Yale University

26 October 2005

Dr. Yves Brun
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Dear Dr. Brun:

I am writing to apply for the position of Assistant Professor in your Department. My research focuses on homologous recombination and the maintenance of genome integrity. I am especially interested in the role of recombination in DNA damage repair and meiotic chromosome segregation.

As a graduate student with Dr. Hideyuki Ogawa (Osaka University, Japan), I worked on DNA damage repair in budding yeast. I showed that Mre11, a conserved protein involved in DNA damage repair, has two separable functions - initiating meiotic recombination and forming single-stranded DNA at double-strand break ends. The defect in the latter function can be suppressed by overproducing Exo1, a 5' to 3' DNA exonuclease.

As a postdoc with Dr. Shirleen Roeder (Yale University), I have studied the mechanisms of homologous chromosome pairing and recombination during meiosis in budding yeast. I identified and characterized a number of key proteins essential for double-strand break repair during meiosis. This work demonstrated a role for meiotic recombination in achieving accurate alignment of homologous chromosomes. I also discovered a novel mechanism to down-regulate the mitotic recombination machinery during meiosis when the meiotic recombination machinery is impaired. A newly identified meiotic protein, Hed1, serves this function by directly binding and inhibiting Rad51, a recombinase that plays a central role in mitotic recombination.

Using yeast genetics as a powerful tool, I plan to focus my future research on the following three aspects of homologous recombination. First, I will investigate double-strand break processing, which is essential for initiating homology searching and triggering the DNA damage checkpoint. Second, I will examine the mechanism responsible for the recognition of homology between DNA molecules, using the meiotic recombination machinery as a model. Third, I will examine the molecular mechanism of Rad51 inhibition by Hed1, thereby gaining insight into a novel mechanism to regulate homologous recombination.

I look forward to hearing from you. If you require additional information, please contact me.

Sincerely,

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Research Accomplishments:

Mechanisms and Regulation of Meiotic Recombination

Meiosis is a specialized cell cycle that produces haploid gametes from diploid parental cells, playing a central role in transmitting genetic material to the next generation. At meiosis I, homologous chromosomes segregate to the opposite poles while sisterchromatids remain associated. This meiosis-specific segregation pattern inevitably requires a mechanism to accurately recognize similarity between chromosomes so that homologous chromosomes align before separation.

Homologous recombination is a mechanism that detects similarity at the nucleotide level between two DNA molecules. Rad51 and Dmc1 are two orthologs of the bacterial RecA recombination protein. Unlike Rad51, which functions in both mitotic and meiotic cells, Dmc1 is produced only during meiosis.

By taking advantage of the powerful genetics available in budding yeast, together with microscopy and biochemistry, my research has focused on understanding the mechanism of meiotic recombination and the role of recombination in homologous chromosome paring and segregation.

(1) The Rad51-Only Pathway Operates in Parallel to the Dmc1-Dependent Pathway during Meiosis

I demonstrated that there are two parallel pathways of meiotic recombination that differ in terms of their dependence on Rad51 and Dmc1 [1]. The pathway that predominates in meiotic cells (the Dmc1-dependent pathway) requires both recombinases, as well as a number of meiosis-specific proteins (as described below). The secondary pathway requires Rad51, but not Dmc1, and is similar to the pathway that operates to repair DNA double-strand breaks in vegetative cells.

(2) Identification of Proteins that Act with Dmc1 in Meiotic Recombination

I characterized a number of proteins that act in the Dmc1-dependent pathway of meiotic recombination. I identified a novel protein complex (the Hop2/Mnd1 complex) that discriminates homologous from non-homologous sequences during meiotic recombination and thus plays a major role in homologous chromosome recognition and alignment [1, 2]. In addition, I found that two meiotic proteins, Mei5 and Sae3, work together with the meiosis-specific recombinase, Dmc1 [3].

(3) Discovery of Hed1, a Protein that inhibits Rad51 during Meiosis

I discovered a novel mechanism to down regulate the mitotic recombination machinery during meiosis when the meiotic recombination machinery is impaired [4]. A newly identified meiotic protein, Hed1, serves this function by directly binding and inhibiting Rad51, when Dmc1 is absent. Furthermore, since *HED1* gene expression in vegetative cells inhibits mitotic recombination, Hed1 may serve as a tool to repress recombination in any cell type (such as higher eukaryotic cell lines and transgenic mice).

References

1. Tsubouchi, H. & Roeder, G. S. The importance of genetic recombination for fidelity of chromosome pairing in meiosis. Dev. Cell 5, 915-25 (2003).
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3. Tsubouchi, H. & Roeder, G. S. The budding yeast Mei5 and Sae3 proteins act together with Dmc1 during meiotic recombination. Genetics 168, 1219-1230 (2004).
4. Tsubouchi, H., Sehorn, M. G., Sung, P. & Roeder, G. S. Budding yeast Hed1 is a meiosis-specific inhibitor of Rad51. Cell, submitted.

Hideo Tsubouchi

Future Research Plans:

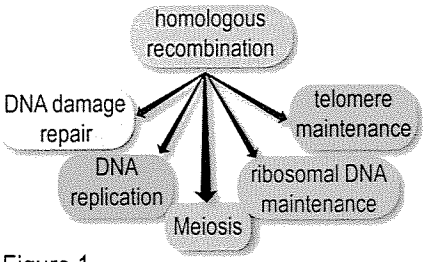

Figure 1
Homologous recombination:
the mechanism to maintain genome integrity

Homologous recombination is a mechanism capable of assessing sequence similarity between two DNA molecules and exchanging strands between homologous sequences [1]. This mechanism is widely used in various contexts to maintain genome integrity (Figure 1): DNA damage repair, replication and meiotic chromosome segregation, to name just a few. Although some variation exists, this process progresses through a series of well-defined steps (Figure 2). Double-strand breaks (DSBs) are established initiators of homologous recombination. Once a DSB is formed, one strand is digested to expose single-stranded DNA with a 3' end, which is used for homology searching. Once homology is found, strand exchange occurs between the invading single-stranded DNA and homologous duplex DNA. DNA is synthesized from the 3' end of the invading strand using the homologous DNA as a template. The newly synthesized strand is then annealed back to the other broken end, and the integrity of the duplex is restored.

My future research will focus on two critical steps in homologous recombination: DSB end processing and homology searching (checked in Figure 2). In addition, I will investigate the regulation of homologous recombination, by studying a novel meiotic protein (Hed1) that conditionally inhibits Rad51 during meiosis.

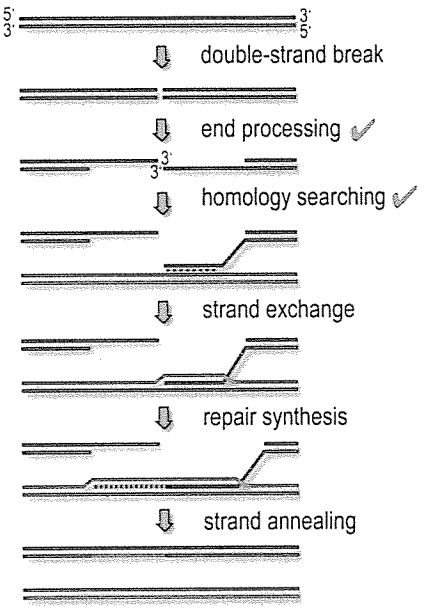

Figure 2
Steps in homologous recombination

(1) Molecular Mechanism for Processing DSB Ends

Forming single-stranded DNA at a DSB end is crucial for multiple purposes. First, single-stranded DNA is essential for recombinases to perform homology searching, without which cells have to rely on a less accurate, end-to-end joining mechanism to repair DSBs. Second, exposed single-stranded DNA is essential for activating the DNA damage checkpoint to arrest the cell cycle until DNA damage is repaired. Despite the significance of DSB processing, the molecular entity that catalyzes this process is still unknown.

Mre11-Rad50-Xrs2 (MRX), a conserved protein complex involved in DNA damage repair, is important in facilitating the digestion of DSB ends [2, 3]. However, it is unknown how this complex is involved in the process [4] (Figure 3) (although Mre11 has a nuclease consensus, the nuclease-negative mutant shows no defect in resecting DSB ends). I hypothesize that MRX is required for facilitating other exonuclease(s) to work on DSB ends. To understand the role of the MRX complex in facilitating processing of DSB ends, I will screen for mutants in MRX that show sensitivity to DNA damaging agents though the complex still localizes to DSBs. Localization will be done under the fluorescence microscope using Mre11-Green Fluorescent Protein. The sites of the mutations will be determined and the biochemical properties of the mutant proteins will be examined.

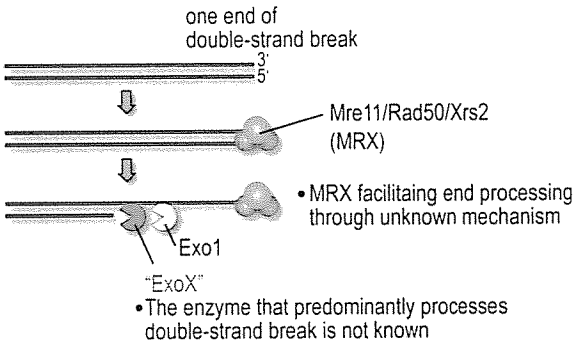

Figure 3
Double-strand break processing

The defect in processing DSBs in the *mre11* mutant can be suppressed by overproducing Exo1, a 5' to 3' DNA exonuclease [5]. However, the absence of Exo1 causes no (or very little) reduction in DSB processing. These results indicate that there is an yet unknown exonuclease involved in the process ("ExoX" in Figure 3). I will perform genetic

screening for a protein that, when overproduced, suppresses the DSB processing defect in the *mre11 exo1* double mutant. A mutation in a gene encoding such an exonuclease is predicted to show a synthetic defect in DSB processing with the *exo1* mutation. Thus, genetic screening will also be performed for mutants that exacerbate the DSB repair defect of the *exo1* mutant.

(2) Understanding the Molecular Mechanism of Homology Searching

The mechanism to assess similarity between two DNA molecules lies at the heart of homologous recombination. However, this molecular mechanism is largely unknown. Meiotic recombination plays an essential role in meiotic chromosome segregation by facilitating recognition between homologous chromosomes [6]. Dmc1 is a potent meiosis-specific homologous recombinase (i.e., bacterial RecA homolog). Dmc1 works in a recombination pathway that predominates in meiotic cells (the Dmc1-dependent pathway) [7]. Genetic and cytological analyses defined two steps necessary for Dmc1 to execute homology searching, one for Dmc1 loading onto single-stranded DNA, and the other for assessing similarity between two DNA molecules [7-9]. Each step shows a unique requirement for certain meiosis-specific proteins. By reconstituting the homology searching reaction *in vitro* with the meiotic recombination machinery as a model, I aim to understand the molecular mechanism underlying homology searching.

Presynaptic Filament Formation Factors: Mei5 and Sae3 act together with Dmc1. Any one of the three proteins is essential for the localization of the other two to meiotic chromosomes, suggesting that Dmc1, Mei5 and Sae3 act together to bind DNA substrates (most likely single-stranded DNAs created at DSB ends), forming a structure prerequisite for homology searching (presynaptic filament). However, this structure is not sufficient for homology searching.

Homology Searching/Assessment Factors: The absence of either Hop2 or Mnd1 results in the accumulation of Dmc1, Mei5 and Sae3 on chromosomes, along with unrepaired DSBs. Furthermore, chromosomes that are not homologous to each other are mistakenly aligned to each other, arguing that the essential role of the Hop2/Mnd1 complex is discriminating homologous from non-homologous sequences.

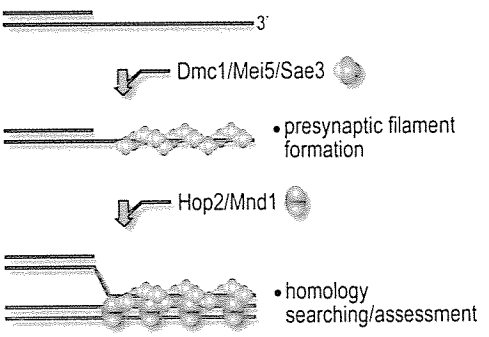

Figure 4
Two-step reconstitution *in vitro* of the homology searching reaction

Based on the above-mentioned working hypotheses, I will attempt to reconstitute *in vitro* the two steps in Dmc1-dependent homology searching (Figure 4). First, the single-stranded DNA-binding activity of the Dmc1-Mei5-Sae3 complex will be examined. Second, the Hop2/Mnd1 complex will be added to the reaction to see if it activates homology searching. Through these studies and analyses of the *in vitro* system, I hope to understand the molecular mechanism underlying homology searching.

(3) Mechanism to Regulate Meiotic Recombination by Hed1, a Meiotic Inhibitor of Rad51

I recently discovered that Hed1 provides a novel mechanism to down-regulate the mitotic recombination machinery during meiosis when the meiotic recombination machinery is impaired [10]. Hed1 serves this function by directly inhibiting Rad51, when Dmc1 is absent. This observation leads to the following key questions.

How does Hed1 inhibit Rad51?: Hed1 physically interacts with Rad51 as well as with Hed1 itself *in vitro*. To understand the significance of these protein-protein bindings, both *hed1* mutants deficient in interacting with Rad51 and *hed1* mutants deficient in Hed1 self-association were isolated. Characterization of these mutants indicates a link between the self-association of Hed1 and the inhibition of Rad51. I will test the biochemical properties of these *hed1* mutant proteins in self-association, Rad51-binding and Rad51-inhibition. At the same time, the phenotype of these *hed1* mutants *in vivo* will be analyzed.

How is Rad51 inhibited by Hed1?: Hed1 binds not only yeast but human Rad51, raising the possibility that Hed1 recognizes a common structure shared by RecA orthologs throughout species. I will screen for *rad51* mutants deficient in interacting with Hed1 to test this possibility. This analysis may also address which properties of Rad51 (e.g., ATP-hydrolysis) are compromised by Hed1 binding. At the same time, the known-biochemical properties of Rad51 (e.g.,DNA-binding, ATP-binding and hydrolysis activities) will be examined in the presence of Hed1.

How does Hed1 sense the meiotic recombination defect to decide to inhibit Rad51?: The mechanism by which Hed1 inhibits Rad51 specifically in the absence of Dmc1 is mysterious. To investigate this phenomenon, I will screen for *hed1* mutants that inhibit meiotic recombination in an otherwise wild-type background. The sites of the mutations will be determined and the biochemical properties of the mutant proteins will be examined.

Hed1 as a tool to regulate recombination artificially: An interesting property of Hed1 is its ability to down-regulate homologous recombination when expressed in vegetative cells. Together with the fact that Hed1 can bind human Rad51, this observation suggests that Hed1 may provide a way to regulate recombination artificially in any cell type, including higher eukaryotic cells and transgenic mice. Controlling the production of Hed1 under a conditional or tissue-specific promoter may provide a way to study the *in vivo* functions of recombination in higher eukaryotes.

Taken together, these analyses will lead to further understanding of the molecular mechanism for regulating recombination.

References

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10. Tsubouchi, H., Sehorn, M. G., Sung, P. & Roeder, G. S. Budding yeast Hed1 is a meiosis-specific inhibitor of Rad51. Cell, submitted.

Teaching Experience / Interests:

As a graduate student, I was a teaching assistant for a genetics laboratory course, in which I taught yeast genetics to undergraduate students. As a Junior Faculty member in Hideyuki Ogawa's lab at Osaka University, I trained four graduate students and one undergraduate student. Although this position was not officially an independent one, I was fully responsible for designing research projects and determining future directions. I conducted regular discussions with my students, organized journal clubs and lab meetings, and helped graduate students writing theses. All four of my graduate students successfully obtained Master's degrees. As a Faculty member, I taught a genetics laboratory course whose subjects included DNA replication, recombination and the cell cycle.

At the undergraduate level, I am interested in teaching general genetics. For advanced courses, I am interested in dealing with subjects related to genome dynamics, including DNA damage repair, replication, recombination, mutagenesis, transposition and cell cycle checkpoints. The best way to understand and teach biology is by doing experiments, so I am eager to train undergraduate students in my lab.