THE OHIO STATE UNIVERSITY

Department of Microbiology

484 West 12th Avenue
Columbus, OH 43210-1292

Phone 614-292-2301
Fax 614-292-8120

Dr. Yves Brun,
Systems Biology/Microbiology Faculty Search,
Department of Biology,
Indiana University, Jordan Hall 142,
Bloomington, IN 47405-7005

October 14th 2005

Dear Dr. Brun,

Dr. Arina K. OMER

Arina Omer is an exceptional scientist, and already an experienced university teacher. Since completing her Ph.D. in 2002, Arina has, in essence, been functioning as a faculty member at UBC, both continuing investigative research and teaching. She is an outstanding candidate for an Assistant Professor position with combination of documented excellence in research in microbial molecular and evolutionary biology, teaching and international experience.

As a graduate student working with Pat Dennis, she made spectacular progress in being the first to detect, and then in impressively characterizing several families of non-coding RNAs in *Sulfolobus solfataricus*. This was seminal and ground-breaking research that elevated her quickly to a leadership position in the world of archaeal molecular and cell biology. Since Pat Dennis left UBC, she has continued to build on these successes and although she has certainly received some long-distance professional guidance from him, essentially all of their recent research progress is really her progress. The international research community is very well aware of her excellence as emphatically underlined by her recent invitations to give presentations at the 2005 Archaeal Gordon Conference (Oxford, UK) and 2005 IUMS-International Congress of Bacteriology and Applied Microbiology (San Francisco, USA), and to contribute a review chapter to the December 2005 edition of Current Opinions in Microbiology. I have had several opportunities to hear her speak, and to discuss research with her. She is a very polished and effective lecturer with an enthusiasm for science and depth of knowledge that is exciting and impressive. She will be an excellent classroom lecturer and she will bring excitement and enthusiasm to your graduate student program. She will also be an excellent graduate student-recruiter.

I hope these comments are adequate for your needs, but please do give me a call if I can add or amplify on any issues (1-614-292-2301). There is no doubt that Arina Omer will be a very productive research scholar, faculty member and professional colleague, and I recommend her to you very highly.

Yours sincerely,

John N. Reeve, Ph.D.
Rod Sharp Professor,
Chair, Department of Microbiology

Patrick P. Dennis
Professor Emeritus
Department of Biochemistry and Molecular Biology
University of British Columbia
Vancouver, British Columbia
Canada
V6T 1Z3

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

October 18, 2005

Re: Arina Omer

Dear Committee,

I would like to recommend my former graduate student, Arina Omer for a faculty position (Assistant Professor) in your department. Arina began in my laboratory in the fall of 1996 as an immigrant from Romania (via France) with a strong undergraduate scientific background and training but with only a rudimentary understanding of oral and written English. Her language skills developed very rapidly and she immediately focused on a challenging and "unsafe" project for her thesis research (see below). In order to supplement her graduate student stipend and to obtain additional teaching experience, Arina applied for and was accepted as a teaching assistant in the undergraduate Biochemistry 301 laboratory course. She preformed this task in a careful, thoughtful and responsible manner. In doing so she attracted several third year students in the course into my laboratory to do summer and honours research. I have been impressed by her ability to relate to, communicate with and provide supervision for these students. She is a bright, and talented experimentalist and in addition is able to efficiently communicate with and motivate students under her direction.

Since September 2003, Arina has held the full time position of Instructor in the Department of Biochemistry and Molecular Biology at the University of British Columbia and has been responsible for teaching the third year Biochemistry Laboratory Courses. It is my understanding that she has totally revitalized this course by introducing new methods and has done an outstanding job in supervising the graduate student teaching assistants and engaging the undergraduate students.

Her research as a PhD student was outstanding. At the time she started in my laboratory, it was known that the eukaryotic protein, fibrillarin, was essential for viability in yeast and associated

with a family of small nucleolar RNAs (snoRNAs) that only recently had been shown to target 2'-O- methyl ribose modifications to specific locations in small and large subunit ribosomal RNAs. The targeting mechanism uses a guide sequence within the snoRNA that is complementary to the target region in rRNA. At the time, two facts were available to us -- that archaeal genomes contain a homologue of the eukaryotic protein fibrillarin and that the ribosomal RNA of one hyperthermophilic archaeal species (*Sulfolobus acidocaldarius*) has a high content of 2'-O- methyl ribose modifications in its small and large subunit ribosomal RNAs. Arina set out to determine if Archaea possess methylation guide ribonucleoprotein (RNP) complexes.

To achieve this objective Arina cloned and sequenced the genes for fibrillarin and a second protein implicated in this process (Nop56) from *Sulfolobus*. She then expressed the genes in *E. coli*, purified the two proteins to homogeneity and used the purified proteins to generate polyclonal antibodies. The antibodies were highly specific and reacted with proteins of the expected size, present in *Sulfolobus* cell extracts. Arina reasoned that if these proteins associated with small RNAs, she might be able to generate a cDNA library from the RNAs that co-immunoprecipitate with the proteins. Initial attempts to construct the library using crude cell extracts failed. Arina guessed correctly that the abundance of the small RNAs was low and that an enrichment of the complexes would be required. Without knowing anything about the nature of the RNP complexes she chose to use glycerol gradient centrifugation. By using western blot analysis she was able to monitor the sedimentation of the two proteins; using fractions enriched for the two proteins, she was able to recover a collection of very low abundance small RNAs (sRNAs), 50 to 65 nucleotides in length. These were used to construct the cDNA library. When Arina began to sequence entries, she immediately discovered small RNAs that had features characteristic of eukaryotic C/D box methylation guide RNAs. She demonstrated by primer extension that the RNAs are present in cell extracts and confirmed that several of the predicted sites of 2'-O- methyl ribose modification within rRNA are indeed modified.

At the time a partial genome sequence of another species of *Sulfolobus* (*S. solfataricus*) became available. Arina performed BLASTN analysis on the sequence to determine it she could recognize homologues between the two species. Although there were a few low probability hits that turned out to be correct, it became clear that these sRNAs were not particularly well conserved between the two organisms. At this time we were contacted by Todd Lowe, then a graduate student with Sean Eddy at Washington University in St. Louis. Todd had designed a snoRNA search program that was highly successful in identifying C/D box snoRNA genes in the sequenced yeast genome. When applied to the archaeal genomes available at the time, the original program failed to find snoRNA-like sequences. Using Arina's cloned sRNAs, Todd retrained the search program to recognize archaeal features and using the new version we discovered more than 250 sRNA genes in sequenced archaeal genomes. This collection establishes archaeal sRNAs as the evolutionary ancestors of eukaryotic C/D box snoRNAs, illustrates the importance of sRNAs as chaperones to guide and stabilize the folding of nascent rRNA during ribosome assembly, and has given us a very clear picture of the structure and evolution of these sRNAs in Archaea.

Work to this point alone would have been sufficient for a very high quality PhD thesis, but Arina was not satisfied; she wanted to know more about the structural composition and function of

these archaeal RNP machines that are the ancestors of eukaryotic methylation guide snoRNAs. Despite numerous efforts, all previous attempts by other investigators to purify and characterize eukaryotic C/D box methylation guide complexes had been and continue to be unsuccessful. Similarly, Arina's earlier experiments had produced the puzzling result that neither purified fibrillarin nor Nop56 were able to bind to an sRNA transcript in spite of the fact that they coimmunoprecipitate as a complex. To solve this conundrum, she set about purifying the particles from *Sulfolobus* cell extracts and identifying all of the components. Highly purified complexes were shown to contain the sRNA, the proteins fibrillarin and Nop56, and a new low molecular weight protein that had been previously annotated as an archaeal ribosomal protein (designated aL7a). This protein turned out to be the key. From N terminal amino acid sequence she cloned and expressed this gene and purified the protein. Next she asked if it was possible to reassemble the RNP complex *in vitro* from purified recombinant proteins and an *in vitro* transcript of an sRNA. Very quickly she found that the aL7a protein binds with high affinity to the C/D motifs in the sR1 sRNA transcript. Moreover, the aL7a protein nucleates the addition of Nop56 and fibrillarin to the complex. At about this time, it became clear that aL7a is a member of a larger family of RNA binding proteins that includes the 15.5kD protein from humans. This human protein had been shown to be a component of the U4snRNP involved in intron splicing and to bind to a RNA motif termed the kink turn. The C/D (or C'/D') box motifs of snoRNAs can fold into this motif and the 15.5 kD protein was proposed to be a component of the eukaryotic C/D box snoRNP as well as snRNP splicosomal complexes.

After having achieved assembly of the RNP complex, the next logical step was to determine if the complex had *in vitro* methylation guide activity. Arina assembled a complex in the presence of a fragment of rRNA that was complementary to the D box guide of the sR1 sRNA and added radio-labeled S-adenosyl methionine (SAM) to the mixture. Radioactivity was rapidly incorporated into the acid insoluble material and shown to require the presence of guide and target RNAs and all three proteins. By site specific mutagenesis, Arina demonstrated that a Watson-Crick base pair between the guide and target at the site of modification was essential for activity. She next constructed two amino acid replacement mutants in the putative SAM binding motif of fibrillarin and showed that the mutant proteins were still able to assemble into RNP complexes but were defective in the targeted methylation reaction. In a final experiment Arina demonstrated by nuclease mapping that methylation was directed to the predicted position (equivalent to U52 in small subunit rRNA) in the target RNA. This work represents the first demonstration of guide directed, *in vitro* methylation and represents the best evidence to date that the fibrillarin protein possesses the methylase activity.

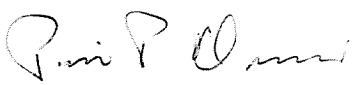
I was on academic leave to the National Science Foundation from September 2000 until December 2002 and resigned my University position in order to stay on as a permanent Program Director at NSF. In my absence Arina has had the full responsibility of running my laboratory on a day to day basis and guiding the activities of a technician, two other graduate students and several undergraduate students in addition to her own work. She has done an outstanding job in this capacity and in so doing has demonstrated the organizational and creative ability to direct her own independent research group. The major part of her work is described above and is in her thesis (defended in January, 2002). Moreover, she has initiated new projects since that time that provide a solid foundation for an independent faculty research program. She is already well on her way to defining the minimal RNA required for complex formation and the nature of the

RNA-protein and protein-protein interactions within the complex. She has established a collaboration with a structural biology group to crystallize RNA-protein complexes and determine their structures. Arina has taken over the full supervision of my one remaining graduate student, Maria Zago. Arina and Maria recently constructed a new cDNA library using immunoprecipitation with antibodies against the L7Ae protein. This library has revealed presence of a very large collection of small non C/D box RNAs in *Sulfolobus* that have very intriguing properties. This system can serve as an excellent model for probing the numerous, complex and critical roles that small RNAs play in many important biological processes. As a faculty member, I am sure that Arina will continue to build on the tools, resources and technology that she has developed over the last few year.

Arina has established her reputation within the international community; she is already well respected and has been invited to present her work at national and international meetings. In the last eight years (since joining my laboratory), she has mastered all of the important essentials of the English language and consistently gives a clear and exciting presentation of her work.

In summary, Arina has been a marvelous student and colleague. From her experiences and reputation as a graduate teaching assistant and Instructor in the department, I know that she will be an excellent teacher at both the undergraduate and graduate levels. She has the ability to relate to students under her direction and to motivate them to become interested and involved in science. She is an excellent teacher. As part of her PhD thesis research, she has solved not one but two "hard" problems and has mastered a wide variety of experimental techniques. She has never been afraid to ask difficult questions and to design and implement complex experiments even when common sense would argue that "it will never work". She has demonstrated extraordinary diligence, imagination and insight. She reads widely and assimilates diverse information which she can then direct to the challenges that she encounters. She has been invited to give oral presentations of her work at an annual meeting of the Canadian Institute for Advanced Research -- Program in Evolutionary Biology (2000), at the RNA Society Meeting (2001), and at a Gordon Conference on Archaea (2001, 2003 and 2005). All of her presentations have been superb and received with great enthusiasm by the scientific audiences. There is no question in my mind that Arina is fully deserving of a position and fully capable of fulfilling the associated academic responsibilities. I believe that she is outstanding and extremely well qualified for an independent faculty position.

Sincerely yours,



Patrick P. Dennis
Professor Emeritus
Department of Biochemnistry and Molecular Biology
University of British Columbia

Present address:
Program Director for Microbial Genetics
Division of Molecular and Cellular Biosciences

National Science Foundation
4201 Wilson Blvd.
Arlington, VA 22230
Phone: 703 292 7145; Fax: 703 292 9061
Email: pdennis@nsf.gov

P.S. Please note that I write this letter of reference as a former mentor and now a collaborator of the Arina Omer at the University of British Columbia and not in my capacity as a Program Director at the National Science Foundation.