Plant DNA repair and recombination on the French Mediterranean

Workshop on Plant DNA Repair & Recombination

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This EMBO Workshop on Plant DNA Repair & Recombination took place between 31 May and 3 June 2007, in Presqu'île de Giens, France, and was organized by B. Hohn, A. Levy, H. Puchta, D. Shippen, C. Well and C. White.

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de Giens in the south of France from 31 May to 3 June 2007 for an EMBO Workshop on Plant DNA Repair & Recombination to share their latest results. Almost 10 years have passed since the previous EMBO Workshop on the topic—organized in 1998 in the Netherlands—so expectations were high. Owing to space limitations, this report cannot be comprehensive and will, instead, reflect meeting highlights by using feedback from the session chairs.

The processes of DNA repair and recombination have been studied for many years using various organisms including bacteria and yeast, and also mammalian cells and plants. This has revealed the subtleties of the molecular mechanisms underlying these processes and has shown on the one hand clear conservation of these processes in evolution, and on the other clear differences in their regulation among the different organisms. Such differences make harnessing the recombination process for precise genetic engineering easily accomplished in yeasts, but (currently) tedious in mammalian and plant cells. The power of plant genetics, especially with the aid of the model organisms *Arabidopsis thaliana* and *Oryza sativa*, is proving to be instrumental in the identification of factors that mediate and regulate recombination. Importantly, it has been shown that plants with mutations in recombination genes that lead to embryonic lethality in mammals can grow and reproduce. This allows the study of the effects of such mutations in a multicellular organism for the first time. The process of meiotic recombination is of course crucial for plant breeding; therefore, a large part of the workshop was devoted to meiosis.

J. Haber (Waltham, MA, USA) opened the meeting with an excellent keynote presentation on the molecular mechanisms of homologous recombination and double-strand break (DSB) repair in *Saccharomyces cerevisiae*. On the basis of recent work he postulated that although crossovers are the default mechanism for meiotic recombination, they are repressed in mitotic cells by a set of DNA helicases. He also discussed competition between homologous recombination pathways and non-homologous pathways, and the implications that understanding such competition has for gene targeting in plants. The perspective of a yeast geneticist was particularly appropriate for this meeting because there has been a recent and highly productive emphasis on analysing the plant homologues of yeast recombination genes. These efforts have

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Introduction

Two factors motivate research in plant DNA repair and recombination today: the unique tools that plants offer for understanding fundamental biological processes, and the promise that a deeper understanding of these processes will facilitate improved technologies for breeding and engineering crops for food, fuel and medicines (Tzfira & White, 2005). With these thoughts in mind, scientists assembled at the Presqu'île

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resulted in groundbreaking knowledge that will facilitate the subsequent examination of plant-specific recombination mechanisms and proteins.

B. Hohn (Basel, Switzerland) followed with a keynote retrospective on her influential work over the past two decades studying DNA repair and recombination in plants. Her group showed that not only DNA damaging agents but also environmental stress—for example, pathogen attack—induces genome instability. The demonstration that plants can pass on ‘molecular memories’ of stress to their offspring—possibly helping them to adapt to their environments—is a challenge to our current understanding of genetic mechanisms. Hohn reported that the progeny of plants exposed to biotic or abiotic stress showed the same genomic instability as their parents, even though the offspring did not experience either stress (Molinier *et al*, 2006). The instability persists for at least four generations, regardless of whether the offspring are subjected to the stress. Hohn indicated that initial experiments hint at an epigenetic mechanism.

Somatic DNA repair and recombination

A. Britt (Davis, CA, USA) discussed the regulation of ionizing-radiation-induced checkpoint responses in a group of mutants of *A. thaliana* called *suppressors of gamma* (*sog*). These mutants do not undergo ionizing-radiation-induced cell-cycle arrest but show a hypersensitivity to hydroxyurea, indicating that they are defective for DNA damage repair; therefore, they harbour mutations in putative checkpoint control genes. T. Cools (Ghent, Belgium) used microarray analysis to show that, on challenge by DNA-replication-inhibiting agents, mutants in the *A. thaliana* cell cycle kinase gene *WEE1* can upregulate DNA repair genes without downregulation of cell-cycle control genes (De Schutter *et al*, 2007). This suggests that *WEE1* is crucial for cell-cycle arrest on activation of the DNA replication checkpoint. P. Genschik (Strasbourg, France) reviewed his work on the CUL4–DDB1–DDB2 (CUL4 for cullin 4; DDB for damage-specific DNA-binding protein) complex—which in mammals is active in protein degradation related to repair and damage response—and its role in ultraviolet (UV)-resistance in plants. However, unlike Cul4 knockdowns in mammals, knockdown in *A. thaliana* did not induce hyper-endoreduplication. W. Xiao (Saskatoon, Saskatchewan, Canada) also reported on a ubiquitin ligase complex that is required for the regulation of protein activity/specificity, rather than degradation. He showed methylmethane sulphonate sensitivity in his *uev1D* mutant. This mutant probably has a defect in processing blocks encountered by the DNA polymerase. RAD51B, RAD51D and XRCC2 (XRCC for X-ray repair cross complementing) have an important role in somatic recombination but not in meiotic recombination, as reported by C. White (Clermont Ferrand, France) who studied the roles of the five RAD51 paralogues in recombination (Bleuyard *et al*, 2005). By contrast, White found that RAD51C and XRCC3 are important for meiotic recombination. New fluorescence *in situ* hybridization (FISH) data indicated that in *rad51C* and *xrcc3* mutants, chromosome pairing in meiotic prophase I was restricted to heterochromatic regions. The work of J. Hays (Corvallis, OR, USA) and A. Sakamoto (Takasaki, Japan) again showed that knockouts affecting DNA stability that are lethal in animals can be tolerated in plants, making them an attractive multicellular system for the study of damage repair and response. Sakamoto showed that mutants in several members of the translesion polymerase ζ

complex of *A. thaliana*—including REV1, REV3 and REV7 (REV for UV-reversionless phenotype)—showed elevated sensitivity to UVB. Interestingly, *rev3⁻* and *rev1⁻* plants had lower mutation frequencies, suggesting a role for polymerase ζ and its associated factors in UV-induced mutations (Takahashi *et al*, 2005). Hays used a root growth assay to show that polymerase ζ and polymerase η single mutants show cell death on UVB irradiation and that double mutants suffered similar cell death even in the absence of UVB treatment. Mutants in *BRCA1* (breast cancer 1) and *BARD1* (BRCA1 associated RING domain 1) homologues were shown by H. Puchta (Karlsruhe, Germany) to be sensitive to the DNA cross-linking agent mitomycin C and to be deficient in somatic homologous recombination, suggesting a functional conservation between mammals and plants (Reidt *et al*, 2006). Furthermore, the *RECQ4A* gene of plants might be a functional equivalent of the Bloom syndrome counterpart in humans, based on the hyper-recombination phenotype of its knockout mutant and its genetic interaction with the gene for endonuclease MUS81. Puchta also showed that *mus81* mutants in *A. thaliana* are sensitive to DNA-damaging agents and partly defective in inducing homologous recombination in somatic cells. The double mutant *mus81recq4a* also had a strong proliferation defect, suggesting that these two genes are involved in two pathways that together are essential for the removal of aberrant replication intermediates.

The moss *Physcomitrella patens* has generated significant interest among researchers with respect to gene targeting in plants because of its high rate of homologous recombination; B. Reiss (Cologne, Germany) and D. Schaefer (Versailles, France) discussed the latest insights into these mechanisms. Surprisingly, Schaefer found that the rate of gene targeting was reduced by two orders of magnitude following *Agrobacterium tumefaciens* mediated transformation, although it had been previously reported—for the yeast *Kluyveromyces lactis*—that *Agrobacterium* transformation is beneficial for gene targeting (Bundock *et al*, 1999). This discrepancy might be related to the sensitivity of the *P. patens* system to heterologous sequences at the DNA ends, as Schaefer found that 20–100 base pair heterologies lead to 10–100-fold lower gene targeting. The sequence of *P. patens* is close to completion and it has been found that, interestingly, it contains two *RAD51* homologues but lacks *BRCA1* and *BRCA2*, as well as *XRCC3*; both *RAD51* genes have to be knocked out to decrease the efficiency of gene targeting. Reiss found that strains mutated in both genes grow slowly, are sensitive to DNA damage and suffer from severely reduced spore formation.

T. Peterson (Ames, IA, USA) gave an update on the mechanisms that *Zea mays* transposons use to generate deletions, inverted duplications, and inversions and translocations with direct and inverted transposon termini. The transposition-based system constitutes an alternative to the Cre–lox system for generating genome modifications (for the associated animations see <http://jzhang.public.iastate.edu/transposition.html>). P. Schloegelhofer (Vienna, Austria) identified the *GR1/COM1* (GR1 for gamma response 1) gene as an *A. thaliana* homologue of the yeast Com1/Sae2 protein, which is essential for the release of the enzyme Spo11 from its complex with cleaved meiotic DNA. Mutants in this gene are resistant to all tested DNA-damaging agents except mitomycin C and are sterile owing to a defect in meiotic pairing. As expected, the SPO11 protein was found bound to DNA and genetic evidence also pointed to activity of *GR1/COM1* downstream from *SPO11* and upstream from *RAD51*.

Chromatin and epigenetics

Several epigenetic marks are now known to influence a myriad of meiotic and mitotic processes. These marks include DNA and histone methylation, phosphorylation and acetylation. J. Paszkowski (Geneva, Switzerland) revealed that methylation 1 (MET1), which is known to direct methylation at CpG sites in plants, is also important for maintaining histone methylation states. Mutants in *MET1* showed a significant decrease in methylation at the lysine 9 residue of histone H3. T. Roldan-Arjona (Cordoba, Spain) presented exciting work on glycosylases that demethylate DNA through base excision. Mutants in *Demeter (DME)* or repressor of silencing 1 (*ROS1*), two such glycosylases, have pleiotropic phenotypes including slow growth, aborted seeds and hypermethylation. The regulation of DNA demethylation in plants will undoubtedly be an exciting avenue for future research.

Chromosome maintenance and architecture

Telomeres protect the natural ends of chromosomes from triggering DNA-damage-response mechanisms. This is achieved by specialized proteins that bind to telomeric DNA and define the unique properties of telomeric chromatin. D. Shippen (College Station, TX, USA) presented data showing that telomere proteins that contain oligonucleotide/oligosaccharide-binding fold domains evolve at a rapid pace. In *A. thaliana*, three divergent *PROTECTION OF TELOMERASE 1 (POT1)* genes were defined. POT1 functions as a component of the telomerase and is essential for enzyme activity *in vitro* and *in vivo*. There is evidence for numerous sites of *POT1* positive selection in *A. thaliana*, supporting the conclusion that it is under significant evolutionary pressure. J. Fajkus (Brno, Czech Republic) has used genetic, biochemical and biophysical methods to map the interactions between *A. thaliana* proteins of the SMH (single myb histone) family and POT proteins. These studies should pave the way for a greater understanding of the complex interactions within telomeric chromatin.

Although telomeres contain a unique set of proteins that distinguish them from DSBs, many proteins that are required for DSB repair are localized to telomeres and have essential roles in chromosome-end protection and maintenance. M. Gallego (Aubière, France) presented data indicating that a deficiency of the RAD1/ERCC1 endonuclease in *A. thaliana* reduces chromosome-end protection in the absence of telomerase, giving rise to an increased incidence of chromosome fusions and extrachromosomal telomeric DNA. K. Riha (Vienna, Austria) showed that Ku70–Ku80 inhibits homologous recombination activities at chromosome termini in *A. thaliana* by suppressing alternative telomere lengthening and resolution of t-loops, which leads to the excision of extrachromosomal circular molecules (Zellinger *et al*, 2007). Such excision events produce a terminally deleted telomere that might destabilize the genome. L. Vespa (College Station, TX, USA) showed that ataxia telangiectasia mutated (ATM) kinase has a vital role in genome stability either by preventing rapid telomere deletions or eliminating cells that have undergone large deletions from further propagation.

Besides their primary role in chromosome stabilization, telomeres are also implicated in chromosome pairing during meiosis. S. Armstrong (Birmingham, UK) observed that, in *A. thaliana*, telomeres associate forming a bouquet-like structure during the meiotic interphase before the onset of general chromosome synapsis (Armstrong *et al*, 2001). These findings suggest that the bouquet facilitates subsequent chromosome alignment and synapsis.

Fig 1 | RAD51 staining in the *Zea mays* pachytene meiocyte. The figure depicts a partial projection of a *Z. mays* pachytene meiocyte taken by conventional fluorescence microscopy. The DAPI stained pachytene chromosomes are shown in red and RAD51 foci at the sites of homologous recombination are shown in green. The projection is approximately 20 µm in diameter. Image courtesy of Zac Cande, Inna Golubovskaya and Chung-Ju Rachel Wang (Berkeley, CA, USA). DAPI, 4',6-diamidino-2-phenylindole.

In plant somatic cells, non-homologous end joining seems to be the predominant pathway for DSB repair, similar to the situation in vertebrate somatic cells. Nevertheless, I. Schubert (Gatersleben, Germany) reported that, in plants, genotoxin-induced chromosome rearrangements in freshly replicated chromatids are frequently attributable to DSB repair through homologous recombination. Furthermore, K. Watanabe (Gatersleben, Germany) showed that, immediately after X-irradiation, *A. thaliana* sister chromatids actively align, apparently for repair by homologous recombination, and that this process is significantly slowed down in *mim* mutants. *MIM* is the *A. thaliana* orthologue of *SMC6*, a member of the structural maintenance of chromosome (SMC) protein family that organize higher order chromosome structure. Watanabe's results suggest that *MIM* is required to ensure correct sister chromatid alignment to facilitate repair processes.

Recombination and meiosis

Homologous chromosomes can occasionally interact during mitosis and suffer accidental breakage events. By contrast, during meiosis they pair efficiently, form a specialized proteinaceous structure—the synaptonemal complex—and undergo programmed DSBs that are repaired by recombination mechanisms. Z. Cande (Berkeley, CA, USA) used both conventional fluorescence microscopy (Fig 1) and new ultra-high-resolution structured illumination microscopy techniques to reveal unprecedented views of chromosome structure and chromatin remodelling during meiosis, including chromosome interlock

(unresolved chromosome entanglements that occur during synapsis) resolutions, which might be the rate-limiting step in completing synapsis. G. Moore (Norwich, UK) described the role of the Ph1 locus in chromosome pairing in wheat. Hexaploid wheat contains three sets of chromosomes and, in order to be fertile, it must pair true homologous chromosomes during meiosis. The Ph1 locus is known to be necessary for the correct pairing of homologous chromosomes (Griffiths *et al*, 2005). Moore discussed data showing that this locus is a cyclin-dependent kinase (Cdk)-like gene complex related to the *IME2* and *CDK2* meiotic checkpoint genes of *S. cerevisiae* and *H. sapiens*. G. Copenhaver (Chapel Hill, NC, USA) debuted a visual assay for meiotic recombination in *A. thaliana* based on transgenic fluorescent markers expressed in the pollen tetrads produced by the *qrt* (quartet) mutant. By using this system, he showed that MUS81 in *A. thaliana* mediates a subset of interference-insensitive crossovers (Berchowitz *et al*, 2007). C. Weil (West Lafayette, IN, USA) has also developed a highly effective visual assay in *Z. mays* based on kernel colour. With this system as the basis for a forward screen he has recovered a collection of hypo- and hyper-recombination mutants as well as crossover interference mutants. T. Gerats (Nijmegen, The Netherlands) used a different large-scale genetic approach—based on differential display in petunia species—to search for genes specific to meiosis, enabling him to develop a stage-specific profile of meiotic transcripts.

Several groups reported on the analysis of individual genes important for meiotic recombination. M. Grelon (Versailles, France) presented data on such a new mutant—*putative recombination initiation defect* (*prd1*)—in *A. thaliana* (De Muyt *et al*, 2007). The *prd1* mutant has markedly reduced crossover frequencies and does not show early recombination markers such as DMC1 (DMC for disruption of meiotic control) foci. The PRD1 protein seems required for DSB formation and seems to interact with SPO11-1 but not SPO11-2. C. West (Leeds, UK) showed that the *A. thaliana* Nijmegen breakage syndrome 1 (NBS1) protein interacts with MRE11 and RAD50, as it does in other organisms, and is crucial for the somatic DNA damage response. Mutations in NBS1 are known to be associated with an increased risk for developing non-Hodgkin lymphoma in humans. In contrast to MRE11 and RAD50, the *A. thaliana* NBS1 protein seems to be dispensable for meiosis—although it seems responsible for the residual fertility of *atm* mutants (Waterworth *et al*, 2007). F. Lhuissier (Wageningen, The Netherlands) reported that Mlh1 associates with only 70% of late recombination nodules on each of the chromosomes in *Solanum lycopersicum*, and that these seem to be subject to CO interference (Lhuissier *et al*, 2007). C. Franklin (Birmingham, UK) presented data that suggests ASY1 helps regulate whether DSBs are repaired by DMC1 or RAD51. DMC1 is the preferred choice in ASY1-proficient lines, although RAD51 can repair them if necessary, but less effectively (Sanchez-Moran *et al*, 2007).

Recombination and related technologies

The most eagerly awaited session—the one dedicated to gene targeting in plants—took place on the final day. Keen interest in these technologies from both academia and the commercial sector has recently driven rapid progress in this field. P. Hooykaas (Leiden, The Netherlands) gave an overview of his laboratory's work on gene targeting and the identification of factors involved in DNA integration through homologous and non-homologous recombination using yeast as a model system. He reported that knocking out genes involved in non-homologous recombination led to a significant increase in gene targeting in yeast and fungi, but only to a limited increase in plants.

That DNA DSBs are entry points for the recombination machinery and that gene targeting can be increased several-fold by introducing a specific DSB at the locus of interest has been previously described. Many speakers described recent improvements in the development of engineered nucleases that can be targeted to specific sites in the genome. V. Shukla (Indianapolis, IN, USA) reported on the use of zinc finger nucleases (ZFNs) to generate a knockout mutation of *IPK1*—a *Z. mays* gene involved in phytate biosynthesis. Although frequencies of gene targeting were not provided, this was the first report of an endogenous plant gene being modified by an engineered endonuclease. T. Tzfira (Ann Arbor, MI, USA) described his efforts to harness ZFNs for gene deletions and precise gene insertions through non-homologous end joining. D. Voytas (Ames, IA, USA) talked about the efforts of the Zinc Finger Consortium to convert ZFNs into a robust platform technology (<http://www.zincfingers.org/>). ZFNs, however, were not the only enzymes discussed for making targeted chromosome breaks. F. Paques (Romainville, France) described recent progress made by his company in engineering meganucleases—endonucleases with large (>12 bp) recognition sites—to recognize specific chromosomal targets. Although very promising, these meganuclease-based techniques will still require a significant investment in the design and evolution of the enzyme—as they sometimes seem to have adverse effects on the cells in which they are present—in order to guarantee that the specific DNA sequences of choice are efficiently and exclusively cleaved. The development of new vectors might also hold the keys to the development of gene targeting tools. One such example is the work of S. Iida (Okazaki, Japan), who had previously developed vectors based on positive-negative selection with two DTa (Diphtheria toxin A)-negative selection markers, one adjacent to each T-DNA border to target the *WAXY* locus in *O. sativa*. He reported that the same strategy can in principle be used for any locus and showed data for targeting at the *ADH2* (alcohol dehydrogenase) locus and some other loci. Thus, gene targeting might now become routine in this organism (Terada *et al*, 2007). Another type of vector was presented by F. Van Ex (Brussels, Belgium); this vector is excised from a genomic locus with the Cre-*lox* site-specific recombination system, where Cre is expressed under a meiotic promoter. Preliminary data of this promising system were presented but further work will be needed to show whether true gene targeting events occurred. This system might hold great promise for plant species that are difficult to transform.

Concluding remarks

The workshop successfully brought together several communities of plant researchers working on meiosis/plant breeding, DNA repair/chromosome stability and biotechnology/transgenesis. It exposed the participants to the latest scientific results in the different areas, and stimulated interaction and cross-fertilization of ideas and techniques. In view of the enthusiasm and positive feedback, it was decided to make efforts to organize such a meeting every two years, alternating between Europe and the USA.

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