In addition, the majority of non-additively expressed genes in the allotetraploids are repressed, and >94% of the repressed genes in the allotetraploids match the *A. thaliana*-specific genes, consistent with the silencing of *A. thaliana* rRNA genes in nucleolar dominance[21] and the overall suppression of the *A. thaliana* phenotype in the synthetic and natural allotetraploids.[42] Moreover, the differentially expressed genes belong to the ontology of various biological pathways including metabolism, energy, cell defense, signaling and aging, and plant hormonal regulation. Notably, few transposon-encoded genes are identified in the AFLP-cDNA and microarray analyses,[43,86] suggesting interspecific hybridization does not induce genome-wide transposon activation. Alternatively, the transposons may be settled in the late generations (S5) of selfing.

Interestingly, altered expression of *HSP90* and related HSP genes in synthetic allopolyploids may provide a buffering capacity for morphological evolution.[87] Furthermore, genes involved in plant hormonal regulation such as ethylene biosynthesis are coordinately expressed in the synthetic allotetraploids, suggesting genome-wide modulation of regulatory pathways.

The formation of *Arabidopsis* allotetraploids by "one-step" hybridization between two autotetraploid species may confound the effects of hybridization and genome doubling. To determine how genome doubling affects gene expression, Wang et al. (2006) analyzed gene expression differences between a diploid and isogenic autotetraploid.[86] Few genes were upregulated or downregulated in the *Arabidopsis* autotetraploids, consistent with the findings in yeast, in which a dozen genes were expressed differently in response to ploidy changes in a series of haploid, diploid, triploid and autotetraploid.[88] The data suggest that during autopolyploid formation the dosage-dependent regulatory mechanisms prevail.[89]

In another study, Hegarty et al. (2005) found dramatic differences in floral gene expression between the allohexaploid hybrid, *Senecio cambrensis*, its parental taxa *Senecio squalidus* (diploid) and *Senecio vulgaris* (tetraploid), and the intermediate triploid (sterile) hybrid *Senecio* × *baxteri*.[90] Therefore, reprogramming gene regulatory networks in interspecific hybrids and allotetraploids is a consequence of mediating transcriptome divergence originating in two species rather than simple genome doubling. To separate the effects of hybridization and genome "doubling," one may compare gene expression variation between F_1 hybrids (hybridization) and allotetraploids (genome-doubling). However, these comparisons may be confounded by the colchicine-treatment and genomic effects on F_1 hybrids.

Non-additive gene regulation appears to be a process that not only reprograms the transcriptome divergence between progenitors that diverged millions of years, but also mediates gene expression variation in maize aneuploids[91] and *Chenopodium* tetraploids[92] and in hybrids between the same species.[93] Auger et al. (2005) found non-additive expression of many selected alleles in diploid and triploid hybrids of maize.[93] The transcript levels in the diploid hybrids correlated negatively with the diploid inbred parents. Genes in the triploid hybrids are expressed non-additively and their expression is also affected by genome dosage. The available data suggest that hybridization between interspecific, intraspecific or inbred lines induces expression variation that exceeds the range between the original parents.[94] We predict that a common theme of gene expression changes in allotetraploids and inbred hybrids is heterotic and/or inter-genomic interactions between the alleles or loci that are dependent on the genetic distances or levels of sequence divergence between the species.[86] These findings may shed light on the relationship between heterosis[94] and evolutionary success of allopolyploids.[22] Indeed, allopolyploidization provides a means of permanent fixation of hybrid vigor between species.[22,40,41]

Mechanisms of gene regulation in allopolyploids

The growing amount of gene expression and genomic data in polyploidy research has stimulated many thorough reviews about the probable cause of gene expression divergence and novel genetic variation in allopolyploids.[13–16] These reviews provide a wide spectrum of insights into genome evolution, genetic and epigenetic regulation, and physiological and morphological responses in polyploids.

The expression changes observed in synthetic allotetraploids and their progenitors include gene repression (or silencing, which is an extreme form of repression), genetic dominance, subfunctionalization and novel activation (Fig. 2B). Some of these changes may be attributed to DNA sequence alterations such as sequence deletion, and interchromosomal exchanges and rearrangements (Fig. 2A), whereas others are mediated at the transcriptional and post-transcriptional levels (Fig. 3). It should be possible to apply the knowledge of chromatin modifications and transcriptional regulation learned from a diploid system to the expression of orthologous genes in an allopolyploid system. However, the critical issue is how the orthologous genes are discriminated for expression changes in the new allopolyploid cells that contain two or more divergent genomes. Once the expression patterns are established, they are maintained by chromatin modifications such as DNA methylation.[95] Blocking DNA methylation by chemical inhibitors[70] or dominant negative regulation of DNA methyltransferase genes[43] leads to activation of the silenced genes. Here we are inspired to propose a few models for the initial determination of locus-specific expression patterns during allopolyploid formation and evolution.

Transcriptional regulation in allopolyploids

During evolution, the selective modification of regulatory networks controlling gene expression is thought to enable the colonization of new ecological niches and respond to developmental programs and environmental cues. The interactions between external effects and internal programming of gene expression networks will determine species specificity. A good example is the concerted evolution of rDNA sequences and RNA polymerase I transcriptional machinery, which is typified by species-specific factors.[96] As a result, human rRNA genes are not transcribed by murine RNA polymerase I extracts, unless human SL1 (a human-specific factor) is present. Consistent with this notion, the enhancer imbalance model[76] suggests that, in *Xenopus* interspecific hybrids, dominant rDNA clusters have stronger enhancers that titrate the available transcription factors so that the rDNA clusters with weaker enhancers are inaccessible to the transcription activators and are not transcribed. Although this model is not supported by transient and in vitro and in vivo transcription assays in plants,[78,80] the involvement of species-specific factors should not be ignored. These factors may represent upstream regulators in a regulatory pathway. If regulatory factors (X and/or X′) (Fig. 3A, A1–4) are compatible to both downstream genes, downstream genes are activated through *trans*-acting effects[69] on *cis*-regulatory elements of X and X′ (Fig. 3A, A1). In this case, quantitative variation and competition for binding affinity between transcription factors and binding sites in the respective promoters may determine the expression levels and fitness of corresponding paralogous genes[18] (Fig. 3A, A1–4). However, if only one factor is present or if X and X′ factors are incompatible with one of the orthologous downstream genes (Fig. 3A, A2–3), a single orthologous gene may be activated primarily through *cis*- and *trans*-acting effects. If both factors are absent or the factors are inhibitory to the downstream genes (Fig. 3A, A4), both loci are expected to be silenced.

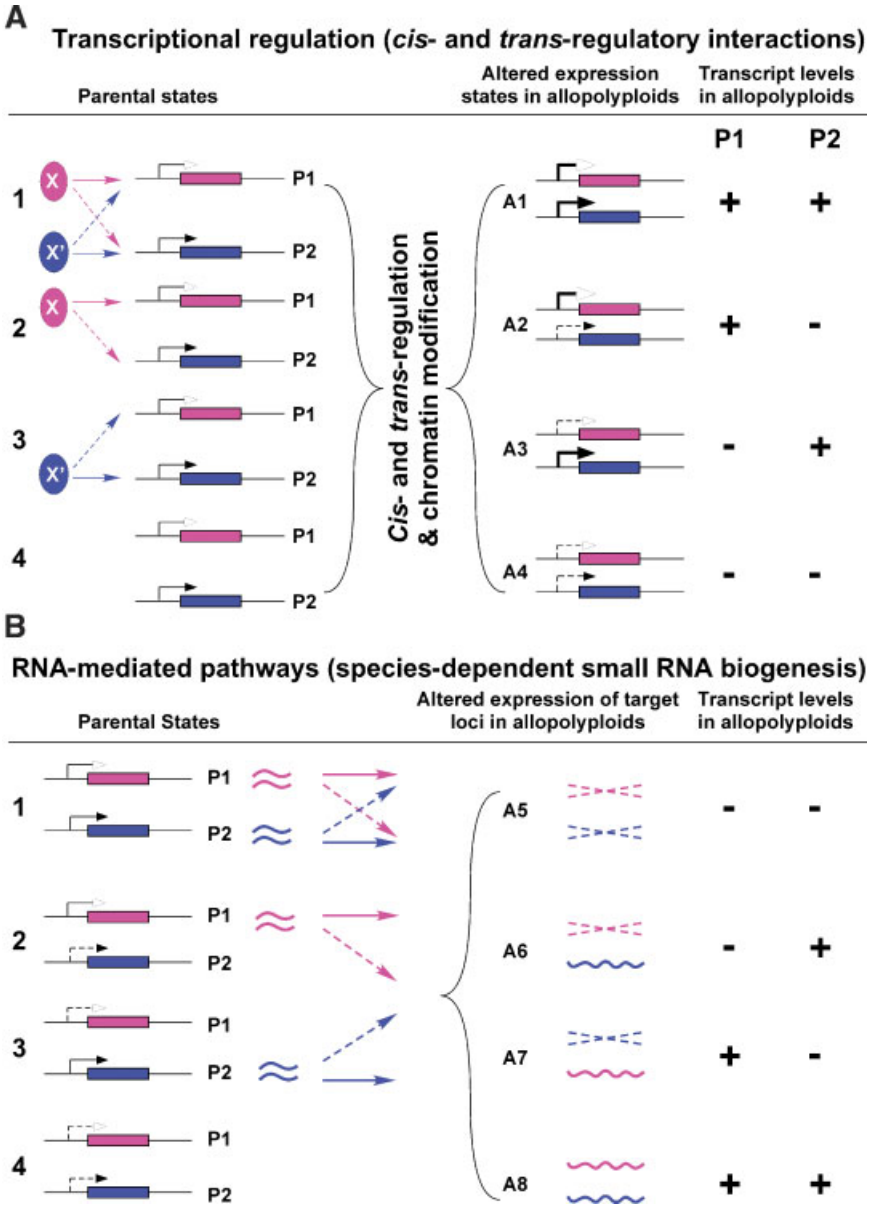

Figure 3. Two models for the gene expression changes observed in the allopolyploids. **A:** The transcriptional regulation model suggests interactive roles of sequence evolution, transcriptional regulation and chromatin modification in modulating the expression of orthologous genes in the allopolyploids. Transcriptional regulators (X, P1 and X′, P2) are compatible with the orthologous loci leading to the expression of both in allotetraploids (A1). Only one regulator (X or X′) is present or compatible with one of the loci leading to the expression of a single locus (A2 or A3). Absence of upstream regulators or incompatibility between the two loci result in silencing of both loci (A4). **B:** The RNA-mediated pathway model indicates differential accumulation of small RNAs that may act as negative regulators for target genes. Production of small RNAs (siRNA and miRNA) from orthologous genes is associated with downregulation of both genes ("cross out" symbols) in allotetraploids (A5). Production of small RNAs from one species results in silencing of one locus (A6 or 7). Absence of small RNAs promotes transcript accumulation of both orthologous loci (A8). Blue and red colors represent protein regulators (ovals), loci (boxes), small RNAs (short waved lines) and RNA transcripts (long waved lines) from parent 1 (P1) and parent 2 (P2), respectively. The blue and red arrows indicate possible *cis* (solid lines) or *trans* (dashed lines) interactions, whereas the arrows on each locus indicate transcription (open head, P1 and filled head, P2, all solid lines) and low levels of transcription (dashed lines). The "+" and "−" indicates accumulation and downregulation of transcripts, respectively, in allotetraploids. Thick arrows indicate upregulation.

The effects of transcription activators or repressors on gene activation or silencing could be mediated through the recruitment of chromatin-remodeling complex to a specific locus.[21] Alternatively, the chromatin may be remodeled in the allotetraploids (Fig. 3A, A1–4) because the inter-genomic interactions between two divergent genomes may induce perturbation of the chromatin structure inherent from the progenitors. One might elucidate the detailed mode of action in this regulatory system using the dominant-negative mutants in chromatin proteins and the upstream regulators, which may overcome genetic redundancy in allopolyploids.[43] Indeed, some silenced genes are reactivated in the *A. suecica met1*-RNAi lines[43] or by blocking DNA methylation,[70] which is correlated with promoter demethylation in these specific loci.

This model also suggests that some key regulators, such as transcription factors or chromatin remodeling complex factors including DNA methylation and histone modification components, may control the expression of many downstream genes in the regulatory pathways.[95] Transcription factors play a role in hybrid lethality in *Drosophila*.[97,98] More than 50% of the transcriptome diverged between *D. melanogaster* and *D. simulans* during the 2 to 3 million years since common ancestry of evolution. The majority of these genes evolved to establish sex-biased gene expression patterns,[97,99] which may cause the lethality of hybrid males or hybrid dysgenesis between the two species unless hybrid rescue mutants are used.[98] Hybrid lethality[98] is overcome by progenitor-dependent *cis* regulation through chromatin modifications, transcription factors such as Myb.[98] By the same token, some transcription factors and chromatin-remodeling components may contribute to the genome-wide non-additive gene regulation and/or species-specific repression in *Arabidopsis* allopolyploids.[86]

RNA-mediated gene regulation in allopolyploids

RNA interference (RNAi) is an evolutionarily conserved mechanism for modulating gene expression. Short anti-sense RNAs are produced by the cleavage of dsRNA precursors to target corresponding RNAs for degradation.[100] As a result, short interfering RNAs (siRNAs) and microRNAs (miRNAs) are negative regulators of target transcript accumulation. Although they share similar pathways of biogenesis involving slightly different sets of RNA processing machineries, siRNAs and miRNAs have different origins and modes of action.[100] siRNAs are produced mainly from transposons, heterochromatic repeats and viral sequences and serve as negative regulators or chromatin modulators of their own sources, whereas miRNAs are encoded in intergenic regions and regulate other loci important for animal and plant development. In addition to functioning as translational repressors as do animal miRNAs, plant miRNAs behave as siRNAs that exert negative *cis*- and *trans*-acting effects[101] on cleavage of the target loci through imperfect matches. Moreover, RNAi pathways operate at the genome-wide scale, which may directly affect epigenetic modification of homologous sequences that induces gene silencing and DNA elimination. Therefore, RNAi pathways are delicately modulated in each species including the progenitors of polyploids.

Evidence for the involvement of RNA-mediated gene regulation came from a study in wheat synthetic allotetraploids.[68] Reactivation of the transposons in the synthetic allotetraploids induces transcript readouts that are correlated with upregulation or downregulation of neighboring genes depending on whether the readout transcripts are in sense or anti-sense orientations.[68] Transcriptome analysis indicates that *Arabidopsis* contains a large amount of anti-sense and sense transcripts with unknown function.[102] These transcripts may play a role in the regulation of target genes as a general mechanism for plant development and/or of the orthologous targets in allopolyploids. Overexpressing double-stranded RNAs can effectively downregulate the expression of endogenous target genes in *A. suecica*.[43,103] Moreover, transgene expression in *Arabidopsis* autopolyploids is regulated by ploidy levels and the transgene that is silenced in diploids and autotetraploids is reactivated in the triploids.[104] The expression levels of endogenous genes vary in response to the "odd" or "even" dosage of chromosomes in maize.[105] The data suggest that, in addition to RNAi, chromosome pairing and paramutation-like interactions[106] may be responsible for gene regulation in polyploids.

At a genome-wide scale, RNAi pathways inherent from progenitors may be disrupted in interspecific hybrids or allopolyploids because of incompatibilities between the two divergent species. The disruption may change or modify the efficiency of RNA biogenesis machineries, accumulation of siRNAs and miRNAs, and specificity of siRNA and/or miRNA targets. One possibility is that siRNA and miRNAs are differentially accumulated in allopolyploids (Fig. 3B, A5–8), which in turn results in the downregulation of the target loci. If small RNAs such as miRNAs are promiscuous for the RNA targets, then the transcripts from both progenitors will be repressed or downregulated (Fig. 3B, A5). If, however, small RNAs have high fidelity of their targets, only one of the orthologous or homoeologous targets is repressed (Fig. 3B, A6–7). Alternatively, the target specificity may be achieved by the ability of *cis* or *trans* acting capacity of the siRNAs and miRNAs. As a result, the target loci are downregulated from one progenitor (Fig. 3B, A6–7), two progenitors (Fig. 3B, A5) or neither (Fig. 3B, A8).

We simply do not know whether and how the siRNAs and miRNAs from different origins are differentially expressed in allopolyploids. We predict that, for miRNAs encoded by precursors in the intergenic regions, differential accumulation of miRNAs may be controlled by transcript levels of the precursors produced, probably mediated by any of the transcriptional regulatory mechanisms discussed above

(Fig. 3A). Alternatively, each species of siRNA and heterochromatin transcripts may be differentially mediated by RNA polymerase IV machinery.[107,108]

There is no reason to exclude other post-transcriptional regulatory mechanisms such as RNA stability,[109] alternative splicing, RNA processing and editing, and RNA cache[110] that may be involved in the differential accumulation of transcript levels in the allopolyploids. For example, the stability of mRNAs varied from species to species and the half-life of mRNAs produced from progenitors may be directly correlated with differential accumulation of transcripts in the allopolyploids.

Conclusion and future perspectives

Allopolyploidy provides a unique system for study of the mechanisms for reestablishing functional biological pathways via genetic and epigenetic interactions between evolutionarily divergent genomes, orthologous genes and their products, and divergent regulatory networks. Imagine how difficult it is to assemble a functional new engine using the parts from different brand models of cars built at different times. Solving the puzzles of divergent regulatory pathways in an allopolyploid species is equally if not more difficult. The observed changes in polyploids may be mediated by genetic (sequence-dependent), epigenetic (sequence-independent) mechanisms or both (Fig. 4). Genetic changes include translocation and transposition, sequence deletion and insertion, non-homologous chromosome pairing and additive gene regulation, whereas the epigenetic phenomena included non-additive gene regulation, transposon activation, silencing of homoeologous genes and subfunctionalization. The underlying genetic and epigenetic mechanisms for these dynamic changes observed in the polyploids may be intricately related (see Fig. 4 legend). The genomic and gene expression data documented in the last decade have provided new insights into the many evolutionary and mechanistic questions that have been posed by polyploidy researchers[2,5,13–22,24,71,89] since Stebbins.[41] For examples, what is the mechanism of choice; i.e. which genes from which progenitors are chosen for transcriptional and/or post-transcriptional regulation? Why are some genes subjected to changes in the allopolyploids, whereas other duplicate genes

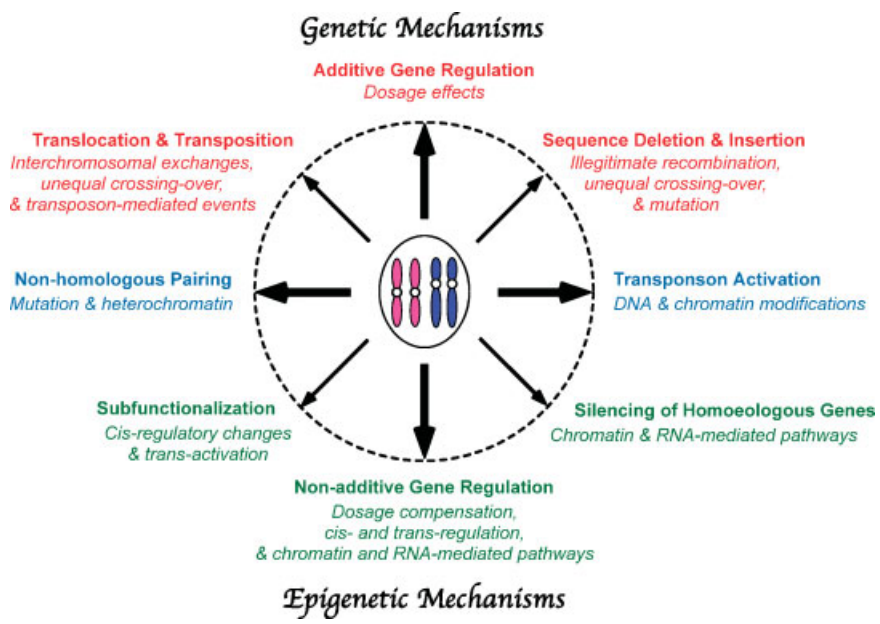

Figure 4. A wheel of the models for polyploidy-dependent genomic rearrangements and gene regulation. Eight types of observed changes (bold-type fonts) and their underlying mechanisms (italicized fonts) were arranged circularly around an allotetraploid cell (center). The changes may be mediated by genetic (DNA sequence-dependent, top and red), epigenetic (DNA sequence-independent, bottom and green) mechanisms or both (middle and light blue). The distinctions among some of the classified changes and underlying mechanisms may not be very clear. For example, changes in DNA methylation may activate transposable elements that may in turn induce transposition or chromosomal breakages and exchanges. Similarly, non-additive gene regulation may include subfunctionalization and silencing of homoeologous genes. The dashed ring represents circular interactions among some, if not all, classified changes and predicted models. Additional research is needed in order to provide experimental data for these models. Note that a polyploid organism may experience some but not all changes listed in the diagram. For instance, genomic sequence rearrangements were predominately detected in *Brassica* and wheat allopolyploids but not in *Arabidopsis* and cotton allopolyploids. The thickness of arrows may correspond to an arbitrarily level of the changes that have been documented in the polyploids.

become "redundant" and "dispensable" in a pathway? Which proportions of gene expression changes are associated with epigenetic regulation and/or genomic rearrangements? Do changes in chromatin structure or RNA pathway affect individual loci or many genes and pathways? What is the role of orthologous genes in developmental regulation or tissue-specific expression? Does gene expression divergence affect the evolutionary fate of duplicate genes? Does the nuclear and cytoplasmic incompatibility affect gene expression variation? How does allopolyploidization breakdown self-incompatibility and overcome inbreeding depression? Do aneuploidy (partial genome duplication) and polyploidy (whole genome duplication) have similar effects on gene expression? How do allopolyploids adjust the cell cycles, mitotic division, chromosome pairing, meiotic segregation, cellular growth and organismal development? Finally, why are polyploids so successful during the evolution? Obviously, there are more questions than answers in the challenging field of polyploidy biology, which provides golden opportunities for young scientists to employ innovative approaches, to develop new methodologies such as genome-wide assays of allelic expression and quantitative analysis of expression changes and phenotypic variation, and to elucidate the mechanistic roles of polyploidy in genome evolution of plants and animals.

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