# The Dynamic Plant Genome

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Our group is interested in understanding how species evolve. Our research focuses on the molecular mechanisms responsible for the plasticity and biodiversity seen in the plant kingdom. We study the mechanisms of homologous recombination, polyploidization, hybridization and transposition. In addition, we are harnessing these mechanisms to develop new tools for functional genomics and biotechnology in plants such as gene targeting and transposon-mutagenesis.

**Polyploidy and Hybridity** are prominent in the plant kingdom. Wheat for example is an hexaploid, whose genome is an hybrid combining the genomes of three diploid progenitors. Polyploidization can occur overnight, for example through inter-specific hybridization followed by genome doubling via unreduced gametes. It is thus one of the most efficient and rapid ways to generate a new species and is a driving force in plant genome evolution. The paradigm to explain the success of polyploidy was that the increased range of gene dosage, the new heterotic interactions between alleles, homeoalleles or genes and the buffering of the mutation load resulting from gene duplication, facilitate the formation of novel genes and the establishment of the new species. While this long-held view is still valid, there are now new twists to the paradigm. Work done in our laboratory, in collaboration with Prof. Moshe Feldman, has emphasized the importance of non-Mendelian processes and described their time course. In these studies, synthetic polyploids were made and analyzed immediately after formation. These studies show that a new, non-additive variation, not previously present in the diploid progenitors, can be induced immediately in the hybrid and upon polyploidization rather than on an evolutionary scale, affecting both coding and non-coding DNA. The basis of this new "reprogramming" of the genome is both genetic and epigenetic. The types of non-Mendelian changes observed were: programmed elimination of sequences (coding and non-coding); gene silencing associated with cytosine methylation and transcriptional activation of retrotransposons. This rapid reorganization of the genome structure and expression is now investigated in wheat as well as in model systems, *Arabidopsis* and budding yeast. In collaboration with Prof. Naama Barkai, we have found that interspecific hybrids of yeast also show heterosis (hybrid growth vigor). We are studying the novel rewiring of genetic networks in yeast hybrids and their possible contribution to heterosis. We are also carrying genetic screens to identify the genes that contribute to heterosis (work in progress).

**Transposons,** or mobile DNA elements, make up to 80% of the plant genome and are major players in contributing to rapid genome evolution. We have studied their transposition mechanism, regulation and evolution. Among the highlights of this research are (i) the discovery that non-autonomous elements are derived from autonomous elements by a mechanism of abortive gap repair; (ii) that transposon activation can interfere with the normal activities of neighboring genes on a genome wide scale, effectively disabling some genes, and turning on other genes when they should be switched off. We showed that transcriptional readout from the termini of transposons can generate antisense RNA for adjacent genes that are in opposite orientation to the readout transcripts. Conversely, readout activity can activate adjacent genes that are oriented in the same direction as the transcriptional readout. (iii) We have developed a new high-throughput system for gene discovery and functional genomics in tomato,

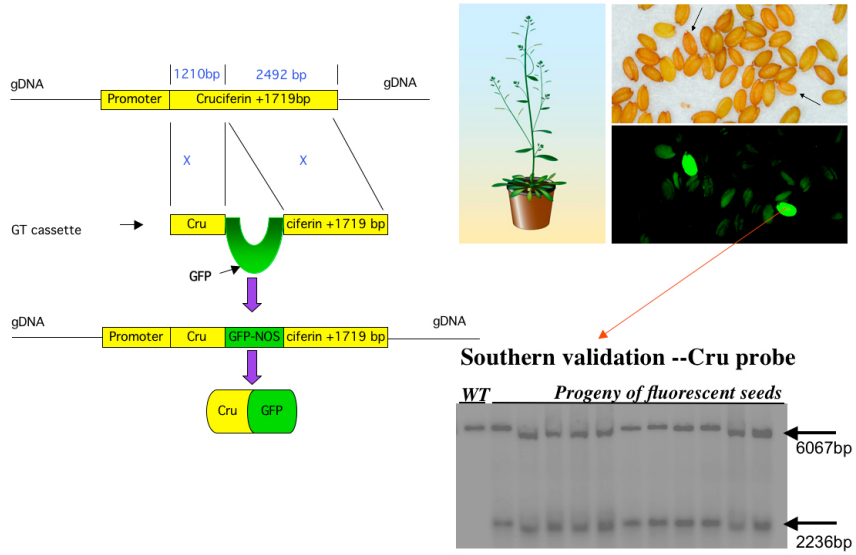


***Fig. 1*** *A new gene targeting assay has been developed (Shaked et al. 2005) whereby the Arabidopsis seed-specific Cruciferin gene is used as a genomic target. The Gene targeting vector shares homology with this locus but has a GFP insertion fused to Cruciferin. Fluorescent seeds can be thus obtained when insertion occurs via homologous integration. This assay enables to identify Gene targeting events and to screen the effect of various proteins and mutants on gene targeting. A variation of this system has been developed whereby mRFP substitutes GFP, thus giving a stronger signal.*

based on transposon mutagenesis in the background of Micro-Tom, a miniature tomato that can be grown at a density of up to 1000 plants per $m^2$ and has a rapid life cycle of 70-90 days. We have introduced features that enable enhancer and promoter trapping and more recently we have adapted the system in collaboration with Dr. Asaph Aharoni for gain-of-function mutagenesis. We showed that this system is powerful for the functional characterization of a variety of genes.

**Homologous recombination** plays two opposite roles, contributing to genomic diversity through the exchange of homologous chromosomal segments and contributing as well to genomic stability through the repair of DNA breaks. We have investigated the control of homologous recombination in plants and we have developed new technologies for the precise engineering of plant genomes (gene targeting). One factor that represses homologous recombination in plants is the divergence between the recombination partners. We showed that a single mismatch can cause a 3-4 fold drop-off in homologous recombination and that the mismatch repair AtMSH2 gene is responsible for this divergence-based repression of recombination in both somatic and meiotic tissues. This work opens the prospect to use mismatch repair mutants for transferring genes across related species. We also showed that plant chromatin remodeling genes controls DNA recombination and DNA damage repair and in some cases can control the maintenance of genetic and epigenetic processes. In addition, we found that the chromatin remodeling yeast RAD54 gene can enhance the frequency of gene targeting in plants by 1-2 orders of magnitude. Current research aims at further enhancing the rates of gene targeting by combining different approaches. We are co-expressing the partners of Rad54 (Rad51 and Rad52) at the same time and location when and where the targeting vector is delivered to improve homology search and strand invasion. In addition, in collaboration with Michael Elbaum, we are using protein chemistry, exploiting DNA-protein interactions, in order to stabilize the physical interactions between the vector and the chromosomal target in order to facilitate gene targeting.

**Contribution to plant biotechnology**

The work done in the Levy laboratory deals with the understanding of genetic mechanisms that can be applied to the development of new tools for plant breeding. Our work on transposable elements has been patented and licensed, for functional analysis of genes in tomato. In addition, our work on DNA recombination, both mitotic and meiotic, enables to exploit a very broad range of genetic diversity in a more efficient manner. It can be used for efficient gene transfer, via sexual hybridization, between varieties from the same species. Our work on mismatch repair genes facilitates the transfer of genes between different, but related (homeologous) species. Our work on gene targeting contributes to the development of improved technologies to introduce genes from any species into plants. This work paves the way for precise genetic engineering in plants, a technology that is expected to have a profound effect on scientific research in plant genetics as well as on the development of GMOs with well defined genetic modifications that would be less prone to gene silencing, easier to regulate and more acceptable to the broad public. Finally, our studies on polyploids and hybrids may help better exploit the heterotic interactions between divergent genomes and develop improved hybrids and polyploids.

**Selected publications**


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