

Yale University

Department of Molecular Biophysics
and Biochemistry
School of Medicine
333 Cedar Street
P.O. Box 208024
New Haven, Connecticut 06520-8024

Campus address:
Sterling Hall of Medicine
333 Cedar Street

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Yves Brun
Systems Biology/Microbiology Faculty Search
Department of Biology
Indiana University
Jordan Hall 142
1001 E 3rd Street
Bloomington, IN 47405-7005

Dear Yves Brun:

I am very pleased to write this letter of recommendation to support Dr. Hideo Tsubouchi's application to your Department as tenure-track faculty. I have read all of Hideo's published studies with interest and admiration, and since my arrival at Yale in June of 2003, I have interacted with him on a regular basis. In addition, during the past year, Hideo has been working with Mike Sehorn, a postdoctoral fellow in my group, on the biochemical characterization of several factors that he has found to be indispensable for homologous recombination and DNA double-strand break repair in meiotic yeast cells. Since I know Hideo and his work well, I feel I am in a good position to provide an informed account of Hideo's potential as an independent faculty.

Hideo has superb research training and is among the most earnest and talented young scientists that I know. Hideo did his graduate work with Hideyuki Ogawa in the area of meiotic recombination, specifically focusing on the role of Mre11 and Exo1 proteins in meiotic recombination and DNA repair. The two first-authored papers that Hideo published with Hideyuki Ogawa in *MCB* and *MBC* enjoy frequent citations and are considered really important contributions by folks working in our field. Hideo did well in picking Shirleen Roeder, a well-recognized leader in recombination mechanism, as his postdoctoral mentor. In the Roeder laboratory, Hideo has carried out very detailed and highly original analyses of several meiotic recombination proteins, including Hop2, Mei5, and Sae3. Hideo's work with Shirleen has already resulted several high impact publications (in *Dev. Cell*, *Genetics*, and *MCB*) and is instrumental in establishing a functional relationship between the aforementioned factors and the meiotic DNA recombinase Dmc1. In collaboration with Mike Sehorn of my group, Hideo has found that Mei5 and Sae3 exist as a stable heterodimer and purified the protein complex to near homogeneity. The purified Mei5-Sae3 complex binds Dmc1 and the Dmc1-related recombinase Rad51 as well. I am confident that the ongoing work of Hideo will delineate the mechanistic role of the Mei5-Sae3 complex in meiotic recombination and DNA repair. In a screen for suppressors of the meiotic defects of the *dmc1* null mutant, Hideo has identified a protein called Hed1 that shows functional interactions with the Rad51

recombinase. Hideo has expressed Hed1 in bacteria, and in collaboration with Mike Sehorn, has purified it to near homogeneity. The Hed1 protein binds DNA and Rad51. Hideo and Mike have already put together a manuscript on the initial characterization of Hed1 and are busy further dissecting the role of Hed1 in Rad51- and Dmc1-mediated recombination reactions using the assay systems that are available in our laboratory. Overall, the studies of Hideo have already made a major contribution toward deciphering the intricacies of meiotic recombination, and his ongoing biochemical work with us promises to yield insights into the mechanistic role of several key factors in this process.

As independent investigator, Hideo will follow the leads that he has generated on the Mei5-Sae3 complex, the Mnd1-Hop2 complex, and the Hed 1 protein by applying a combination of genetics and biochemistry to define the roles of these factors in Rad51- and Dmc1-mediated reactions. In addition, he will investigate the influence of chromatin structure on meiotic recombination and continue his efforts in identifying new players in meiotic recombination. Given the importance of meiotic recombination in chromosome segregation and genome maintenance, these planned studies of Hideo have extraordinary significance. Having served on various NIH study sections that review applications in this area of research, I believe that Hideo's grant applications will have little trouble getting funded.

Hideo is an extremely personable, outgoing individual and has an excellent command of the English language, a relatively rare gift among scientists from Japan. What also impresses me about Hideo is the considerable foresight that he has. For instance, knowing that having a biochemical component will allow him to address problems at the mechanistic level, he has insisted on learning from us all the protein purification and biochemical techniques that are germane for his studies and has been doing a really admirable job at it. Hideo attends our laboratory meetings often, and it is always a pleasure to have him around, asking thoughtful questions and providing his insights about different research problems.

In conclusion, Hideo Tsubouchi has ample intellect, training, and commitment to excel in a competitive environment. Since I believe that Hideo will be a truly fabulous addition to your faculty, I wish to recommend him to you with the highest possible enthusiasm. In fact, if a similar position were to become available in any Department here at Yale, I would surely provide Hideo with my wholehearted support for such a position as well.

Sincerely,



Patrick Sung, D. Phil.

Professor, Molecular Biophysics & Biochemistry