HHMI
HOWARD HUGHES MEDICAL INSTITUTE

October 21, 2005

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

Dear Dr. Brun:

I am writing on behalf of Hideo Tsubouchi in support of his application for the position of Assistant Professor in the Department of Biology. I could not be more enthusiastic in my endorsement of Hideo.

As a postdoctoral fellow in my lab, Hideo's productivity has been truly remarkable. Over the years, I have been fortunate to have many wonderful postdocs in my lab at Yale, but Hideo clearly outshines them all. His accomplishments are quite extraordinary. Hideo's postdoctoral research has focused on the mechanisms and pathways of meiotic recombination using budding yeast as a model organism. He has made a number of significant findings, which have been published in three substantial research articles, with a fourth manuscript in preparation.

Hideo's first publication as a postdoc was published in Molecular and Cellular Biology in 2002. Previous work in my lab had shown that the Hop2 protein functions to prevent synaptonemal complex formation between nonhomologous chromosomes. Hideo used a clever genetic approach to identify the Mnd1 protein as a Hop2-interacting protein. He carried out an extensive characterization of the *mnd1* mutant, demonstrating that it is defective in (i) meiotic cell cycle progression, (ii) synaptonemal complex formation (as determined by immunofluorescence analysis of surface-spread chromosomes), (iii) homologous chromosome pairing (as assessed by fluorescent in situ hybridization), and (iv) meiotic double-strand break repair and recombination (as determined by a physical Southern blot assay). He presented evidence indicating that the Hop2 and Mnd1 proteins interact with each other. These proteins colocalize to foci on chromosomes, and their localization is mutually dependent. Furthermore, Hop2 and Mnd1 co-immunoprecipitate from meiotic cell extracts. Sequence analysis indicated that the Hop2/Mnd1 complex is conserved across species. Hideo's studies of the Hop2/Mnd1 complex have inspired molecular and biochemical studies of this complex in mammalian cells.

Shirleen Roeder, Ph.D.
Investigator

Yale University
Department of Molecular, Cellular & Developmental Biology
P.O. Box 208103
New Haven, Connecticut 06520-8103
203.432.3501 • Fax 203.432.3263 • shirleen.roeder@yale.edu

Hideo's second paper, published in Developmental Cell in 2003, provided insight into the mechanism by which the Hop2/Mnd1 complex ensures pairing and synapsis between homologous chromosomes. Through a series of elegant molecular and cytological experiments, driven by classical genetic reasoning, he convincingly showed that Hop2/Mnd1 acts downstream of the Dmc1 and Rad51 recombinases in the meiotic recombination pathway. Hideo thereby demonstrated an important role for the recombination machinery in promoting accurate homolog alignment. The importance of recombination in pairing, relative to recombination-independent mechanisms such as telomere clustering, had previously been difficult to assess. Hideo's data clearly demonstrate a vital role for recombination in promoting (not just stabilizing) interactions between homologous chromosomes. This work was featured in an article in Nature Reviews Genetics.

In a screen for multicopy suppressors of the *hop2* mutant defect, Hideo identified the *RAD51* gene. Subsequent studies revealed that Rad51 overproduction also suppresses the meiotic defects of *dmc1* mutant. Based on these observations, Hideo postulated the existence of two parallel pathways of meiotic recombination. One pathway, referred to as the Dmc1-dependent pathway, involves Dmc1, Rad51 and the Hop2/Mnd1 complex. The other pathway, designated the Rad51-only pathway, requires Rad51, but not Dmc1, Hop2 or Mnd1. The Dmc1-dependent pathway is the predominant pathway operating in wild-type cells. These results indicate, unexpectedly, that Rad51 is fully capable of promoting meiotic recombination, including those aspects of recombination that are unique to meiotic cells. This work was also described in Hideo's Developmental Cell paper.

In his third paper, published in Genetics in 2004, Hideo presented evidence that the Mei5 and Sae3 proteins act as accessory factors to the Dmc1 RecA-like protein. Although the *mei5* and *sae3* mutants had previously been shown to be defective in recombination, nothing was known about their functions at the molecular level, and it was not clear whether their effects on recombination were direct or indirect. Hideo showed that the *dmc1*, *mei5* and *sae3* mutants all have the same phenotype, in a multitude of assays. Furthermore, the Dmc1, Mei5 and Sae3 proteins act in the same pathway as determined through the analysis of double and triple mutants. Most important, Hideo showed that the Dmc1, Mei5 and Sae3 proteins colocalize on chromosomes, and their localization is mutually dependent. Despite the fact that Dmc1 is a RecA homolog, attempts (in many different labs) to demonstrate Dmc1-mediated strand exchange activity in vitro have failed. Hideo's observations raise the possibility that Mei5 and Sae3 are essential accessory factors to Dmc1 and suggest obvious biochemical experiments, which Hideo has initiated.

Hideo's most recent findings are by far the most exciting, and I predict they will have enormous impact on the field. He has identified and characterized a novel protein called Hed1. Several observations indicate that Hed1 serves as a meiosis-specific negative regulator of the Rad51 strand exchange protein. First, the absence of Hed1 allows meiotic double-strand breaks to be repaired efficiently in the *dmc1* and *hop2* mutants, and this repair depends on Rad51. Second, the Hed1 protein colocalizes with Rad51 to meiotic chromosomes, and Hed1 localization depends on Rad51. Third, production of Hed1 during vegetative growth results in a deficiency in double-strand break repair, by specifically inhibiting Rad51-dependent recombination events. Fourth, the purified Hed1 and Rad51 proteins associate strongly with each other. Finally, purified Hed1 protein inhibits the in vitro strand exchange reaction catalyzed by Rad51. Thus, the inefficiency of meiotic DSB repair in the *dmc1* mutant

can be explained by inhibition of Rad51 by the Hed1 protein. The existence of a meiosis-specific Rad51 inhibitor has never even been postulated, and there is no precedent in any other system. I therefore predict that Hideo's paper, which has been submitted to Cell, will be met with considerable interest and surprise. Because of the major role that Rad51 plays in mitotic recombination and DNA damage repair (in addition to its role in meiosis), I believe that this manuscript will be of interest to a very broad audience. Also, Hed1 will be a valuable tool in the molecular biologist's toolbox since it provides a means to inhibit recombination even in cells in which genes encoding recombination enzymes cannot easily be knocked out.

Hideo's path to progress has met with its share of obstacles and challenges, but he has shown remarkable perseverance and determination. For example, three of the genes he has studied, *MND1*, *SAE3* and *HED1*, were incorrectly annotated in the yeast genome database. Thus, it was necessary to sequence the gene, clone and sequence the corresponding cDNA, and identify intron/exon boundaries, before successful epitope tagging strategies could be devised for localizing the proteins. The purification of the Hed1 protein was a veritable tour-de-force and totally dependent on techniques with which neither Hideo nor anyone else in my lab had any prior experience. He spent many long hours in Patrick Sungs' lab at Yale, learning new techniques, and transferring that technology to my laboratory.

Hideo is truly a jack-of-all-trades. He moves seamlessly from modern genetic approaches, to sophisticated cytological methods, to classical biochemistry. He is willing and able to use whatever strategy or technique is most likely to shed light on the questions he seeks to answer. His technical competence is truly remarkable. His experiments work almost first time every time. His data are invariably of high quality.

Hideo has presented his research at a number of national and international meetings. When seminar speakers visit Yale and ask to meet with me, I usually arrange for Hideo to present his research. The response to these presentations (both public and private) is unanimous. Everyone is extremely impressed by the enormous amount of research Hideo has accomplished, by the novelty and importance of his findings, and by the clarity and thoughtfulness of his presentations. Based on these responses, I predict that Hideo will be very competitive in the job market this year, both in the US and abroad.

Hideo is without question the most valuable member of my research team, both in terms of research productivity and in terms of his intellectual contributions. Ever since he arrived in my lab, Hideo has functioned completely independently in all aspects of his research, from experimental design through the interpretation of results. He is a critical thinker and an unusually creative experimentalist. All of Hideo's experiments are designed to test specific hypotheses; they take clear and direct aim at a better understanding of molecular mechanism.

During his time in my lab, Hideo has supervised the research of two technicians (at different times), and he has been very helpful in advising the graduate students in my lab. He is extremely generous with his time and knowledge, as well as with his reagents and protocols. He quickly gains the respect and the cooperation of those who work with him. I predict that he will be a wonderful advisor and mentor to the graduate students and postdoctoral fellows in his own lab.

Hideo's postdoctoral research has established a firm groundwork for future studies in a number of areas. His studies of Hed1, Hop2/Mnd1 and Mei5/Sae3 have progressed to the point where a biochemical approach is feasible; he has already initiated such studies with the Hed1 protein. Thus, Hideo is in a position to dissect in considerable molecular detail the mechanisms of action of these proteins. In addition, Hideo has begun to study the Pch2 protein, which promises to shed important new insights into the role of chromatin structure in meiotic double-strand break formation. Furthermore, I am confident that Hideo will continue to identify new meiotic genes using clever genetic methods similar to those used to identify Mnd1 and Hed1. There is no question that Hideo has many exciting projects ready and waiting for his future graduate students and postdocs. As a new investigator, Hideo is certain to hit the ground running. He will be reaping the fruits of his postdoctoral research for many years to come.

Of the many outstanding graduate students and postdoctoral fellows I have been lucky to have in my lab, there is no one in whom I have greater confidence of success as an independent investigator. Indeed, to a large extent, Hideo has been functioning at this level from the moment he entered my lab. He arrived with clearly defined questions he wished to address. All I have done is to provide the space, supplies and freedom for him to bring his ideas to fruition. Hideo has the rare combination of intelligence, creativity, technical excellence, managerial abilities, interpersonal skills, enthusiasm, perseverance and self confidence necessary to succeed as a faculty member. Moreover, he is extremely hard working and unwavering in his commitment to a career as an independent investigator. I am certain that he will succeed. I think he has the potential to be a real superstar.

Sincerely,

Shirleen Roeder

Shirleen Roeder, Ph.D.
Investigator, Howard Hughes Medical Institute
Eugene Higgins Professor of Molecular, Cellular and Developmental Biology,
Yale University
Professor of Genetics, Yale University School of Medicine