JOHNS HOPKINS
UNIVERSITY

Department of Neuroscience
School of Medicine / 725 North Wolfe Street
Baltimore MD 21205-2185

October 3, 2005

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

Re: Dr. Yingbin Fu

Dear Dr. Brun,

I am writing this letter to offer my strongest support for the application by Dr. Yingbin Fu for the faculty opening in your department.

To put it simply, Yingbin is an excellent young scientist and an extremely nice person. He graduated at age 20 from Beijing University, the top university in China, and was very productive in his Ph.D. work on plant biochemistry at Michigan State University. He joined my lab immediately after his degree. My laboratory at the time was working largely on the physiology of visual transduction in the retina, but, with Yingbin's training in biochemistry, I decided to try something different. A colleague at Hopkins had just identified in the retina two new small G proteins, Rin and Rit, which are homologous to Ras and, unusual for this family of proteins, have a Ca-calmodulin-binding site. Because my lab was doing some work on calmodulin at the time, I suggested to Yingbin to figure out what these proteins do. We quickly found that Rin and Rit could interact with MEKK1 (Mitogen-activated protein kinase (MAPK) kinase kinase) through a yeast-two-hybrid assay, but the next step of the problem turned out to be much more difficult (not unusual for the yeast-two-hybrid approach!). Despite Yingbin's hard work and persistence using a variety of methods such as co-immunoprecipitation and neurite outgrowth assays on PC12 cells transfected with active and dominant-negative forms of Rin and Rit, we were unable to obtain conclusive evidence for their functional role in the MEKK1-mediated signaling pathway. Despite the road block, it gave me a chance to observe closely how Yingbin worked, and his attitude impressed me. In addition to his hard work and persistence, he was optimistic and cheerful throughout. He is truly a delightful person to be around.

I decided next to return to familiar territory. Yingbin had also absorbed a lot of what the rest of the laboratory was doing in sensory transduction. One major scientific question in vision

at the time was the cellular mechanisms underlying the much lower sensitivity and faster response kinetics of retinal cones compared to rods. One popular belief was that these rod-cone functional differences resulted from the fact that the active conformation of cone pigment has a lifetime only a tenth that of the rod pigment (rhodopsin). To address this fundamental question, Yingbin proposed to express cone pigment in rods by making transgenic animals, so that the rod and cone pigments could be compared side-by-side in the same photoreceptor. Besides transgenic mice, Yingbin also rallied for the transgenic-frog model because transgenic frogs could be generated relatively quickly. I took his suggestion, which turned out to be a great idea. With help from Nick Marsh-Armstrong, an expert in transgenic-frog technology at Hopkins, Yingbin produced animals that expressed red cone pigment from human or salamander. In collaboration with Vladimir Kefalov, a postdoctoral fellow in the lab who is an electrophysiologist, Yingbin showed that, surprisingly, rod and cone pigments signal downstream identically, thus overturning the previously held idea by others based on the short lifetime of the active conformation of cone pigment. Instead, Yingbin's work suggests that any difference between rod and cone transductions must lie in steps downstream from the pigment. In addition to this fundamental finding, Yingbin also showed that, at least in amphibians such as Xenopus and salamander, red cone pigment has a much higher rate of spontaneous (thermal) isomerization than rod pigment. This thermal isomerization in darkness is equivalent to the presence of real light, thus triggering light-adaptation mechanisms in cones and engendering lower sensitivity and faster response kinetics even in darkness. The success of this work, which is considered a classic in the field, has owed much to Yingbin's insight and persistence. It appeared in Nature two years ago with Yingbin as a co-first author.

A parallel piece of work that Yingbin has completed is the generation and study of transgenic mice that expressed red cone pigment in their rods. One obvious advantage of these mice is that, by breeding them to the rhodopsin-knockout line, we were able to obtain mice in which the rhodopsin in rods was completely replaced by cone pigment, making some of the data analysis a lot simpler. These experiments have confirmed the conclusion from the transgenic-frog experiments that rod and cone pigments signal identically in a given cell environment. Moreover, the mouse experiments provided another important insight about the role of retinaldehyde (the chromophore) in the pigment. Unlike Xenopus and salamander, which use vitamin A2 (11-cis dehydroretinaldehyde) as chromophore for their pigments, mouse (like human) use vitamin A1 (11-cis retinaldehyde) as chromophore. Yingbin has found that, whereas A2-cone pigment isomerizes spontaneously at a high rate, A1-cone pigment is much more quiet, and does not constitute a major factor in the low sensitivity of cones. Thus, depends on whether A1 or A2 chromophore is used as chromophore (the typical dividing line being land-based versus aquatic/amphibian species), the thermal isomerization of cone pigment may or may not contribute to the low cone sensitivity. This work has been written up with Yingbin as co-first author, and will be submitted to Neuron soon.

A third project by Yingbin, also attributed entirely to his originality, was on a newly discovered retinal photoreceptor – the melanopsin-expressing, intrinsically photosensitive retinal ganglion cells – a photoreceptor that has caught the fancy of many scientists these days. Unlike rods and cones, which mediate acute, image-forming vision, these unique ganglion cells mediate non-image-forming visual functions such as circadian photoentrainment and the pupillary light reflex. Melanopsin is an opsin-like protein that we have demonstrated, based on a gene-

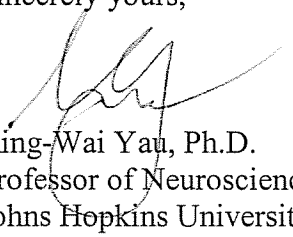
knockout approach, to be absolutely required for these unusual cells to be photosensitive. However, there was disagreement about whether melanopsin was indeed the photopigment of these cells or merely a photoisomerase required for the regeneration of 11-cis retinaldehyde required for the function of a still-unidentified pigment. Yingbin came up with the idea of using a mouse line (RPE65 knockout mouse) in which the supply of 11-cis retinaldehyde in the retina is extremely low owing to a defect in its regeneration (so that all of the chromophore is bound up in the ester form). The rod sensitivity of these animals is down by many orders of magnitude compared to normal. By using the pupil reflex as an assay for the function of the intrinsically photosensitive retinal ganglion cells, Yingbin found that the photosensitivity of these ganglion cells also went down dramatically in RPE65 -/- mice. Nonetheless, this defect in the RPE65-/- ganglion cells could be rescued by an intraperitoneal injection of exogenous 11-cis or 9-cis retinaldehyde into the animal. In parallel, in vitro electrophysiological recordings from the melanopsin-expressing ganglion cells of RPE65 -/- animals also indicated a substantial decrease in photosensitivity, which could be rescued by superfusing the cells with a 9-cis-retinal-containing solution. In contrast, the complete loss of photosensitivity of melanopsin-knockout ganglion cells was unaffected by 9-cis retinal injection, as assayed by the pupil reflex or single-cell recordings. These results immediately suggest that melanopsin is indeed the pigment, and not just a photoisomerase. Interestingly, Yingbin found that the low photosensitivity of the pupil reflex in RPE65 -/- mice was also rescued by the injection of all-trans retinal. This fascinating result indicates that melanopsin in fact embodies the properties of both a pigment and a photoisomerase (i.e., it is a bistable pigment, which so far has only been found in invertebrates). Although independent experiments by others based on heterologous expression of melanopsin in cell lines also led to the same conclusion at about the same time, Yingbin's experiments were more elegant because it was an in vivo study. The work recently appeared in Proc. Natl. Acad. Sci. with Yingbin as the first author.

Finally, Yingbin has been centrally involved in a collaborative project between our lab and Janis Lem's at Tufts University School of Medicine, examining the role of transducin $\beta\gamma$ subunits in phototransduction. Yingbin has found exciting evidence for a role of these subunits in the termination of the light response, a property hitherto unexplored in the field. This work will have implications about trimeric G protein signaling that goes beyond vision. The work is largely complete, and shall be targeted to Neuron with Yingbin as co-first author.

In the past several years, Yingbin has truly blossomed into an excellent independent scientist. He is widely read, has very good original ideas, shows good scientific judgment, possesses a keen sense of curiosity, and is highly observant as an experimentalist. It is always a pleasure to discuss science with him. On top of these fine scientific qualities, Yingbin is also one of the nicest individuals one could come across. He is mature, wise, caring and helpful. Above all, he is sociable and has a great sense of humor. He always strives to reconcile, making him a wonderful figure in the laboratory. He is also an excellent teacher: patient and generous in sharing his knowledge and expertise. For more than a year now, he has been supervising an undergraduate round the year, and they have an excellent working relation. Finally, Yingbin has very good communication skills. He writes well and, despite some native accent, gives clear and effective oral presentations.

In summary, I think Yingbin will make a fine independent investigator. His broad expertise in biochemistry, molecular biology and cell biology – and even some working knowledge in physiology – makes him pretty all-round. His fine personality and sunny demeanor will also endear him to colleagues and students alike. I support his application with the highest enthusiasm.

Sincerely yours,



King-Wai Yau, Ph.D.
Professor of Neuroscience
Johns Hopkins University School of Medicine