

October 18, 2005



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Dear Dr. Brun:

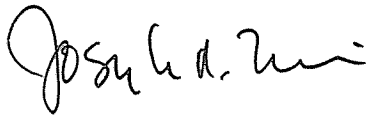
I write to support the application of **Dr. Marito Araki** for a position in your department. Let me say at the outset that I think Marito is an absolutely superb young scientist, one with a very impressive record of achievement, and one who I believe will develop a very productive independent research career. Marito is a postdoctoral fellow in the laboratory of Dr. Maki Asano, a close colleague of mine in the Department of Molecular Genetics and Microbiology. Indeed, I have had a considerable amount of contact with individuals in her lab and Robin Wharton's lab with whom Maki interacts closely, and this includes Marito. I thus have had the opportunity to become familiar with his science and to get to know him personally.

Although Maki and Robin can speak more clearly and specifically as to Marito's accomplishments, I can say that his work on Orc protein degradation is absolutely first-rate and represents a major accomplishment in the understanding of mechanisms that control cell cycle progression, not to mention the underlying specificity of protein degradation. His recent work, described in an EMBO paper and now the Genes & Development paper, has very clearly detailed the role of the APC complex in targeting Orc1 destruction as well as identifying a unique motif within Orc1 that confers the specificity of this regulated degradation. This involved a very careful set of experiments that established the experimental system, methodically detailed the temporal control of Orc1, and then identified APC as the mediator of the degradation. He then went on to carefully dissect Orc1 sequences responsible for the targeted degradation and firmly established the role of this motif in controlling Orc1 accumulation. These two papers are examples of science done extremely well and with a focus on addressing the big picture questions.

In addition to this success in his work here at Duke, I will also point out that Marito came with a very impressive track record from his PhD studies with Fumio Hanaoka in Osaka. This involved work focused on molecular mechanisms of nucleotide excision repair that resulted in a substantial collection of papers that characterized the components of the repair machinery. In my experience as Chair of a department engaged in faculty recruitment, I always placed high value on not only the most recent accomplishments of a candidate but also the track record that provided evidence of continued productivity and significant accomplishment. I believe this is a critically important indicator of future success – that is, an individual who has been successful through multiple steps in their career is likely to continue in this fashion. I haven't seen too many other examples as impressive as Marito in this regard.

Finally, let me say that from my personal knowledge of Marito, you couldn't look for a better person. He is an exceedingly warm and interactive person, one who is ready to discuss issues and to offer help where possible. His English is excellent and his presentations are clear and superb. Indeed, he presented his work that is described in the Genes & Development paper at our most recent department retreat just this last month and it was probably the best presentation at the retreat. Obviously, it helps to have terrific data which he has but he also did a superb job in presenting the work in a clear and concise manner. All of this goes as evidence for his ability to be an effective teacher and departmental colleague. He has my very highest enthusiasm and recommendation. I would urge you to invite him for a visit to look for yourself.

Sincerely yours,

A handwritten signature in black ink, appearing to read "Joseph R. Nevins". The signature is fluid and cursive, with the first name "Joseph" being the most prominent.

Joseph R. Nevins

JRN/th

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SOUTHWESTERN MEDICAL CENTER
AT DALLAS

Hongtao Yu, Ph.D.
Michael L. Rosenberg
Endowed Scholar in Medical Research

Department of Pharmacology

October 14, 2005

Yves Brun, Faculty Search
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Dear Dr. Brun:

I am writing to strongly support Dr. Marito Araki's application for an Assistant Professor position in your department. Marito received his B.S. degree from Tokyo University and his Ph.D. degree from Osaka University in Japan. His Ph.D. thesis research deals with the mechanism of a form of DNA repair in mammalian cells, called nucleotide excision repair (NER). Marito is currently a postdoctoral fellow in the lab of Robin Wharton at Duke University Medical Center. His postdoctoral research has been focused on the mechanism of DNA replication in the fruit fly, *Drosophila melanogaster*.

To maintain genetic stability, multiple mechanisms restrict DNA replication to once-and-only-once during each cell division cycle. One such mechanism is the cell-cycle regulated degradation of proteins necessary for the licensing and initiation of DNA replication. Marito showed that, in *Drosophila*, the degradation of ORC1 (the largest subunit of the origin recognition complex) requires the anaphase-promoting complex or cyclosome (APC/C), a large multi-subunit ubiquitin ligase. My lab investigates the structure, function, and regulation of APC/C. Among other things, we have reconstituted the ubiquitination of several APC/C substrates using immunopurified APC/C and other necessary ubiquitination reaction components. Zhanyun Tang in my lab then helped Marito to demonstrate that ORC1 efficiently ubiquitinated by APC/C in this *in vitro* assay. These results were published in Marito's **EMBO J** paper.

Soon after the paper was published, Marito contacted me and asked whether it would be possible for him to come to my lab and learn the APC/C assay. I agreed, and Marito stayed in my lab for about a month. During this period, Marito prepared all the ingredients for the APC/C assay himself and successfully learned how to do this assay. In fact, his assays were more beautiful than those done by some of my postdoctoral fellows who have much more experience with this. He then brought this assay back to Duke and used it to define the O-box, a novel cis-element within ORC1 that is required for APC/C ubiquitination *in vitro* and degradation *in vivo*. Using the consensus of the O-box, Marito searched the fly genome for more potential APC/C substrates. This allowed him to identify a novel APC/C substrate, Asp (abnormal spindle, a

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protein involved in spindle pole organization). These results have recently been published in **Genes Dev.**

Once he secures his independent position, Marito plans to continue his studies on APC/C-mediated protein degradation in *Drosophila*. Most of the studies on APC/C have been done in yeast, in *Xenopus* egg extracts, and in mammalian tissue culture cells. It has becoming increasingly clear that APC/C has important functions outside the cell cycle. For example, APC/C is active in terminally differentiated cells and is required to prevent axonal growth in neurons. APC/C is also involved in the degradation of proteins in the TGF β pathway. Thus, it is absolutely essential to study these types of APC/C functions in a multicellular organism that is genetically tractable. This is exactly what Marito intends to do. His proposed studies on the functions of APC/C in *Drosophila* will significantly advance our understanding of this important protein machine. In fact, he has already had a very promising start. In his unpublished work, Marito has identified bicoid, a homeobox transcription factor essential for embryonic patterning, as an APC/C substrate. This implicates APC/C in the development of early fly embryos. Marito's expertise in both fly genetics and biochemistry will serve him well in his future studies.

I got to know Marito well when he came to Dallas to do experiments in my lab. We also met each other at scientific meetings. Marito struck me as a very intelligent, mature, and meticulous scientist. Marito has a warm and outgoing personality. For a person educated in Japan, he speaks and writes well in English. While here in Dallas, Marito attended our departmental seminars and actively participated in the discussions following the lectures. He asked many intelligent questions and left a strong impression with my colleagues during his short stay. Many of colleagues had mistakenly thought that Marito was a new regular member of my lab and congratulated me for having landed such a talented postdoctoral fellow!

As a collaborator and a friend, I believe that Marito will be able to set up and run a successful independent research program. He is uniquely positioned to make important contributions to our understanding of regulated protein degradation in cell division and development of multicellular organisms. I support his application enthusiastically.

Sincerely,



Hongtao Yu

Associate Professor of Pharmacology