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20 September, 2005

To whom it may concern,

I am pleased to offer my very strongest support of Jian-Qiu Wu for a faculty position in your Department. I am the Welsh Distinguished Term Professor of Biology at UNC-Chapel Hill and was on Jian-Qiu's thesis committee. I thus followed his progress closely, having both read his first papers and having had periodic updates at committee meetings. I have also kept in touch since he joined the Pollard lab. Jian-Qiu is a terrific scientist, and has a great future ahead of him. . I am confident he will be one of the next generation of leaders at the interface between cell and developmental biology.

Jian-Qiu did his Ph.D. in the lab of John Pringle. John is one of the leaders in the field of cell polarity. However, John is not a hands-on mentor, and as a result, students in his lab have to be both very independent and also very persistent to be successful. Jian-Qiu has both of these quantities in abundance. Upon entering John's lab, he designed his own first project. He undertook to search the then existing version of the *S. pombe* database, looking for homologs of actin-associated proteins. He discovered a homolog of alpha-actinin,, which is absent in budding yeast, and decided to learn about its function in *S. pombe*. He designed and carried out a knockout, and found that this mutant had a surprisingly mild phenotype. Being both persistent and intelligent, he developed a hypothesis that another protein was partially redundant with alpha-actinin. While no obvious sequence paralog existed in *S. pombe* , he noted that there was a gene related to fimbrin, which from its cell biological properties had functional similarities to alpha-actinin. He thus knocked out the fimbrin gene, and noted that this also had only mild effects. However, when he made the double-mutant, he saw a dramatic effect on the actin cytoskeleton. I was exceptionally impressed by his work. It was technically outstanding and the data generated was of the highest quality, as expected and required from John's lab. However, most impressive was how he proceeded through the thought process as would an excellent post-doc, thinking through at each step what the next necessary task was, and how the data fit into the big picture. This work was published in the Molecular Biology of the Cell, in a very nice paper. I would note that Jian-Qiu had the draft of this paper done 15 months before submission, but it was delayed by having to go through the Pringle paper mill, the wheels of which often grind quite slowly. He also co-first authored a quite important methods paper describing vectors he and a post-doc developed which have proven very useful to *S. pombe* workers, he co-authored a review from the Pringle lab, and he has several publications from his masters work in China.

Jian-Qiu subsequently pursued a difficult but quite interesting problem. Previous work in John's lab revealed that the septins, which play essential roles in cell division in organisms as diverse as budding yeast and *Drosophila*, do not appear to be essential for cytokinesis in *S. pombe*. Jian-Qiu has focused on what alternate mechanism *S. pombe* uses to allow it to cytokinese in the absence of septin function. In doing so he characterized an additional (presumably the last) septin, showing that it plays a role in sporulation. More importantly, he designed and implemented a synthetic lethal screen to identify putative genes that act together with septins. He has identified a number of such genes, and is characterizing them using genetic and cell biological approaches. It is likely these will encode proteins that may well function in other organisms in cytokinesis, cell polarity and other septin functions. This work will be incorporated into two additional first-author or co-first author publications. One of these, which has existed in manuscript form for more than three years,

describes very exciting experiments that suggest that septins are act in parallel with both actin-myosin ring regulation and a cell wall pathway. Further, they suggest that in *S. pombe* these pathways may be semi-redundant for cytokinesis completion. John has promised to submit this paper later this week.

Jian-Qiu is clearly the scientific equal of the very brightest graduate students with whom I interacted with during my own graduate work at Harvard Medical School, or during my postdoctoral work at Princeton. I would compare him favorably to another Pringle lab graduate student, Hanna Fares, who after a post-doc with Iva Greenwald accepted a faculty position at the University of Arizona, or with Geraldine Seydoux who I knew as a graduate student in Iva Greenwald's lab and who is now a tenured professor at Johns Hopkins. In addition to his intellectual and scientific qualities, Jian-Qiu is a mature and very likeable individual, who is an outstanding lab member. He is very articulate and thoughtful, and his presentations to his thesis committee have always been enjoyable in their clarity and productiveness. He has also presented his work in several local, national and international meetings. Finally, he works as hard as any graduate student I have ever met.

He chose an outstanding lab in which to do his post-doc. Tom Pollard is one of the leading figures in cell biology worldwide. In his lab Jian-Qiu is adding rigorous training in biochemistry to his repertoire. His work in the Pollard lab has already gone very well. I visited Yale a couple of years ago to Yale to give a seminar and was gratified to hear Dr. Pollard's enthusiasm for Jian-Qiu's contribution to the lab and to the field, and to learn that this was the work he is presenting in talks around the nation. He has carried out a remarkable survey of the localization of all known actin binding proteins or proteins thought to be involved in cytokinesis in fission yeast, using state-of-the-art imaging technology. This resulted in the creation of a "wiring diagram" of cytokinesis, describing the order in which various proteins arrive at the contractile ring. This is a remarkable achievement, resulted in a very high profile publication in Developmental Cell and led to an invitation to present a talk about it at this month's ASCB Meeting. He followed this with a tour-de-force effort to bring together cell biology and biochemistry, measuring for the first time the concentrations of 28 different cytoskeletal and signaling proteins in fission yeast cells. This is a landmark achievement—these sorts of data are essential to allow one to begin to truly model the complex apparatus that mediates cytokinesis, yet most (including me) thought this was beyond our technical abilities. This work is now in press in Science. He has also co-authored a paper in Molecular Biology of the Cell. Thus he has done exceptionally well in terms of the usual measure sof success for a post-doc. However, even more important, his work is truly novel and is re-shaping both our view of cytoskinesis and also our view of what one can accomplish with microscopy.

In summary, I recommend Jian-Qiu to you most highly. I would be happy to answer any questions you might have.

Sincerely yours,

Dr. Mark Peifer

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September 27, 2005

Yves Brun
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Dear Dr. Brun:

I am pleased to recommend Dr. Jian-Qiu Wu, an outstanding candidate for a faculty position in your department. Wu is a remarkable experimentalist, with a bright future. He has already done groundbreaking work and should be one of the leaders of his generation in experimental cell biology.

As a postdoctoral fellow Wu has made two lasting contributions to our understanding of cytokinesis. First, he mapped out the temporal and spatial pathway for a dozen proteins participating in the assembly and constriction of the contractile ring in fission yeast. Second, he perfected methods to measure the global and local concentrations of more than two dozen cytoskeletal and signaling proteins in live cells as they march through the cell cycle.

The first study involved incorporating fluorescent protein tags into the genes for 15 different proteins and establishing that the fusion proteins produced from their native promoters were fully functional by phenotypic analysis and genetic crosses. Using cells with one marker on the spindle pole body, as an internal marker for the progress of the cell cycle, and another marker on the protein of interest, Wu established the precise time that each protein concentrates at the equator of dividing cells and the changes in the structures that each forms. He also filled in gaps in the genetics experiments that others had reported. The resulting synthesis established that the assembly pathway is the same as the genetic pathway, thus revealing for the first time the underlying biochemical pathway for the assembly and constriction of the contractile ring during cytokinesis. Previous work had created a vague and inconsistent pathway, so Wu's work brought great clarity to the field and laid out the research agenda for the field for years to come.

His second project on counting proteins in live cells is a monumental piece of work that sets the stage for a much deeper understanding of cytokinesis. Prior to this work we did not know the concentration of any protein in the cytokinetic contractile ring. Wu developed a method with fully functional YFP-fusion proteins integrated into the genome under the control of their native promoters to measure the global and local concentrations of about 30 cytokinesis proteins and signaling proteins. He showed that the fluorescence measured by confocal microscopy or flow cytometry is directly proportional to the number of fusion proteins in the live cell. This calibration allowed him to chart the concentrations of 30 proteins in live cells across the cell cycle in fission yeast. As you can appreciate, this was a staggering amount of work involving construction of dozens of genetically engineered yeast strains, careful phenotyping to rule out

artifacts, 3D confocal microscopy, biochemical measurements of the concentrations of a sample of these proteins and analysis of a massive data set. Now he can count the number of protein molecules at any place in the cell. He has measured for the first time the stoichiometry and concentrations of the constituent proteins in the small cortical patches that are the precursor of the contractile ring, in the forming and constricting contractile ring, spindle pole bodies and cortical actin patches. No other data is more important for understanding the molecular mechanism of cytokinesis and designing key biochemical experiments to test new hypotheses. This work will appear shortly in Science.

In work being prepared for submission Wu has characterized the early stages of contractile ring assembly and also clarified that actin patches are important for septation but not for contractile ring assembly. In the time that he has left in my lab Wu plans to help students begin a systematic analysis of the molecular stability of cytokinesis structures with photobleaching experiments and to move on to biochemical analysis of the top components of the pathway, polo kinase and anillin (Mid1p). His planned experiments should reveal how polo kinase triggers the release of Mid1p from the nucleus and how Mid1p organizes the other pioneering proteins for the contractile ring. This is important, groundbreaking work.

This work is part of Wu's ambitious plan to analyze the mechanism of cytokinesis, the final step in cell division. Wu brings to this project tremendous energy, a critical mind, a healthy skepticism, rigorous standards of evidence and a wealth of knowledge about genetics and microscopy. As a graduate student with John Pringle at the University of North Carolina he developed widely used methods to manipulate the genes of fission yeast and carried out elegant work on septins. Owing to Dr. Pringle's deliberate approach to writing (no fault of Wu's), some of that excellent work is only now being submitted for publication.

Wu is a shrewd and tireless experimentalist, well suited to his ambitious long term plans to study cytokinesis. He also has the vision to look beyond genetic dissection of the pathways. He will use cellular experiments to identify key molecular interactions to study by biochemical analysis. Given his talent, this range of skills will allow Wu to establish a leading research program with a broader base than scientists with a narrower focus.

Wu is a generous, collegial individual. He has been an outstanding mentor for several postdocs and graduate students beginning work on fission yeast in my lab as well a number of undergraduates who have carried out senior thesis research under his direction. As one postdoc with a biochemical background declared after working with Wu for a month: "Just do what Jian-Qiu says and it will work." Thus he is an outstanding teacher in a laboratory setting and should do well teaching small classes.

Yours truly,

Tom Pollard

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September 30, 2005

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Dear Colleagues,

It is a great pleasure to write in support of **Jian-Qiu Wu**'s application for a faculty position in your department. Jian-Qiu arrived in Chapel Hill by virtue of a contact between a phycology professor that he had in China and a member of the UNC faculty (since retired) who worked on red algae. However, he quickly decided (or maybe knew before he came) that he wanted to work in an area that was a more active focus of modern cellular and molecular biology. He worked in Ralph Quatrano's lab for a while, but this situation was unsettled because Ralph was already contemplating the move that he subsequently made to Washington University. When Jian-Qiu approached me about a possible rotation, I was a bit doubtful, because his English wasn't too good and it wasn't really clear how talented he was. Thus, I didn't agree to take him on for a rotation until he volunteered to work through the summer without a stipend – I didn't let him do this, but I was sufficiently impressed by the gesture to take him into the lab. I then set him to working with Jürg Bähler (an outstanding postdoc in the lab at the time, now at the Sanger Center) in our small *S. pombe* subgroup. Jürg and I were both amazed at how quickly Jian-Qiu picked things up and by his very rapidly developing skill at the bench, and I soon agreed, with enthusiasm, to take him on for his thesis work. This proved to be one of the best personnel decisions of my career.

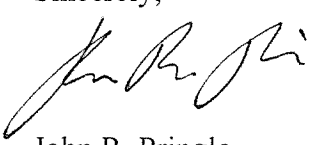
To put it simply, Jian-Qiu was a model graduate student. He worked phenomenally hard, putting in very long hours during which he worked with intense concentration. I've known some other hard workers, but I'm not sure that I've ever seen Jian-Qiu's equal. He also worked with great skill at the bench, and he mastered a wide variety of genetic, molecular biological, cell biological, and *S. pombe*-specific methods. When things were slow at the bench, he didn't just sit around playing games on the computer (like so many of the students these days), but instead dug into the literature, scanning for papers of interest and then reading them carefully, so that he developed a wide and deep knowledge of the information related to his interests. He thought for himself and had his own ideas, and although I sometimes disagreed with these, I always appreciated the effort, and I did notice that sometimes it was he, rather than I, who was on the right track! Jian-Qiu also worked very hard at his English, both spoken and written, and both improved dramatically. His spoken English became perfectly fluent and clear (both speaking and understanding), and he learned to give an excellent talk about his work. In working on his papers, he studied very carefully – and sometimes challenged! – the changes that I had made. As a result

of this effort, by the time he left he was a pretty decent writer for someone at his career stage, and I'm sure that he has improved further in working with Tom Pollard. In addition to all of these other positive attributes, Jian-Qiu was consistently pleasant and cooperative and got along very well with others in the lab.

In his lab work, Jian-Qiu completed several very nice projects. First, he played a major role (along with Jürg Bähler) in the extension of PCR-based gene-targeting methods into *S. pombe* (published in *Yeast*); these methods and the associated plasmids have now been adopted by essentially all *S. pombe* laboratories around the world. Second, he completed (almost entirely on his own) the first study of *S. pombe* α–actinin and fimbrin (published in *Mol. Biol. Cell*). Third, he made major contributions to our efforts to elucidate the still-mysterious conserved role of the septin proteins in cytokinesis. Although the septins are essential for cytokinesis in *S. cerevisiae* and some animal cell types, they are not essential for cytokinesis in *S. pombe* or in some other animal cell types, and they have not been found in plants or *Dictyostelium*. On the hypothesis that the nonessentiality in some cell types might reflect functional redundancy with some other system, Jian-Qiu undertook a synthetic-lethal analysis of the problem in *S. pombe*. He identified several mutants that have given us our first really useful insights into the role of the septins in this organism, and we expect the continuation of this screen (now being carried out by a new student in the lab) to yield additional informative mutants. We have been slow to publish this work, first because Jian-Qiu kept improving the story by doing additional experiments in his spare time after he had moved to the Pollard lab, and more recently because we wanted to publish it together with the full description of the *S. pombe* septin family. However, the latter paper has been very slow to get written (it covers work done by many people over many years), so that we finally decided to send in the synthetic-lethal paper without it and then try to follow with the other as quickly as possible. While working on the synthetic-lethal project, Jian-Qiu also helped me tremendously by filling in the many gaps that had been left by previous students and postdocs in our other studies of the *S. pombe* septins, so that he will also be a co-first author on this rather massive paper.

When Jian-Qiu left my lab, I was confident that he would be an absolutely terrific postdoc, and he has clearly lived up to this expectation – both of his major papers so far from the Pollard lab are of the highest quality and importance. In addition, I believe that he has taken advantage of the opportunity to broaden his training in biochemical and biophysical approaches to cell biological problems. The resulting breadth of training, taken together with Jian-Qiu's great ability, outstanding work ethic, and the congenial personality that helps to make him a highly effective co-worker and collaborator, makes it seem certain to me that he will go on to have a highly successful independent career. In short, I recommend him most highly and without any reservations. Please feel free to contact me if I can provide any additional information.

Sincerely,



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October 10, 2005

Mr. Yves Brun
Systems Biology/Microbiology Faculty Search
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Dear Mr. Brun:

I am writing in support of Dr. Jian-Que Wu who you are considering for a faculty position. Dr. Wu is familiar to me through meetings with him at conferences and through his research publications, but I have not worked closely with him.

Dr. Wu's research interests are in understanding the mechanism and regulation of cytokinesis, using the fission yeast Schizosaccharomyces pombe as a model system to investigate these processes. His most important work falls into two areas. In the first, he has defined the timing of appearance and spatial location of a large number of proteins required for the formation of the actin contractile ring, a key step in cytokinesis. Using fluorescent tags for the proteins of interest, he established that there were four sequential stages to the assembly and contraction of the actin ring. Actin building proteins were eliminated from the ring as it contracted whilst myosin became more concentrated. The sequence of events defined by this cytological analysis was confirmed by conventional genetic dependency experiments. More recently, he has shown that the anilin-like protein Mid1 recruits myosin, formin and other proteins into a broad band pre-cursor of the actin ring. Acting with profilin these proteins stimulate actin filament assembly and subsequently coalescence of all the components into a tight, compact actin ring. These studies represent one of the most complete descriptions of the molecular basis of cytokinesis in any organism and are of the highest quality.

His second area of study has been more technical, and has aimed at determining the numbers of particular molecules involved in the process of cytokinesis. By extremely careful quantification using a confocal microscope and fluorescent cytometry, he has determined the concentrations of over 25 proteins in the contractile ring. This not only established the quantities of components required for cytokinesis, measurements essential for more accurate modeling of the processes involved, but also provides a general approach for the quantification of many components within a cell. This work is a technical tour-de-force and is of great importance for techniques in cell biology.

Dr. Wu is an outstanding research worker, intelligent, committed, technically able and extremely hard working. He is carrying out two great projects and has the ability to pursue them both to good conclusions. I rate him very highly and recommend him strongly for your position.

Yours sincerely,

Paul Nurse

Paul Nurse
President