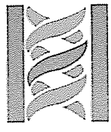

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10 October 2005

Yves Brun
Systems Biology/Microbiology Faculty Search
Department of Biology
Indiana University
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Dear Dr. Brun:

It is with the greatest enthusiasm that I recommend **Richard Gardner** to you for an assistant professor position at Indiana University. Rich is the most impressive post-doctoral fellow to have ever studied with me, and ranks among the best that I have seen during my time at the Hutchinson Center and the University of Chicago.

Rich came to my lab after earning his Ph.D. in the laboratory of Dr. Randy Hampton (UCSD) where he studied ubiquitin-mediated degradation of proteins at the endoplasmic reticulum. I will leave it to Hampton and others at UCSD to extol Rich's considerable accomplishments as a student, but I will mention that I was quite impressed with Rich's application for a post-doc position in my lab. Not only were his letters of support exceptionally positive, but his letter of inquiry was also memorable. The letter was several pages long, and in it he detailed a number of ideas that he considered as potential projects during a post-doc. He considered investigating different areas of biology (including protein quality control, chromatin regulation, protein trafficking, evolution of protein structure/function) with the common theme of viewing these areas through the lens of ubiquitin modification of protein and carrying out the studies in the model system *Saccharomyces cerevisiae*. His letter reflected some of his inherent strengths that I have come to appreciate on a daily basis. He has an expert knowledge of the ubiquitin field, a broad understanding and interest in biology, he can think deeply about a variety of biological problems and how to solve them experimentally, and he is always well-prepared when making a written or oral presentation.

After Rich decided to join my lab, we discussed his ideas and he refined some of them into three different projects. I typically encourage post-docs to try a couple of different things with the intention that one of the ideas will actually pan out. Here is one of the ways in which Rich is unique – he made all three projects work!

In the first project, Rich was interested in asking whether there was a protein quality control degradation system in the nucleus that protected it from the accumulation of aberrant proteins – such as those known to arise in some diseases with protein aggregates in the nucleus. This idea piqued his interest not only because it was unexplored territory, but also because the nucleus represented a cellular compartment in

which defective proteins would arise post-production. This contrasted with the cytoplasm, endoplasmic reticulum, and mitochondria, where protein quality control was thought to work primarily at the production level – as a polypeptide is being synthesized and undergoes maturation. Hence he thought that something novel about protein quality control recognition might turn up by studying the nucleus.

Rich initiated this project by taking a clever "21st century scholar's" approach to thinking about the problem – he took advantage of the vast array of literature and available databases to test a hypothesis. He began searching for nuclear protein quality control degradation pathways in yeast by working under the assumption that temperature-sensitive mutant nuclear proteins might be substrates for such a pathway. This idea was based on the observation that some temperature-sensitive proteins in bacteria are recognized as "aberrant" and destroyed by protein quality control machinery, despite the fact that the mutant proteins often possess normal activity. In these cases, the temperature-sensitive phenotype results from reduced steady-state levels of the protein and can be suppressed by inhibiting the protein's degradation, thus allowing the mutant protein to accumulate to functional levels.

Rich speculated that if a general nuclear-localized protein quality control degradation system exists, then the temperature-sensitive phenotypes of different mutant nuclear proteins with disparate normal functions would be suppressed by a common extragenic mutation. So he searched for temperature-sensitive alleles of nuclear protein genes that had been previously identified in yeast, and then looked at all those for which a genetic suppressor screen had been done. From the search, which took only about a week, he found two independently conducted genetic analyses that had a common suppressor: the *SAN1* gene. The short story is that Rich's intuition and method were right on target. As published earlier this year [*Cell* 120:803-15 (2005)] – he went on to show that San1 is an ubiquitin protein ligase that facilitates ubiquitin-mediated protein degradation of a variety of temperature sensitive alleles of distinct nuclear proteins. San1 is restricted to the nucleus and defines a protein quality control pathway that is involved in post-production quality control. The San1-pathway recognizes and degrades only "aberrant" proteins; the wild type counterparts of these mutant proteins are stable and unaffected by San1. While there was no obvious growth phenotype in *san1*Δ cells, Rich's thorough experimentation and thinking about its role in cellular function led to his discovery that in the absence of *SAN1*, cells are under a chronic "nuclear stress" that elicits a specific response, which appears to be directed primarily toward stress in the nucleus. How the San1 pathway recognizes "aberrant" proteins, how the cell senses problems in the nucleus, and how San1 and its metazoan homologues may mitigate "diseases of the nucleus" are interesting questions that Rich plans to pursue in the future.

It is worth mentioning that others had interpreted the original findings of San1 differently, but Rich had the confidence in his ideas to test, and ultimately prove, his hypothesis about San1's function. Rich's discovery of this pathway and his resulting interpretations have been enthusiastically accepted and noted by the ubiquitin and protein quality control fields; his work has been highlighted in several recent reviews and he has been invited to speak at three international meetings (two Gordon Conferences and the International Congress of Biochemistry and Molecular Biology in Japan).

The second project Rich embarked on was based on earlier work in our lab. We had identified Dot4 as a ubiquitin protease involved in chromatin-mediated silencing in yeast. We knew that it bound one of the silent chromatin proteins, Sir4, but we were unable to determine the substrate from which Dot4 removed ubiquitin. In his original letter to me, Rich speculated that it might be a histone, specifically histone H2B – based on various bits of data from our lab and others. At the same time that Rich initiated the *SAN1* project he also began to test his hypothesis about Dot4. The short story is that Rich was right again! This work was also recently published [*MCB* 25:6123-39 (2005)] and like the *SAN1* strory, it was a very thorough and complete analysis. Typical of a good piece of work, it answered key issues in an elegant manner. He used the combined power of yeast genetics and biochemistry – allele specific activities – to dissect out the contributions of Dot4's regulation of histone H2B ubiquitination and its impact on silencing, as well as discovering that it played an additional role in gene regulation elsewhere

in the genome.

I need to point out that Rich was working on the San1 and Dot4 projects concurrently. In the process he not only demonstrated incredible organizational skills at the bench, but like an ambidextrous athlete, he developed his intellectual prowess in both areas. Besides his accomplishments in ubiquitin-mediated protein quality control, he became, and still is, incredibly well versed in the chromatin field. An example of this is his ability to remember the myriad of histone modifications, their regulation and phenotypic consequences and be able to use this information to synthesize cogent models about chromatin structure and function. In addition, he showed technical cleverness on these projects. One of the proteins, Sir4, which was fortuitously examined in both projects, is difficult to detect – available antibodies react poorly and tags on the COOH- or NH_3-terminus result in a loss of function. Rich came to an elegant solution to be able to monitor Sir4 – he scanned the protein sequence for regions that were similar to epitopes of commercially available monoclonal antibodies. He then made a couple of residue changes in Sir4 to make these regions a perfect match to a particular epitope, then used the relevant monoclonal to detect the Sir4 protein (the modified version still functioned like the wild type protein). Since then, this method has been used repeatedly for "tagging" fussy proteins – by our and other labs.

The third project Rich has been working on is development of a technical method to ask a simple question – what are all the substrates for any partiicualr ubiquitin enzyme (e.g. ligase or protease)? (Yes, this was done concurrent with the other two projects!) For instance, Rich would like to determine all proteins that are normally ubiquitinated by San1 in the cell. The plan is simple in concept – replace normal ubiquitin with a tagged version of ubiquitin that allows purification of all peptides covalently attached to ubiquitin. Identification of the peptides is done by mass spectroscopy – a method that we've been using in the lab for a while. I won't describe all the technical issues that Rich successfully dealt with, but will share one that illustrates his biochemical savvy. Rich began this project by using an available reagent – ubiquitin with the common 6x-histidine tag that allows purification of ubiquitin by chelation of the tag to a Ni^{+2} column. While the column and attached ubiquitin complexes could be washed with fairly stringent conditions such as chaotropic denaturants (6M Urea), Rich quickly realized that there were a very large number of peptides co-purifying with the ubiquitin that were not covalently linked to ubiquitin. In essence, the 6M Urea was ineffective in disrupting many non-covalent protein complexes. In order to get around this problem, Rich had the neat idea to increase the affinity of the tag so he could use even higher stringency washing conditions. He accomplished this by methodically increasing the number of histidines in the tag (7-12) and testing them for *in vivo* function and relative affinity to Ni^{+2} *in vitro*. He found that 8x-histidine on ubiquitin was functional *in vivo* and bound so well to Ni^{+2} that he could wash columns with 8M urea and 0.1% SDS, which disrupted *all* non-covalently attached peptides. This has put Rich in the driver's seat for determining the "ubiquitin-ome" - identifying the complete substrate list of specific enzymes and synthesizing a ubiquitin-mediated regulatory network. Recently, two other groups have published their versions of detecting all ubiquitinated proteins, but they used the 6x-histidine tag, and consequently their data are quite limited and suffer from the issues Rich avoided. I'm sure when Rich's work is published it will set a new standard.

In the process of working on these various projects, Rich has demonstrated expert skills in molecular genetics, biochemical purification and enzymology, mass spectroscopic analysis and computational approaches. He has a great sense of knowing what experiments are key for developing new understanding and combines that with the fearlessness and skill to carry out whatever experimental approach is necessary to forge ahead. He is an awesome experimentalist.

It is rather unusual for a post-doc to successfully work on distinct projects concurrently, and while it meant he didn't make as rapid progress on any one of them if he had "focused", Rich was more interested in enriching his breadth of experience during his post-doc training. I believe this was a successful experiment in its own right, because Rich has clearly demonstrated that he has the inherent

ability to run his own multi-faceted research lab, asking interesting questions and answering them. I also wish to note that while I expect Rich and I may publish one or two more manuscripts together from the work that is ongoing in the lab, all these projects are his. When he begins his own lab he has no worry that my lab will compete with him on his proposed research.

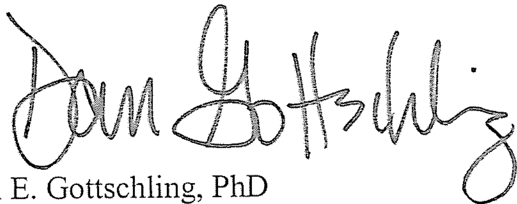
Rich is also a good writer. In the manuscripts we wrote together, he always presented me with a complete first draft that was clearly written and demonstrated his meticulous attention to detail and scholarship. During our writing process I also learned of his receptiveness to criticism as well as his graciousness in giving it. Furthermore, Rich's command of the literature and ability to synthesize it are so exceptional that I rely on his input for much of the manuscript reviewing I do for journals. He is a clear thinker, insightful and helpful with comments, and very fair in reviewing.

By now it should be obvious that Rich has great enthusiasm and ability for science. But another trait that makes him a real jewel is his willingness to share his time and abilities with others. He has been a positive influence on the work of each student and postdoc in the lab, with ideas and technical expertise. This generosity extends to other labs here at the Center as well. In fact, Rich has "converted" three postdocs in non-yeast labs to use yeast in their studies, and has served as their tutor. He also is highly-regarded by all the yeast labs here; in fact, he has been a weekly participant in Sue Biggins' lab meetings, a colleague here at the Hutch, for the past couple of years.

Rich is also an excellent teacher. He is effective at teaching in one-on-one situations in the lab, whether it be teaching someone new methods, training a technician, or getting another post-doc or student to think differently about their experiments. He's equally effective in front of an audience. When Rich first presented his San1 story at the Center's weekly in-house seminar, several of my colleagues remarked on how impressed they were not only by the significance of Rich's findings, but also by how clearly he presented it. Another aspect of Rich that makes him an effective teacher is that he has interests in biology beyond what he is studying. This not only makes him fun to talk with, but highly sought after by others for comments on manuscripts and discussions about new ideas.

In summary, I have no doubt that Richard Gardner will continue to make important contributions into basic biological processes of the broadest significance. He is unusually bright and clear thinking, possesses great energy and enthusiasm for his work, and is a wonderful colleague in every sense of the word. I recommend him with the highest possible enthusiasm and without reservation for a position in your department. Please contact me if I can provide any further information.

Sincerely,



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October 3, 2005

Dear Search Committee:

Dr. Richard Gardner, who earned his Ph. D. in my laboratory, has asked me to write a reference letter for his application for a faculty position. This has got to be one of the easiest tasks I have faced in a long time. Richard's talents are monstrous (in the good sense) and multifaceted; they cover all the obvious varieties including scientific ability, intelligence, and passion for research. But Richard also has vision, initiative, and leadership skills that make him the rarest of rare, even in the high performance world of newly-minted PIs. He is going to be one of sciences' leaders, and you will do your department, colleagues, institution, and students a huge favor by hiring him at (almost) any cost. (I won't tell him that last sentence, so as not to bollix up the bargaining process).

As for pure scientific talent, I have never had a grad student like Rich, and seriously doubt I will ever see another like him. He is unbelievably organized and driven, capable of an astonishing number of simultaneous projects and endeavors, but always instilled with creative and critical thinking. One of our earliest interactions provided a delightful harbinger of his talents: when Richard decided to join the lab, he marched into my office with an organized, incredibly detailed, and beautifully laid-out plan for the project that he wanted to develop. That is rare even for a postdoctoral candidate, let alone a first year graduate student! The starting place of his project was a desire to understand the differences in degradative behavior shown by the two yeast isozymes of the medically important, cholesterol pathway enzyme HMG-CoA reductase, Hmg1p and Hmg2p. Since these very different behaviors occur in two very similar isozymes, a molecular biological analysis (swapping domains, etc.) was called for 325 (yes, that's three-two-five) mutants later Rich had analyzed the problem to a point where literally, unless we had an X ray structure, there was little else to do. On the way, he produced two beautiful papers from these studies, and more importantly, a number of very surprising observations that demonstrated recognition of integrated structures as a mode of specificity in degradation, as opposed to only little peptide "degrons" that may well be the exception rather than the rule.

Although this alone would have been a fine thesis project, Rich went on to do several watershed studies, and in doing so he revolutionized the way we think about the system we study. He developed an *in vivo* cross linking assay that allowed examination of the intimate biochemistry of regulated degradation of Hmg2p, or any protein for that matter. Beyond the obvious biomedical utility of unraveling a novel

mechanism of sterol regulation, his studies also more generally informed us about the nature of protein quality control in the endoplasmic reticulum, a process of broad medical and basic interest. Wait, there's more! As a "side project" Rich also devised a molecular biological approach for analyzing the physiologically relevant, small molecular signals from the sterol synthetic pathway that control Hmg2p stability. Since these signals are directly important for understanding sterols sensing and regulation, the broader utility of this separate avenue is also quite apparent.

In total, Richard is an author on 7 papers from my lab, 6 of which he is first author, and he completed his thesis a year and a half earlier than the other two (very talented) students in his class who joined the lab at the same time. Just for fun, I went to the database in the laboratory, and learned that he made, and I am not kidding, over 900 plasmids in my lab during his stay here. Really. But in addition to his prodigious productivity, I need to also address the more general features of Richard's impressive talents, and this is perhaps the most important part of the letter. Along with his obvious technical prowess, drive, and organizational skills, he also maintained an unflagging interest in the bigger issues such as the nature of leadership, the societal impact of technology, and integration of different aspects of science. This "bigness" of style emerged both in his casual interactions, and in numerous formal endeavors. While doing all those experiments, Richard also instructed several undergraduates at the bench, and he was always thoroughly engaged, fair and creative in the teaching of each. He similarly participated in several formal avenues of teaching and instruction of teachers offered by UCSD. These activities, all done completely for his own edification with almost no fanfare, indicate that Richard will be a fantastic faculty member and teacher along with being a stellar researcher. Beyond that, he is an avid environmentalist, he is politically aware, and very interested in the directions and responsibilities of our society. To be honest, I am not sure how Richard did all these things so effectively. One model consistent with his molecular biological prowess is that he has cloned several versions of himself, and each did a fraction of the total endeavor package. Whatever the mechanism, it is the remarkable blend of attention to critical detail combined with a larger vision and interests that insure Rich's place in the highest echelons of science, and possibly beyond.

Since Richard left my lab, he has thickened the “proof pudding” with his work in Dan Gottschling’s laboratory. There he discovered an entirely new protein quality control pathway, which is one of the more exciting and unexpected developments in this very active field. That work resulted in his recent Cell paper, and lots of secondary attention from journalists and such. Bottom line: wherever Richard goes, his brilliant science, vision and educational leadership will come along, and that bodes well for anyone who hires him as a new faculty member.

In summary, let me say this. You don't have to give Richard Gardner a job. Because someone will and it doesn’t have to be your institution. But if your goal is to get the strongest possible candidate in this year’s pool, someone who is absolutely independent, completely original, incredibly motivated, and scientifically mature beyond his training time, not to mention a person who will grow into a top leader of tomorrow's science, then he’s your person!

Sincerely,

Randolph Y. Hampton, Ph. D.

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September 30, 2005

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Dear Search Committee,

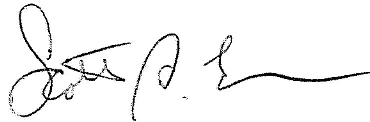
I am writing to provide my enthusiastic support of Dr. Richard Gardner for a faculty position in your Department. I served as a member of Richard's thesis committee for the 4 years while he was a graduate student in Randy Hampton's lab here at UCSD. Richard and I met together in official thesis committee meetings once each year as well as numerous other times informally at seminars and in the hallway. Richard's major project as a graduate student was directed at characterizing the mechanism for regulated degradation of HMG-CoA reductase in the endoplasmic reticulum. As you can quickly see from his cv and publication list, Richard was no ordinary graduate student. He was among our very best. Richard's thesis represented an incredibly thorough and important set of accomplishments. In addition to being bright, interactive and **highly motivated**, Richard **exudes a confidence and creative style** not seen in most scientists. He independently designed most of his experiments and he also developed and successfully carried out many new experimental techniques in the Hampton lab. Randy Hampton was an Assistant Professor when Richard was in his lab and Richard was largely responsible for the achievements that resulted in Randy's promotion and tenure.

As a postdoc in Dan Gottschling's lab, Richard discovered a novel protein quality

control system that functions in the nucleus. He demonstrated that he could suppress the *ts* mutant phenotypes of nuclear protein defects by a common extragenic mutation. Richard identified the *san1* mutation as a suppressor of two distinct nuclear *ts* mutants. Richard demonstrated that San1 is a ubiquitin ligase that functions in the nucleus to target nuclear proteins for ubiquitination and subsequent degradation by the proteasome. San1 appears to be specific for aberrant misfolded proteins. Numerous protein chaperones that function in the nucleus are upregulated in a *san1* mutant. Richard plans to extend this work as an Assistant Professor to develop a full understanding the San1 pathway and the nuclear quality control system.

Richard is **fearless** and he is able to identify and focus on key questions and experiments. Obviously, Richard also is not afraid to put in the long hours and hard work needed to be a successful scientist. He was a model for the graduate students here at UCSD. Richard reads the literature extensively and he thinks, writes and speaks clearly and concisely. His lectures are well organized and his presentation style is lively and engaging. I would rate Richard among the **top 2-5%** of the graduate students an postdocs I have known. I am confident he will continue to be very productive, creative and successful as an independent scientist.

Sincerely yours,



Scott D. Emr
Professor