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Yves Brun
Systems Biology/Microbiology Faculty Search
Department of Biology
Indiana University
Jordan Hall 142
1001 E 3rd St
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Re: Zefeng Wang, Ph.D.

Dear members of the search committee:

I am very pleased to recommend Zefeng Wang for a position in your department. Zefeng was an absolutely outstanding graduate student and he ranks among the very best that we see here at Johns Hopkins. In this letter I will focus mainly on his work in my laboratory

Zefeng received his undergraduate training in Beijing where he received both B.S. and M.S. degrees from Tshingua University. He had a lot of research experience in biophysical chemistry, and he published several papers while in China. He came to my lab in 1998 and his progress was spectacular. Our lab studies the molecular biology of parasitic protozoa with our main focus on African trypanosomes. When another lab reported that RNA interference works in trypanosomes, Zefeng decided to investigate this process and its utility in trypanosome gene silencing. He developed a vector that expressed double-stranded RNA in a stem loop form after induction with tetracycline. He then used this vector to study the function of a mitochondrial topoisomerase II that we thought was involved in the replication of kinetoplast DNA (kDNA), the trypanosome mitochondrial DNA. kDNA is a network containing several thousand small DNA circles, known as minicircles, which are topologically interlocked. We had previously found that minicircles do not replicate when attached to the network, but instead are released from it (by a topoisomerase) so that they can replicate as free minicircles. The progeny reattach to the network periphery, a reaction also mediated by a topoisomerase. When Zefeng used RNAi to silence the gene for a mitochondrial topo II, he found, surprisingly, that over the course of a week the kDNA network gradually shrunk in size and then disappeared, followed by cell death. In investigating the mechanism for kDNA loss, he found that progeny free minicircle replication intermediates accumulate, indicating that they cannot be reattached to the network. The fact that RNAi does not affect release of minicircles, but does inhibit reattachment, explains why the kDNA network shrinks and then disappears. Based on these and other experiments, Zefeng concluded that the function of this topo II must be to reattach progeny minicircles to the network. He published this work in EMBO Journal.

Zefeng then followed up this work with another project that is equally interesting. The kDNA network contains several thousand minicircles each of which encodes guide RNAs essential for RNA editing. His goal in this study was to identify factors controlling the size of the network. He decided to shrink the network, in a relatively controlled manner, by RNAi silencing of the topo II, to see what would be the minimum size compatible with viability. He found that he could conduct RNAi for two days and then clone out a cell line with considerably smaller networks. What he found is that despite the clonality the

networks in the population were heterogeneous in size. To explain this he found that when the replicated networks undergo division they divide asymmetrically, to form one network near normal size and a very small sister network. The cells with the larger network survive and those with the small network are sacrificed. Zefeng's manuscript describing these and other experiments was also published in EMBO Journal.

During the course of these studies, Zefeng turned to a new venture. He collaborated with Mark Drew (a graduate student in our lab) and Jim Morris (a post-doc), to develop new methods for applying RNAi to trypanosomes. The three developed a powerful new vector, pZJM, for RNAi. In this vector a ~500 bp segment of a gene is inserted between opposing T7 promoters each regulated by a tetracycline operator. Transfection of this vector into trypanosomes allows integration by homologous recombination into the non-transcribed rDNA spacer region. After selection for stable transformants, dsRNA synthesis can be induced by addition of tetracycline. The dsRNA quickly causes silencing of the gene of interest. This vector has been extremely useful for research in our lab and in many other labs (we have sent it to well over 50 other scientists, and many have reported great success with it). Therefore, this work has had a major impact on the field. Using pZJM one can test the function of a gene in as little as a month. It is much easier to use than the stem-loop vector that Zefeng used for his topo II experiments. The description of this vector was published in JBC with Zefeng, Mark, and Jim as co-first authors.

Since this work, Zefeng, Mark, and Jim continued to collaborate and together they developed RNAi-based genomic libraries. They have inserted random fragments of genomic DNA into pZJM and transfected the library into trypanosomes. Genomic DNA can be used because trypanosome genes generally do not have introns. After induction of RNAi, they can select or screen for a phenotype of interest. After cloning the cells of interest, they can identify the relevant gene by PCR. They have so far used this library to identify genes that are responsible for sensitivity to the lectin concanavalin A and for sensitivity to tubercidin (a toxic adenosine analog). These are the first example of a forward genetic approach in trypanosomes. One paper, with Zefeng, Mark, and Jim as co-first authors, was published in EMBO Journal, and a second, with the same co-first authors, was published in JBC.

Zefeng was a truly outstanding graduate student. He is very smart and unusually creative. He is unafraid to tackle the most difficult experiments and he has a knack for getting them to work. He is full of enthusiasm for science, and he has been unusually productive. He has excellent personal qualities and he was a close friend of everyone in the lab. He speaks English very well and he gives good talks. His writing skills are way above average for a Chinese student.

He should be a very top candidate for a faculty position.

Sincerely,

Paul T. Englund

Paul T. Englund
Professor of Biological Chemistry