Research News

Genomic change and gene silencing in polyploids

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Combining the genomes of two species through hybridization and chromosome doubling can create a new allopolyploid species virtually overnight. Although common in Nature, such genetic mergers might not be easy. Recent studies, mostly in plants, suggest that polyploidization can induce a flurry of genetic and epigenetic events that include DNA sequence elimination and gene silencing.

Without warning, in through the front door of your little house comes the family of a distant relative, toting all their belongings and unleashing their unruly dogs into your tidy dwelling. And they've come to stay. Surprise! The premise for a new television comedy? Possibly; but in this case, an analogy for those fertilization events in Nature that bring together related, but not identical, nuclear genomes to create an interspecies hybrid. The success and permanence of these sudden genetic mergers is hardly guaranteed. Unlike hybrids formed by crossing two members of the same species, interspecific diploid hybrids are typically evolutionary dead-ends (e.g. mules, the sterile hybrids of horses and donkeys). Differences in chromosome number or organization tend to disrupt chromosome pairing and assortment during meiosis, yielding defective gametes and hence sterility. Doubling the chromosomes of each parent, either before or after the hybridization event, can overcome the problem by providing each chromosome with a precise pairing partner, thus allowing fertility and persistence of the hybrid (Fig. 1a). The formation of such polyploid hybrids, known as allopolyploids, is a powerful evolutionary process in the creation of new species, especially in plants, having produced such familiar crops as cotton and bread wheat[1–5]. Despite their success in Nature, the initial formation of an allopolyploid must be a shock. The resulting crowded genome, like a dwelling made cramped by relatives who will not leave, presents a number of challenges, not the least of which is the potential for conflict because of different instructions being given for the same tasks. Also, transposable elements, the troublesome hounds of the genome, are often unleashed in new hybrids (for reasons that are not yet clear) and can roam their new environment causing damage. And, swapping partners when it's time to pair up can only bring trouble and strife (Fig. 1b). Evidence is emerging 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.

A record of success

Polyploidy can result from doubling a single species' genome (autopolyploidy) or from bringing together two or more different genomes (allopolyploidy). Fusion of unreduced gametes that contain a diploid, rather than haploid, chromosome complement is the most probable route to both types of polyploidy. In the plant kingdom, ~70% of angiosperms (flowering plants) and ~95% of pteridophytes (ferns) are thought to have undergone at least one episode of polyploidization in their evolution[6,7]. Plants are not unique in this respect. The yeast (*Saccharomyces cerevisiae*) genome reveals evidence of an ancient duplication event[8] and in vertebrates, one or two rounds of polyploidization have been suggested to explain the two- to four-fold increase in the number of paralogous genes compared with invertebrates[9–11] (although tandem gene duplications might also explain gene redundancy in vertebrates, making the role of polyploidization controversial[12]). One might expect that autopolyploidy would not have many immediate phenotypic or genetic consequences, having merely doubled the existing genetic information. However, in isogenic yeast strains that differ only in ploidy, DNA microarray

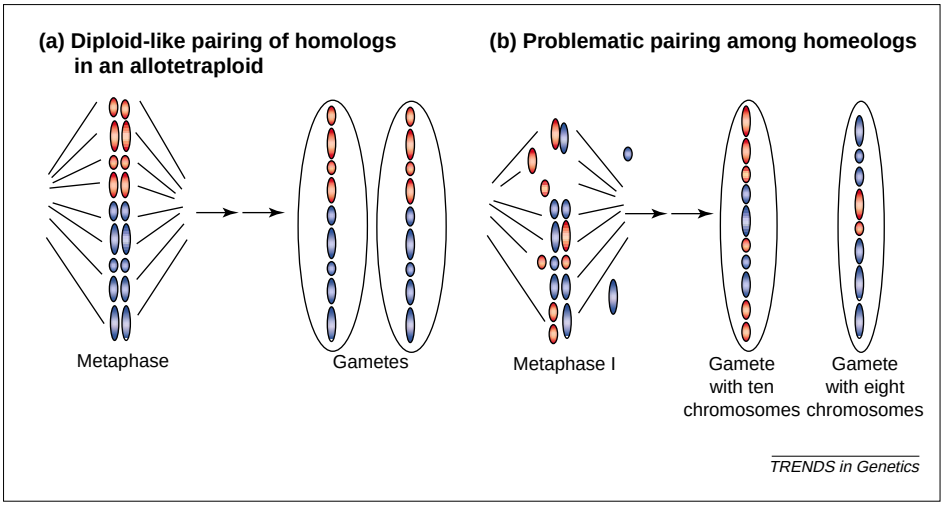

Fig. 1. Chromosome pairing and segregation in natural and newly formed allotetraploids. (a) A chromosome complement of a natural allotetraploid (a species created by the merger of diploid chromosome complements from two progenitors) at metaphase I of meiosis. Chromosomes derived from one progenitor are colored red and those derived from the other progenitor are colored blue. Only homologs pair at metaphase, such that each of the four gametes ultimately receives a complete blue set and a complete red set of chromosomes, perpetuating a state of permanent heterozygosity. (b) In a newly formed allotetraploid, the equivalent chromosomes of each progenitor (homeologous chromosomes) can be similar enough that they can pair with one another. Chromosome rearrangements that had occurred in the progenitors in the time since their divergence from a common ancestor can lead to different chromosome numbers, as in the example shown. As a result, a chromosome of one progenitor can share large tracts of similarity with more than one chromosome derived from the other progenitor and thus pair with multiple chromosomes. As a result, chromosome segregation is disorderly and fertility suffers.

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studies reveal that the expression of some genes changes (either increasing or decreasing) in response to changes in ploidy[13]. Affected genes include cyclins, whose decreased activity might explain the larger size of polyploid cells. Other affected genes alter growth characteristics such as clumping and the ability to invade a semi-solid growth medium. A recent study using a PCR-based method to display mRNA transcript profiles (i.e. cDNA-amplified fragment length polymorphism; cDNA-AFLP) also revealed differences in isogenic diploid versus autotetraploid *Arabidopsis thaliana* plants[14]. Thus, in the short term, autopolyploidy can alter gene expression and possibly modify an organism's phenotype while providing a duplicate set of genetic instructions to buffer against deleterious mutations. On a longer timescale, duplicated genes typically evolve independently and can acquire specialized functions that increase the complexity of the species and have the potential to improve fitness. Alternatively, they could be lost.

By combining genomes of different species, allopolyploids represent genetic entities that are more obviously remarkable than autopolyploids, having succeeded in bringing together genes of progenitors whose phenotypes and life histories can differ substantially. A unique feature of allopolyploids is that their fundamentally hybrid nature can be maintained indefinitely. The reason is that in stable allopolyploids, homologous chromosomes derived from each progenitor will pair and recombine at metaphase, but pairing between homeologous chromosomes (i.e. equivalent chromosomes inherited from the different progenitors) typically does not occur (Fig. 1a). The result is a form of 'permanent heterozygosity' in which one set of alleles from each progenitor is transmitted in each gamete. By contrast, in an autotetraploid, the essentially identical sister chromatids can pair in any permutation, recombine and segregate randomly such that recurrent backcrossing or self-fertilization can ultimately result in homozygosity of alleles, just as with a diploid. Permanent heterozygosity might be a significant advantage in a stable allopolyploid compared with its progenitors, allowing inbreeding or self-fertilization without suffering inbreeding depression (the decrease in fitness experienced by normally outcrossing species when they are inbred; the converse of hybrid vigor).

Making chromosomal infidelity taboo

Precise pairing of homologs is generally not a feature of newly formed allopolyploids. As a result, some chromosomes pair with more than one other chromosome, or fail to find a partner and are left behind when paired chromosomes line up at the metaphase plate (Fig. 1b). These mistakes can result in high rates of chromosome loss, infertility and even spontaneous tumors (for an excellent review, see Ref. 1). So how does a new allopolyploid avoid these problems and achieve stability? Genetic mapping and fluorescence *in situ* hybridization studies comparing chromosomes of natural allopolyploids with (the modern descendants of) their probable progenitors reveal numerous changes. These include DNA sequence elimination, heterochromatin expansion, reciprocal chromosome segment translocations and inversions, all of which can help differentiate homologs from homeologs.

When do genetic rearrangements begin to happen in allopolyploids? The surprising answer in several cases appears to be 'immediately'. An initial hint came from studies of synthetic *Brassica* allotetraploids analyzed three to five generations after their formation[15]. Using randomly chosen genomic and cDNA clones as probes for Southern blotting, progeny often displayed restriction digest patterns that were similar among allotetraploid siblings, but different from either diploid parent, suggesting that genetic mutations or epigenetic changes, such as *de novo* methylation, had occurred. Indeed, Southern blots using restriction endonucleases that recognize the same sequences but that differ in their sensitivity to methylation (e.g. *Hpa* II and *Msp* I), revealed that changes in cytosine methylation had occurred in the allotetraploid progeny relative to the diploid parents. In retrospect, it is possible that all of the changes reported might be attributable to methylation differences, given that all of the restriction endonucleases used were susceptible to inhibition by cytosine methylation. Thus, the conclusion that rapid genetic changes (sequence alterations) had taken place needs to be verified using methylation-insensitive enzymes, DNA sequencing or PCR-based approaches that cannot be confounded by methylation. Regardless, the demonstration that allotetraploid progeny differ from their progenitors, whether by DNA modification or genetic change, was a significant insight in this landmark study.

Strong evidence that elimination of low-copy number DNA sequences can occur in the very first generation after hybridization or polyploidization is provided by two new studies using allotetraploid wheat as an experimental system[16,17]. Using AFLP analysis to survey genomic DNA loci, an astonishing fraction (as high as 14%) of AFLP bands from one parent were lost in new hybrids and allotetraploids. Band loss could not be explained simply by heterozygosity in the parents and the inheritance of only one of the alleles, because the progenitor species were highly inbred and molecular tests failed to detect polymorphisms at the loci examined. Using parental AFLP bands as probes for Southern blotting confirmed the loss of these sequences in multiple independent siblings and also showed that the eliminated sequences were typically present in the genome in low copy numbers. The authors conclude that sequence elimination results from intrachromosomal deletions and that these events might represent steps towards ensuring diploid-like chromosome pairing (homologs with homologs; Fig. 1a).

Genomic mayhem in new hybrids and allopolyploids might not always occur. A study that also used AFLP to analyze the genomic DNA content of newly formed cotton allopolyploids surveyed ~22 000 genomic loci but failed to detect any evidence for rapid genomic changes[18]. Instead, the simple addition of loci from both progenitors was detected in both newly formed allotetraploids and allohexaploids.

Epigenetic influences

In newly formed allopolyploids of *Brassica*, wheat and *Arabidopsis*, changes in cytosine methylation patterns occur frequently within genes and transposable elements[15,17,19]. Although methylation can affect gene activity, there have been few documented cases of redundant genes being silenced early in

polyploid formation. An exception has been the phenomenon of nucleolar dominance, which describes the silencing of rRNA genes inherited from one progenitor in a hybrid or allopolyploid, independent of maternal or paternal effects[20]. Synthetic allotetraploids that re-created four naturally occurring *Brassica* or *Arabidopsis* allotetraploid species displayed the same patterns of nucleolar dominance observed in natural allotetraploids, beginning as early as the F1 generation and becoming completely established by the F2. Silenced rRNA genes subjected to nucleolar dominance are derepressed by 5-aza-2′deoxycytosine (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[21,22].

Two recent papers extend the finding that chromatin modifications can silence redundant genes in allopolyploids to include protein-coding genes, including presumptive transcription factors[14,19]. Both studies used cDNA-AFLP to survey the expression of parental transcripts in newly formed[19] or natural[14] strains (accessions) of allotetraploid *Arabidopsis suecica* and its progenitors, *A. thaliana* and *Arabidopsis arenosa* (also known as *Cardaminopsis arenosa*). Silencing of the orthologous genes of 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. Cloning and sequencing of cDNA-AFLP bands revealed them to be genes encoding transcription factors and enzymes or (in two of 13 cases) transposable element sequences. For two protein-coding genes, Lee and Chen verified uniparental silencing using an RT–PCR assay. The silent genes were derepressed by treating plants with aza-dC, suggesting that they had been silenced by methylation and not by mutation or transposon insertion. A survey of five contiguous genes suggested that silencing was not regional, but was established on a gene-by-gene basis. The significance of uniparental ortholog silencing in determining the phenotype of newly formed allopolyploids is not yet clear, but it is likely to be important given that aza-dC treatment can induce a variety of homeotic transformations and bizarre phenotypes not observed in either parent[1].

Conclusions

Emerging data suggest that a combination of genetic and epigenetic events take place quickly upon formation of a new polyploid, presumably helping to stabilize the genome and formulate coherent gene expression programs. How sequence elimination occurs and is targeted to specific sequences in wheat chromosomes is a mystery. Perhaps there are similarities to the mechanisms of chromosome diminution and programmed DNA rearrangements in flies, nematodes and ciliates[23–25]. The demonstration that epigenetic silencing is frequent in allopolyploids also raises interesting questions. Are the same genes derived from the same progenitor species always silenced in independent natural accessions or synthetic strains of an allopolyploid? If so, how are orthologous genes discriminated? Is 'hybrid vigor' the consequence of having more of a good thing, selective silencing (or elimination) of what would otherwise be too much of a bad thing, or both? We can expect answers to at least some of these questions within the next few years.

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