OMB No. 0925-0001 and 0925-0002 (Rev. 11/16 Approved Through 10/31/2018)

BIOGRAPHICAL SKETCH

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NAME: Chloe Girard

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POSITION TITLE: Postdoctoral Researcher

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
AgroParisTech School of Life Sciences Paris, France	Master	06/2011	Agronomy
Université Paris Sud Orsay, France	Master	06/2011	Plant Biology
Université Paris Sud Orsay, France	PhD	10/2014	Biology
Stanford University Stanford, CA, USA	Postdoc	current	Developmental Biology and Genetics

A. Personal Statement

Throughout my research career I have been puzzled and fascinated by how cells maintain their genome stability throughout meiosis, the special cell division that allows the formation of sperm and eggs during sexual reproduction. During meiosis, chromosomes proceed to two rounds of segregation after one single round of DNA replication; the first division allows the separation of homologous chromosomes, which relies in particular on crossover formation in most species; while the second division segregates the sister chromatids. Defect in CO formation can provoque errors in chromosome segregation during meiosis, which lead to aneuploidy, *i.e.* embryos with abnormal numbers of chromosomes, the leading cause of miscarriages in humans. Crossover occurence relies on the formation of double-strand breaks (DSBs) induced early in meiosis. DSBs are the most deleterious type of DNA lesions that endanger genome stability, and improper repair leads to chromosome rearrangements (e.g. translocations, deletions and insertions), that in turn cause cell death and malignancies. Meiotic cells must therefore ensure a thight control over DSB repair to ensure both CO formation and genome stability. My research has primarily focused on two aspects of meiosis: regulation of the cell cycle (to allow the two divisions) and regulation of DSB repair (to promote CO formation while avoiding chromosome rearrangements).

My undergraduate and Master research contributed to the characterization of the meiotic cell cycle in the plant *Arabidopsis*, and my contribution was acknowledged in two publications in peer-reviewed journals. My interest was then drawn toward understanding the process of meiotic crossover formation and its regulation, and during my PhD studies I worked with several members of my research group to identify factors that influence DSB repair pathway to limit crossover formation during plant meiosis. My contribution led to the publication of three research papers, two of these as first author.

During my doctoral research, I enjoyed thinking about the broader implications of my results, and I was particularly interested in the extent to which the mechanisms we uncovered in *Arabidopsis,* represent general features of meiotic recombination and DNA repair across all organisms. I was also keen on working on an animal model organism, and this led me to pursue postdoctoral research investigating the mechanisms and regulation of DSB repair and CO formation during meiosis in a metazoan model, the nematode *Caenorhabditis elegans*, in Dr. Anne Villeneuve's laboratory in Stanford University.

Dr. Villeneuve has been leading the meiosis community with elegant approaches and major breakthroughs, including assays to closely monitor DSB repair outcome and discovery of the robust marker for CO formation in *C. elegans*, COSA-1.

Since I joined the Villeneuve lab, I have completed a first project on the role of the MRN complex in DSB repair pathway choice during meiosis (see C4), and the manuscript will be submitted for publication shortly. During this process, I successfully acquired valuable expertise in experimental methods for investigating *C. elegans* meiosis, including genetic mapping, strain construction, screening, cytology and genomics techniques that are at the heart of our research. This skill set complements my graduate training in genetics and genome-wide analysis of recombination. I am now shifting to a genome-wide approach to understanding the process of DSB formation and repair at meiosis, looking at the chromosomal features associated with these processes. My goal is to detect specific chromatin environments opportune for DSB formation, and the chromatin reorganization events associated with their repair as COs. My work could provide the foundation for the identification of risk factors associated with elevated chromosome breakage and defect in DSB repair. I am also in collaboration with one member of the Villeneuve lab, Chantal Akerib, to conduct a forward genetic screen to uncover new regulators of meiotic recombination in *C. elegans*. The nematode is an excellent model to pursue this project, as CO control is remarkably tight compared to other species, with only one CO formed per chromosome pair. Many well-established genetic, cytological, and molecular tools exist to investigate meiotic events, and most of the meiotic machinery is conserved across metazoans. I will take advantage of this unique framework to identify new anti-CO factors implicated in the regulation of meiotic CO formation and new potential candidates to tackle causes of infertility in other species including humans.

B. Positions and Honors

Oct 2011-Oct 2014 - PhD thesis, mentor: Raphael Mercier at INRA de Versailles, France – diploma delivered by Université Paris Sud, Orsay, France.
Nov 2014-Present - Postdoctoral Fellow, mentor: Anne Villeneuve at Stanford University, CA, USA.

2011-2016 - INRA de Versailles, France Young Scientist Fellowship from INRA, granted to 7 individuals nationwide per year, for a 3-year PhD and a 2-year postdoctoral training.

2015 - Young Researcher Prize from the Bettencourt-Schueller Foundation, which rewards every year fourteen young doctors in Science or Medicine, "*enabling them to become postdoctoral fellows in leading foreign laboratories"*.

C. Contributions to Science

C.1) Deciphering the meiotic cell cycle regulatory network in *Arabidopsis thaliana*

To generate haploid products, a meiocyte must replicate its DNA, enter meiosis I, proceed through meiosis I to meiosis II without re-replicating its DNA, and exit meiosis II. Errors in these cell cycle transitions may lead to parthenogenesis or teratoma formation, or to the production of diploid gametes that can give rise to ploypoid progeny. As polyploidy has played a key role in the evolution of many fungal, plant, and animal lineages, understanding how polyploidy arises and how it is prevented during normal meiosis is of considerable interest*.*

In my undergraduate and Master research, I contributed to research that identified a regulatory network governing three key steps of meiotic cell cycle progression: entry into meiosis I, the meiosis I to meiosis II transition, and the exit from the meiotic cell cycle. We identified a key meiotic cyclin (CYCA1;2/TAM) required for the transition from meiosis I to meiosis II, and showed that failure to express TAM leads to a high frequency of diploid gametes and subsequent polyploid progeny (D'Erfurth *et al*., 2010). Further, we showed that OSD1, a potential inhibitor of the anaphase-promoting complex/cyclosome (APC/C), functions in conjunction with TAM to regulate the transition from prophase to the first meiotic division. Finally, we identified a potential APC/C subunit (TDM) that is essential for meiosis II exit and required to prevent additional rounds of chromosome segregation (Cromer *et al*., 2012). In addition to elucidating the mechanisms that govern crucial cell cycle

transitions, these results also have potential benefits for plant breeders by providing means to facilitate manipulation of ploidy and meiotic progression in crops (*e.g.* for the production of seedless fruits).

d'Erfurth I, Cromer L, Jolivet S, **Girard C**, Horlow C, et al. The cyclin-A CYCA1;2/TAM is required for the meiosis I to meiosis II transition and cooperates with OSD1 for the prophase to first meiotic division transition. PLoS Genet. 2010 Jun 17;6(6):e1000989. PMCID: PMC2887465.

Cromer L, Heyman J, Touati S, Harashima H, Araou E, **Girard C**, et al. OSD1 promotes meiotic progression via APC/C inhibition and forms a regulatory network with TDM and CYCA1;2/TAM. PLoS Genet. 2012;8(7):e1002865. PMCID: PMC3406007.

C.2) Identification of mechanisms that limit meiotic crossover formation in *Arabidopsis thaliana*

Meiotic crossovers (COs) shuffle parental alleles in the offspring, introducing genetic variety on which selection can act. COs also participate in creating physical linkages between homologous chromosomes that are crucial for the proper segregation of homologs at meiosis I . Despite an excess of recombination precursors, most species only form very few CO per chromosome pair. The remaining intermediates are repaired either as non-crossovers (NCOs) involving non-reciprocal transfer of genetic information, or by using the sister chromatid as a repair template. Mechanisms underlying this limitation in CO number are poorly understood.

We used genetic screens in *Arabidopsis* to identify three pathways that limit meiotic CO formation in plants. The first involves the conserved DNA helicase FANCM (Crismani *et al*., 2012) as well as two helpers, MHF1 and MHF2 (Girard *et al*., 2014). Mutation of FANCM leads to an unprecedented 3-fold increase in CO formation. This pathway is thought to channel recombination intermediates towards the formation of NCOs, therefore preventing a whole pool of intermediates from becoming COs. The second pathway involves the AAA-ATPase FIDGETIN-LIKE-1 (FIGL1), and our data support a role for FIGL1 in disassembling early recombination intermediates (Girard *et al*., 2015). The third one depends on the highly conserved BTR complex (BLOOM-TOP3 - RMI1-RMI2 in human and Sgs1-Top3-Rmi1 in budding yeast) as a major NCO promoting factor (Séguéla *et al.*, 2016*)*. All pathways are parallele to each other, and our results suggest that all three represent successive barriers to CO formation, which when are lifted, leads to a large increase of COs without impairing meiotic progression. Our work reveals that CO number can be substantially increased without impairing chromosome segregation or causing any other immediate reduction in fitness. This raises an interesting question from an evolutionary perspective: why is recombination naturally limited?

Crismani W, **Girard C**, Froger N, Pradillo M, Santos JL, et al. FANCM limits meiotic crossovers. Science. 2012 Jun 22;336(6088):1588-90. PMID: 22723424.

Girard C, Crismani W, Froger N, Mazel J, Lemhemdi A, et al. FANCM-associated proteins MHF1 and MHF2, but not the other Fanconi anemia factors, limit meiotic crossovers. Nucleic Acids Res. 2014 Aug;42(14):9087-95. PMCID: PMC4132730. PubMed PMID: 25038251

Girard C, Chelysheva L, Choinard S, Froger N, Macaisne N, et al. AAA-ATPase FIDGETIN-LIKE 1 and Helicase FANCM Antagonize Meiotic Crossovers by Distinct Mechanisms. PLoS Genet. 2015 Jul;11(7):e1005369. PMCID: PMC4498898. PubMed PMID: 26161528;

Séguéla-Arnaud M, Choinard S, Larchevêque C, **Girard C**, Froger N, Crismani W, Mercier R. RMI1 and TOP3α limit meiotic CO formation through their C-terminal domains. Nucleic Acids Res. 2017 Feb 28;45(4):1860-1871. PubMed Central PMCID: PMC5389728. PubMed PMID: 27965412;

C.3) Transfer of knowledge to plant breeders: increasing crossover frequency in crops

Sexual reproduction allows, in theory, the association of different alleles at many loci in a virtually infinite number of genotypes. In most organisms, however, the number of COs is relatively low, in the range of 1 to 3 events per chromosome pair. The number of allelic combinations accessible to selection is therefore constrained by the tight control on CO formation, and the introgression of new genetic traits during agricultural breeding is hindered. Moreover, some genomic regions are refractory to recombination, hence decreasing further the number of accessible genotypes. The possibility of increasing genetic recombination frequency has consequently drawn much interest from crop breeders seeking to increase the genetic diversity in breeding programs, and to improve the power of mapping and positional cloning efforts (Crismani *et al.,* 2013).

Our discovery of major regulators of crossover frequency, namely the helicase FANCM and the AAA-ATPase FIDGETIN-LIKE-1, opens new perspective for plant breeders to create new varieties, which is largely about sampling many 'recombinants' to find individuals that have heritable advantages compared to their

parents. Two patents have been filled and form the basis of a call for bids where the French National Institute for Agricultural Research (INRA) has agreed to fund the transfer of knowledge to species of agronomic interest (*e.g.* tomato, rice and canola) where there is a desire to increase meiotic recombination. Several private companies are in negotiations to purchase licenses to work with the patents.

Crismani W, **Girard C**, Mercier R. Tinkering with meiosis. Journal of Experimental Botany. 2013; 64(1):55-65. PMCID: PMC3711010

Mercier R**.**, Crismani W. (2013) Increase in meiotic recombination in plants by inhibiting the FANCM protein. US 20140289902 A1/WO 2013038376 A1 “A method to increase meiotic recombination in plants”. INRA Patent n°1158262. Co-authors : Froger N., **Girard C**.

Mercier R., **Girard C.,** Crismani W. (2015). Augmentation of the meiotic recombination by inhibiting the FIDGETIN-LIKE-1 protein – WO2015001467 A1 . Co-author : Froger N

C.4) Deciphering the role of the MRN complex in DSB formation and repair in *C. elegans* meiosis
Crossovers arise from the repair of double-strand breaks (DSBs) by the homologous recombination (HR) machinery. Shortly after DSB formation, the DNA 5' ends are resected by the MRN/X complex composed of the highly conserved MRE11 and RAD50 proteins and a more poorly conserved subunit termed NBS1/XRS2. This resection is essential to engage the HR repair pathway and to prevent repair through non-homologous end joining (NHEJ). In *C. elegans*, MRE-11 and RAD-50 have been implicated previously in both formation and repair of DSBs during meiosis. However, no nematode homolog of NBS1 had yet been identified. While screening for mutants with altered patterns of COSA-1 foci (a very robust cytological mark of COs in *C. elegans*), the Villeneuve lab identified a mutant with aggregates of GFP::COSA-1, a phenotype indicative of impaired recombination. Through mapping, whole-genome sequencing and the generation of new alleles by CRISPR, I showed that the causal mutation disrupts the previously unrecognized *C. elegans* ortholog of NBS1. Using yeast two-hybrid, I showed that NBS-1 interacts with its cognate partners, namely MRE-11 and the MRN associated protein COM-1/CtIP. Interestingly, while MRE-11 and RAD-50 are required both for formation and repair of DSBs in nematode meiosis, my data implicate NBS-1 only in DSB repair. Using a newly developped methods of nuclear spreading along with structured illumination microscopy, we were able to show that NBS-1 is essential to counteract the NHEJ pathway and to promote repair through HR, and is therefore crucial to achieve error-free DNA repair and promote CO formation during meiosis. Our model is that MRE-11 and RAD-50 first contributes to DSB formation, and are joined later on by NBS1 and COM-1 to promote timely and efficient resection to ensure CO formation and genome intergrity.

Girard C, Roelens B, Zawadski K, Villeneuve A (in prep), NBS-1-dependent and –independent roles of MRE-11 and RAD-50 ensure faithful chromosome inheritance during *C. elegans* meiosis

D. Additional Information: Research Support and/or Scholastic Performance

2011-2016 Young Scientist Fellowship (*Contrat Jeune Scientifique,* CJS) from the French National Institute for Agricultural Research (INRA) in Versailles, France (ran until 09/30/2016).