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

Dear Dr. Brun:

I am writing to apply for the tenure-track faculty position in your department posted in the September 2, 2005 issue of *Science*. I am doing postdoctoral research in Tom Pollard's laboratory at Yale University. As described in my curriculum vitae and research statement, I have a strong background using cell biological, molecular, biochemical, genetic, and microscopy approaches to investigate systematically the mechanisms and signaling of cytokinesis in fission yeast. I have laid the foundation for my long-term research program by establishing the temporal and spatial pathway for the assembly and constriction of the cytokinetic contractile ring and by measuring the global and local concentrations of the main cytokinesis proteins.

I enjoy teaching graduate and undergraduate students, both in the classroom and the laboratory, because not only do I enjoy communicating with students but I also find their views of biological questions both stimulating and refreshing.

I have included the names of four references (Tom Pollard, Yale University; John Pringle, Stanford University; Mark Peifer, UNC-Chapel Hill; Paul Nurse, Rockefeller University) at the end of my curriculum vitae and arranged for the letters to be sent to this address. Please do not hesitate to contact me if you have any questions regarding my application. Thank you very much for your consideration.

Sincerely,

Jian-Qiu Wu, Ph.D.

Research Interests

Jian-Qiu Wu

Cytokinesis, the final step in the cell-division cycle, partitions cellular constituents into two new daughter cells. When coordinated with cellular asymmetry, cytokinesis can generate diverse cell types in multicellular organisms. Thus, cytokinesis plays a crucial role in cell reproduction and differentiation. Uncontrolled cell division is a defining characteristic of cancer. Therefore, an understanding of the mechanism of cytokinesis will undoubtedly contribute to our understanding of cancer. The fission yeast *Schizosaccharomyces pombe* has emerged as one of the leading models for the analysis of cytokinesis. Not only is it genetically tractable and favorable for microscopic analysis, but it also has the smallest fully sequenced eukaryotic genome and carries out cytokinesis much like animal cells. Beginning with Nurse's pioneering work on the cell cycle, geneticists have identified more than 50 genes contributing to cytokinesis in *S. pombe*, but biochemical and mechanistic studies have lagged behind the genetics. In this post-genome era, I think it is important to thoroughly investigate the individual proteins, but it is more important to investigate how all the proteins in a process or pathway work together. **Thus, my current research and long-term goal is to investigate systematically the molecular mechanism of cytokinesis.**

I. Past studies as a Ph.D. student with Dr. John R. Pringle

I initially was involved in the development of a PCR and homologous recombination-based method for gene disruption, modification, and tagging in the fission yeast *S. pombe*. The significance and popularity of the paper can be seen by the 386 citations it received so far. I then cloned and characterized an alpha-actinin-like protein and a fimbrin. I found that these two actin cross-linking proteins have overlapping and essential functions in cytokinesis. To pursue my interest in cytokinesis further, I studied the functions of *S. pombe* septins. We cloned and characterized seven septin genes in *S. pombe*. I identified seven mutants from a synthetic-lethal screen in a septin-deficient strain. One mutation is in a myosin essential light chain. Five mutations fall into two genes (one of them is a novel gene *rgf3* that encodes an essential GEF for Rho GTPases) that are in the cell-integrity pathway. These works culminated in four first-author papers (two published, one submitted, one in preparation).

II. Past postdoctoral research with Dr. Thomas D. Pollard

As a postdoctoral fellow, I laid the foundation for my long-term research program by establishing the temporal and spatial pathway for the assembly and constriction of the cytokinetic contractile ring, and by measuring the global and local concentrations of the main cytokinesis proteins in fission yeast.

1. Temporal hierarchy of the assembly and constriction of the contractile ring. I made fission yeast strains expressing native levels of >30 different fully functional fluorescent fusion proteins by integrating mCFP, mEGFP, or mYFP into the genome and used strains expressing pairs of fusion proteins to map out the time course of contractile-ring function (Wu et al., 2003. Dev. Cell 5: 723-734). I discovered that the contractile-ring assembles and constricts in four precisely timed, sequential stages involving the progressive assembly of more complex structures (Fig. 1). During ring constriction, actin-binding proteins are shed in proportion to the loss of contractile-ring volume, while myosin-II becomes more concentrated. Genetic dependencies confirm the temporal pathway. This hierarchy sets the stage for future mechanistic studies at the cellular and molecular levels.

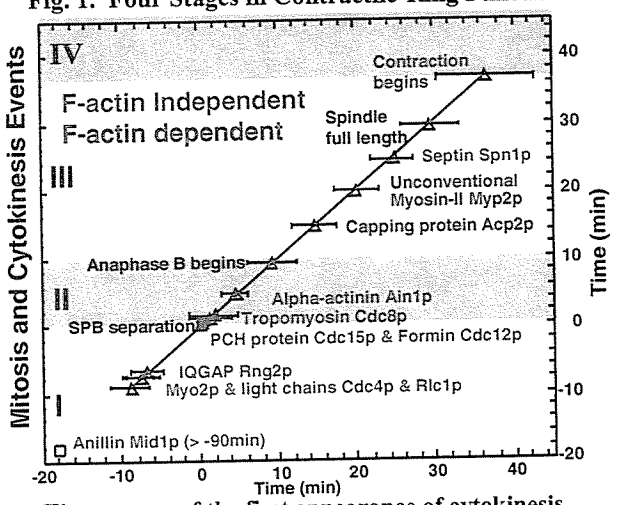
2. Global and local concentrations of 28 cytoskeletal and signaling proteins. I measured the fluorescence of cells expressing YFP-fusion proteins by microscopy or flow cytometry, and found that the fluorescence is directly proportional to the fusion protein concentration measured by quantitative immunoblotting (Wu and Pollard, 2005. *Science* **310**: 310-314). This standard curve enabled me to measure the global concentrations of 28 proteins relevant to cytokinesis and to calculate their local concentrations in the contractile ring, spindle pole bodies, and actin patches (Fig. 2). These quantitative measurements establish the stoichiometry of the proteins in these structures and will be essential for mathematical modeling of cytokinesis in the future. My method can be used to count proteins in other cells even when homologous recombination is not possible. YFP-fusion protein can be expressed from

plasmids and the ratio of tagged and untagged proteins be measured by immunoblotting for the protein. Knowing this ratio, one can use the fluorescence to measure local concentrations of the protein.

3. Spatial pathway of contractile-ring assembly (Wu et al., in preparation). I used pairs of fluorescent fusion proteins to establish the spatial hierarchy of the contractile-ring assembly. Anillin-like protein Mid1p established a cortical broad band of 50 to 100 small dots around the equator at G2/M, then conventional myosin-II (Myo2p heavy chain with two light chains Cdc4p and Rlc1p), IQGAP (Rng2p), PCH protein (Cdc15p), and formin (Cdc12p) were recruited to the broad band and mostly colocalized with Mid1p, all independent of actin filaments but dependent on Mid1p. Cdc12p and profilin Cdc3p stimulate the assembly of actin filaments, a prerequisite for these small dots to coalesce laterally into a sharp, compact ring around the equator prior to anaphase B. These results argue against the popular models suggesting that the contractile ring assembles by extension of a leading cable around the circumference of the cell from a single myosin progenitor or a Cdc12p-Cdc15p spot.

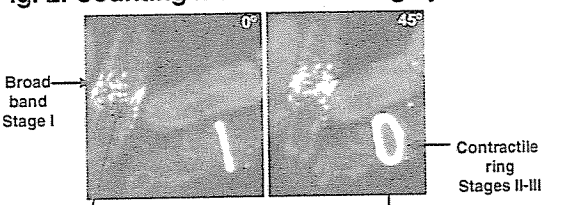
4. Roles of Arp2/3 complex and its activators in cytokinesis (Wu et al., in preparation). Arp2/3 complex and its activators type I myosin Myo1p and WASp Wsp1p begin to concentrate in actin patches near the well established contractile ring early in anaphase B, but are not incorporated into the contractile ring itself, as reported. These patches are highly dynamic with lifetimes of ~20 sec and are involved in septum formation and cell separation, but not in the assembly of the contractile ring as reported by others.

Fig. 1. Four Stages in Contractile-Ring Function



Time course of the first appearance of cytokinesis proteins at the cleavage furrow

Fig. 2. Counting molecules during cytokinesis



Mean molecules per dot	
Mid1p anillin	21
Cdc4p ELC	289
Myo2p dimer	22
Rng2p IQGAP	23
Cdc15p PCH	22
Cdc12p formin dimer	2

	Molecules	Concentration
Actin (EM)	76,000	460 μM
Cdc12p formin dimer	150	3 μM
(Each actin filament ~1.4 μm long)		
Myo2p myosin-II	1450	20 μM
(180 Myo2p minifilaments: ~1 per actin filament)		

Local accumulation and concentrations of mYFP fusion proteins at the cell-division site measured by fluorescence microscopy. Fluorescence micrographs are from cells expressing GFP-Myo2p at three stages observed by spinning disk confocal microscopy.

III. Current and future studies in the Pollard laboratory

1. Analysis of the interactions of anillin Mid1p and Polo kinase Plo1p with each other and other cytokinesis proteins. Detailed biochemical characterization of purified contractile-ring proteins is required to understand molecular mechanisms, and to learn how each protein contributes to signaling or to structure. I am examining Polo kinase Plo1p (0.29 μM, 6,600 molecules/cell) and anillin Mid1p (0.09 μM, 2,100 molecules/cell), proteins at the very top of the assembly pathway for the contractile ring. Plo1p is a multi-functional protein essential for both mitosis and cytokinesis. Its potential substrates include Cdc25p (phosphatase for Cdk Cdc2p), Ras GTPase Spg1p in the SIN pathway, and Mid1p. Mid1p has domains similar to anillin. During G2 Mid1p leaves the nucleus and is the first protein to localize to the medial cortical dots (containing ~20 Mid1p molecules in each dot) around the future cleavage site. This movement might depend on Plo1p, but we do not know the domains of Mid1p required for forming cortical dots, how Mid1p binds to the cortex, or how Mid1p recruits Myosin-II and IQGAP Rng2p to the broad band of dots. I will test whether purified recombinant Plo1p phosphorylates purified Mid1p. I will utilize proteolytic digestion and mass spectroscopy to identify the phosphorylated residues and confirm these sites by mutagenesis of Mid1p. I will utilize Mid1p-YFP or YFP-Mid1p

fusion proteins with truncations to identify which domains of Mid1p are required for nuclear export, targeting to cortical dots, and recruitment of downstream proteins.

I have made all the necessary strains and constructs for expression and have worked out purification procedures for 6His-Mid1p from *E. coli*. I will characterize purified Mid1p using the well-established biochemical assays in the Pollard lab. I will measure the affinity of Mid1p for actin monomers by fluorescence anisotropy, and the affinity for actin filaments by cosedimentation. I will use pyrene actin to test the effect of Mid1p on actin polymerization. If Mid1p binds actin monomers or filaments, or stimulates actin polymerization, I will use Total internal reflection fluorescence (TIRF) microscopy to study the mechanism of action at the level of single actin filaments.

2. Dynamics of cytokinesis proteins at the cleavage furrow by quantitative FRAP-in vivo biochemistry in fission yeast. Fluorescence recovery after photobleaching (FRAP) makes it possible to study biochemical processes not using purified proteins but instead in a living cell. By measuring mobile fraction and diffusion constant, FRAP can be used to address protein dynamics. Dynamics studies of cytokinesis proteins are rarely available. With more than 30 genes tagged with CFP, GFP, and YFP under the control of their native promoters, I am in a perfect position to do FRAP studies systematically in fission yeast. I will do quantitative FRAP in wild type background at 25°C. During ring constriction, actin-binding proteins are shed in proportion to the loss of contractile-ring volume, while myosin-II becomes more concentrated. It will be very interesting to learn whether they have different dynamics. It was reported that septin ring oscillates between a frozen (immobile) and a fluid (dynamic) stage during the cell cycle in budding yeast. Thus, I want to know if different cytokinesis proteins in fission yeast display different dynamics during each stage of cytokinesis. These measurements of mobile fractions and diffusion constants will be invaluable quantitative information for the computational modeling of cytokinesis by collaborators or others in the field.

IV. Future studies as an independent researcher - short term

Aim 1. Dynamics of cytokinesis proteins at the cleavage furrow in mutant background by quantitative FRAP. Building on the FRAP results in wild-type background obtained in the Pollard laboratory and my rich collections of both conditional and deletion mutants, I will investigate the dynamics of cytokinesis proteins under mutant backgrounds at different growth conditions (e. g. permissive and restrictive temperatures). Anillin-like protein Mid1p specifies the division site and recruits the downstream proteins for the assembly of the contractile ring, but leaves the division site before the ring constriction (Wu et al., 2003. Dev. Cell 5: 723-734). It will be interesting to investigate the kinetics in the presence and absence of Mid1p at different stage of cytokinesis. Septin deletion mutant *spn1Δ* in which no other septins can localize is another promising candidate. Septin double rings may maintain a diffusion barrier for the proteins in the contractile ring. Thus, the lateral mobility might be very different in *spn1Δ* from those in wild type. This study may also address the still mysterious roles of septins in cytokinesis. Under the defective signaling in temperature-sensitive mutants in the SIN pathway, the contractile ring forms but does not constrict. I will test if a change in the protein dynamics triggers the ring contraction.

Aim 2. Investigate protein interactions in contractile ring and identify novel proteins by TAP tagging. The spatial and temporal pathway and genetic dependencies for cytokinesis suggest that the contractile ring assembles by specific molecular pathways involving the formation of large protein complexes (Wu et al., 2003. Dev. Cell 5: 723-734). To identify proteins associated with anillin-like Mid1p, IQGAP Rng2p, and PCH protein Cdc15p (three pioneer proteins for the contractile ring), I will utilize TAP tagging (tandem affinity purification) and mass spectrometry to identify tightly associated proteins. TAP tagging will not only detect the physical interactions among the known cytokinetic proteins but may also reveal novel proteins involved in cytokinesis, such as membrane proteins that are postulated to anchor Mid1p in the cortex. With a completely sequenced genome, it will be easy to identify the components of the complex by mass spectrometry. I will characterize the identified novel proteins both in vivo and in vitro, using the approaches mentioned above.

V. Future studies as an independent researcher - long term

Aim 1. Investigate the functions of septins in vitro and in vivo. All seven septins in *S. pombe* contain a GTP-binding site, and five of them also contain a coiled-coil domain that may be involved in protein-protein interactions. The localization of septins as double rings during cytokinesis is interdependent, and thus they might form a complex in vivo. I will investigate the four septins (Spn1p – Spn4p) that localize to the cleavage furrow. Small size (~50 kD), relative abundance (0.6 μM, 10,000 molecules/cell), no overexpression phenotype, and mild deletion phenotype make septins ideal candidates for both in vivo and in vitro studies. I will overexpress and then purify GST- and/or His- tagged fragments and full-length septins from fission yeast or from bacteria if necessary. Once purified from bacteria, I will test if septins form filament in vitro as septins from animal cells, as well as the kinetics of filament formation, the ratio of each septin subunit in the filament. I will observe the septin-filament formation by immunofluorescence microscopy and the filament structure by negative-stain EM. I will measure the affinity of septins for actin filaments by cosedimentation. I will also make truncation mutants using the gene targeting method to study the roles of each domain in cytokinesis and filament formation.

Aim 2. Investigate dynamic protein-protein interactions in the contractile ring using FRET. Fluorescence resonance energy transfer (FRET) occurs if the acceptor and donor fluorophores are less than 10 nm apart, making it possible to investigate dynamic protein-protein interaction beyond the resolution of light microscopy. I will attempt FRET using CFP and YFP fused to potential molecular partners to define the physical relationships between contractile-ring proteins.

More than 50 proteins are known to be involved in cytokinesis, and more will be identified in fission yeast by screening a genome-wide knockout collection. I have investigated about 30 of these proteins, but understanding the mechanism of cytokinesis will require characterization of all the proteins. As a result, I am looking forward to a number of fascinating projects.

Teaching Statement

Jian-Qiu Wu

Since I came to the United States in 1996, I have gained a tremendous amount of teaching and presentation experience. As a teaching assistant at the University of North Carolina at Chapel Hill, I participated in teaching Plant Biology and Laboratory Experiments in Genetics. I have been invited to give talks at four international meetings and many other talks at UNC and Yale. During my time in the US, I trained two technicians, a senior student, and one rotation graduate student. Currently, I am supervising two Yale students for their senior projects. I am committed to teaching students at all levels because not only do I enjoy communicating with students in itself, but I also find their views of biological questions both stimulating and refreshing.

My teaching philosophy is constantly evolving along with my teaching, presentation, and research activities. Just like the cytoskeleton of a cell, science is highly dynamic and undergoes constant restructuring. It is therefore crucial that students appreciate the reality that although most facts found in textbooks are well tested and supported, they do not represent the final word. With the availability of new technologies, some facts and hypotheses will eventually be made obsolete. To understand a certain scientific process, though we need to understand the roles of each component, it is even more important to see how all the components function together. The students need to see both the trees and the forest. My highest priority, then, is to stimulate students to be open-minded and think critically, quantitatively, and systematically in order to creatively solve new problems.

To execute this plan, I will use both high-quality textbooks and original research papers in the classroom. My broad training and teaching experience allow me to teach a variety of biological courses. For a cell biology related course, for example, in addition to a textbook, I will ask students to read both classic papers from the "Landmark Papers in Cell Biology" (ed. Gall and McIntosh) and recent quantitatively based biology papers. I will request anonymous and frequent evaluations from the class in order to get feedback from my students about syllabus, papers, and teaching techniques, so that if necessary, I can improve my teaching. In evaluating students, I will use two formats. One is a closed-book test assessing the basic knowledge most essential for every student to have, regardless of his or her future plans. This test will ensure that they learn systematically. The other evaluative component will be to write a grant proposal in the NIH format after an in-depth study. They will be asked to choose a specific scientific problem covered in the class and draft a grant proposal consisting of the following sections: Introduction, Significance of the Research, Materials and Procedures, Aims, and Pitfalls. The percentage that each evaluation contributes to the final grade will depend on the level of the course.

Above all, laboratory experience for students is paramount if they are to become good scientists. It is essential to the process of enlightening those students about the real world, and teaching them to think critically and quantitatively. Along with some classic experiments that are integral to students' mastery of a broad range of techniques, I will also challenge the students to perform some real research. I want them to share in the excitement of discovery, which will nurture their interest in scientific research. The research can be any small project that applies well-established techniques and has a high probability of success. I will assign the research project according to their aptitude and skills, paying close attention to their diverse academic background, level of prerequisite knowledge, and interest.

I am very confident that my passion and dedication will serve me well when teaching in your supportive environment, as they have successfully done for my current teaching and research. I very much hope that I will be able to contribute to your university and your students.