The dynamic plant genome

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Our group investigates the molecular mechanisms that drive the rapid evolution of plant genomes, e.g. DNA repair, homologous recombination, microsatellites instability, transposons and polyploidy. In addition, we are harnessing these mechanisms to develop new tools for functional genomics and biotechnology in plants such as gene targeting and mutation detection methods in transposon-mutagenized populations.

Our work makes use of model species, each of which having its particular advantages: i) Arabidopsis has a fully sequenced genome, a small size, short generation time, easy transformation and a broad collection of mutants; (ii) Wheat, a major crop is hexaploid in the case of bread wheat or tetraploid in the case of durum (pasta) wheat. It is a model for studies on polyploidy. It has several wild diploid and tetraploid relatives and synthetic polyploids can be readily be made to mimic the evolutionary event of speciation via polyploidization. iii) Tomato, a well-characterized and transformable genetic system, is a model for studies on the development and metabolism of fleshy berry fruits and has several well characterized wild relatives that can be used to study the control of gene flow across species.

Genetic divergence and homologous recombination

Genetic divergence is known to hinder recombination between distantly related species in bacteria and fungi. e found that a single nucleotide divergence between two 618bp repeats can cause a rapid drop-off of homologous recombination between these repeats in Arabidopsis. his antirecombination effect was independent of the position of the nucleotide polymorphism or of the types of mismatched nucleotides. These data suggest that the presence of mismatched bases in recombination intermediates inhibit recombination and furthermore, that the length of recombination intermediates in Arabidopsis is in the range of the repeat size. We are further testing this model by assessing the antirecombination effect of DNA divergence in the background of mismatch repair (MMR) mutants in Arabidopsis. A series of MMR Arabidopsis mutants were isolated. *S*ome of them show partial sterility and alterations in radiation sensitivity. These mutants are being tested for their effect on homologous recombination between divergent substrates, namely between repeats in cis, between homologous chromosomes during meiosis and between a chromosomal target and an extrachromosomal vector during gene targeting.

Chromatin remodeling and homologous recombination

We have prepared a series of mutants and RNAi lines for 13 Arabidopsis genes similar to the yeast RAD54 gene. hese *At-RAD54*-like genes form a subfamily of the broader SWI2/SNF2 family of chromatin remodeling genes. In yeast *RAD54* plays a primary role in mitotic recombinational repair. It contains a helicase signature and is a dsDNA-specific ATPase. everal RNAi lines and mutants showed severe radiation sensitivity and some of them were defective in intrachromosomal recombination supporting their role in recombinational repair in plants. We are overexpressing the yeast and plants *At-RAD54*-like genes and testing their effect on homologous recombination between various substrates as described above.

Genetic variation released upon polyploidization

The discovery of polyploidy was followed by quantification of its prominence and by contemplating the reasons for its success as a driving force in plant evolution. The paradigm was

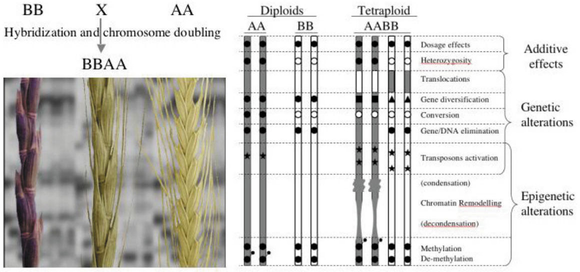

Fig. 1 *On the left, is an example of the formation of a new polyploid species (Genome BBAA) resulting from hybridization between two diploid wild wheat species (Genomes AA and BB) followed by chromosome doubling. On the right examples of genetic and epigenetic alterations resulting from polyploidization are shown*

that polyploid species are successful because of the increased range of gene dosage; the new heterotic interactions between alleles, homoeoalleles or genes; and buffering of the mutation load, as a result of gene duplication, facilitating the formation of novel genes and of recombinant genomes. While this long-held view is still valid, there are now new twists to the paradigm. Recent studies, many of which done in our lab, in collaboration with Prof. Moshe Feldman, have emphasized the importance of non-Mendelian processes and described their time course. These studies show that new variation, not previously present in the diploid progenitors, can be induced rapidly upon polyploidization rather than on an evolutionary scale. The basis of this new variation is both genetic and epigenetic. The types of non-Mendelian changes observed were: programmed elimination of sequences (coding and non-coding); gene silencing; transcriptional activation of retrotransposons and silencing or activation of genes adjacent to retrotransposons. We are currently investigating the mechanism of programmed DNA elimination as well as the mechanisms whereby transposons sense the genomic shock of polyploidy.

Selected Publications

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Kashkush, K., Feldman, M. and Levy, A.A. (2003) Transcriptional activation of retrotransposons alters the expression of adjacent genes in wheat. *Nature Genetics*, 33, 102-106.

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