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October 20, 2005

Dear Search Committee,

Please find enclosed my application for the Assistant Professor position at Indiana University. Currently I am a postdoctoral researcher working with Professor James Broach at Princeton University. My goal is to maintain an active teaching career and competitive research laboratory. Using yeast genetics and novel biophysical techniques, my proposed research program is poised to greatly increase our understanding of DNA repair in the cell. The enclosed research summary and CV will demonstrate my diverse and rigorous training in chemistry and biology. This training has provided me with a broad repertoire of skills and qualities that enable me to address meaningful topics in contemporary biology. The yeast mating type switch is an excellent system to study cellular differentiation as the cell morphs from one mating type to another. Further my research proposal will clarify our understanding of the signaling networks which underpin the DNA damage response. I consider myself a strong candidate for the position advertised.

I obtained a B.S. in Biochemistry from Auburn University where I did undergraduate research in Organic Chemistry. As a graduate student at the University of Texas at Austin, I examined the biochemistry of homologous recombination proteins in yeast and *T4 bacteriophage* with Professor Thomas Kodadek. I spent 6 months exploring the biochemistry of the Rad54 protein with Patrick Sung at the UT-Health Science Center in San Antonio, Texas.

As a postdoctoral researcher, I have used *Saccharomyces cerevisiae* as a model organism to study mechanistic details of the directed chromosomal rearrangements associated with mating type switching. As part of this project, I have developed a novel flow cell system allowing the dynamics of homologous recombination to be observed in a live cell for the first time! Using this technology, I have shown that homologous pairing intermediates in recombination are reversible until subsequent elongation by a DNA polymerase holoenzyme. This represents a key new insight into the understanding of how the outcome of DNA damage is managed by the cell. This insight suggests that many possible manifolds of repair are in equilibrium with each other that may be dictated by restriction points. Such restriction points need to be identified and represent one aspect of my proposed research. Together, this background has given me extensive interdisciplinary training in chemistry, molecular biology, biochemistry, molecular genetics, quantitative cell biology, bioinformatics and microscopy, which can be combined into systems biology.

As my research proposal details, I plan to maintain a program that incorporates many of these skills. I will develop the technology for examining fluorescence based reporters in large arrays of yeast cells using existing microarray reader confocal tools. To demonstrate the utility of this approach, I will identify DNA damage signaling components by creating reporter vectors that contain promoters of genes identified as being implicated in this pathway through microarray analysis. I will use synthetic genetic array (SGA) methods, which have been important in identifying a network based on genetic interactions, to introduce mutants by judicious crossing and marker selection. Mutants with a reduced signal in the reporter will be characterized to better understand the respective gene's function in the DNA damage signaling network.

During the time I have spent as a postdoctoral fellow and graduate student, I have directed undergraduate, graduate students and technicians in a variety of classroom and laboratory settings. I have taught core advanced Organic Chemistry, Advanced Biochemistry and Molecular Biology laboratory courses and literature precepts, and successfully trained and supervised laboratory personnel including graduate students and technicians.

My research has always been funded and my results were the key to the renewal of grant applications. My curriculum vitae, including contact information for five references, summary of my research accomplishments and interests are enclosed. I have asked my references to forward letters directly to your department. If you require any additional information please feel free to contact me or refer to my website.

Thank you for consideration of my application. My background, skills, viewpoint and experience would be an asset to your department. I look forward to hearing from you soon.

Sincerely,

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Research Accomplishments

The primary findings of my doctoral research at UT Austin were centered on the mechanism of recombination in eukaryotes. Here I identified the Rad54 protein as an important factor specific to the eukaryotic strand exchange system. I found that the *RAD54* gene encodes a double stranded DNA dependent ATPase, which binds the Rad51 protein. Interactions of Rad54 protein with the Rad51 protein could facilitate the search for homology or other functions associated with strand invasion (Jiang et al., 1996). For instance, Rad54 protein function has been implicated in the removal of Rad51 protein from late strand exchange intermediates before the loading of a DNA polymerase (Solinger et al., 2002).

In addition, I developed a simple real-time fluorescence based assay for double stranded DNA unwinding. This assay is necessary for the detailed analysis of the mechanism of protein mediated DNA unwinding activity, helicase, and could identify the mechanism of action of these enzymes or be an important tool for drug discovery based on the design of inhibitors in a high throughput manner (Houston and Kodadek, 1994).

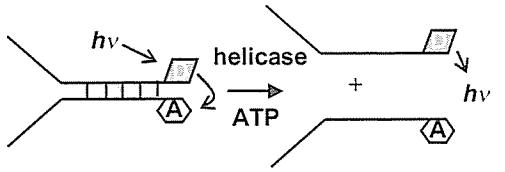

Figure 1. Fluorescence-based assay of DNA unwinding. In the duplex DNA the donor acceptor pair are in close proximity and the donor is quenched. Upon melting with a DNA helicase, the ATP donor is free and emission increases.

As a postdoctoral researcher I examined the donor preference in yeast mating type switching. Yeast has the remarkable ability to change mating type, at *MAT*, through homology directed gene conversion and biased selection of silent mating type donors, *HMLα* and *HMRa,* see Figure 2. Three different strategies were employed: a hetero-allelic reporter system, a live cell homologous pairing assay and a genetic screen to identify genes involved in donor selection.

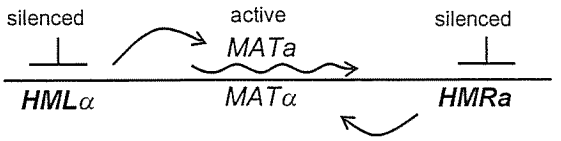

Figure 2. Schematic representation of donor selection in the yeast mating type switch. Two silenced donors are selected to donate mating information in a biased fashion: *MATa* selects *HMLα* and *MATα* selects *HMRa.*

Hetero-allelic recombination reporter system- I developed an *ARG4* prototroph based recombination reporter system to examine the rate of recombination between the mating loci that is governed by a recombination enhancer, *RE*. The *RE* is a *cis*-DNA binding region that controls recombination both positively and negatively (Szeto et al., 1997; Wu et al., 1998). It contains tandem DNA binding sites for α2 and Mcm1 proteins, which are required for selection of *HMR* and *HML,* and acts 14 kilobases away to effect recombination between *MAT* and *HML*. This is the only known long range DNA interaction in yeast and the mechanism of how these loci interact could serve as a model for enhancers in higher eukaryotes. Measured recombination frequencies between the mating loci indicate that the *RE* operates in an intra-molecular fashion but not an inter-molecular fashion for hetero-allelic gene conversion (Houston et al., 2004).

Live cell homologous pairing assay- Complementary to this hetero-allelic recombination approach, I have developed a system utilizing fluorescence based tagging methodology in living cells that enables us to observe loci undergoing recombination. Using a GFP-mediated tagging strategy, one can express LacI-GFP and TetR-GFP fusions in order to observe *lacO* and *tetO* arrays as bright dots in the nucleus of live yeast cells (Michaelis et al., 1997; Straight et al., 1996), see Figure 3 and 4. When the arrays are placed at the mating loci, which are known to undergo gene conversion during mating type switching, the details of the recombination process can be examined. I showed that no observable difference in the relative location of the mating loci exists under normal conditions. However, upon initiation of mating type switching by the expression of the HO endonuclease that cuts at *MAT*, the proper loci became coincident (Simon et al., 2002). This result demonstrates that proximity of the proper loci does not control the bias in donor selection and the

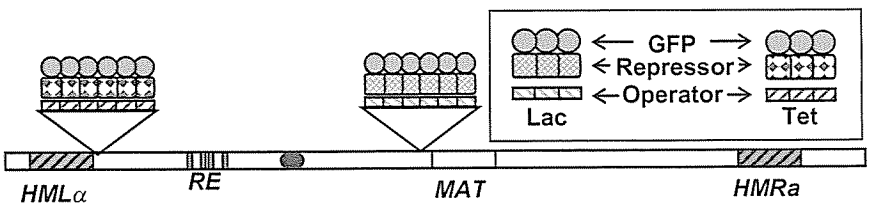

Figure 3. Observation of specific DNA in live cells. The integration of *lacO* and *tetO* arrays at the mating loci with expression of LacR-GFP and TetR-GFP chimeras illuminates bright dot in the cell, which corresponds to the labeled loci.

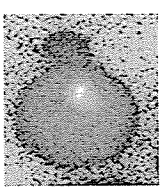

Figure 4. Yeast cells expressing GFP fusions, GFP localizes to the arrays as a discrete spots.

controlling step happens at the commitment to recombination. I have developed a flow cell technology in which I can examine live cells performing the mating type switch by high performance deconvolution microscopy. By interrogating numerous cells every minute for 180 minutes I can observe a statistically significant difference in the HO-mediated cut versus the uncut control distribution. The effect indicates that there are more persistent associations in the cut control that correspond to the recombination events, see Figure 5. The events start to occur 80 minutes after induction of the HO cut and persist for 90 minutes while the duration of the events is less than 20 minutes and there is more than one event per cell. The results indicate that the recombination is quick, with the association events happening on average in 10 minutes, and over a range of about 2 hours. Since there is more than one event per cell, I concluded that the strand exchange intermediate is in equilibrium with the unpaired state. I have extended this approach to examine several mutant yeast strains. Since I observed the same effect in the *rad54Δ*, a mutant that does not complete the recombination process, I could rule out that rapid cleavage and repair is occurring during these associations.

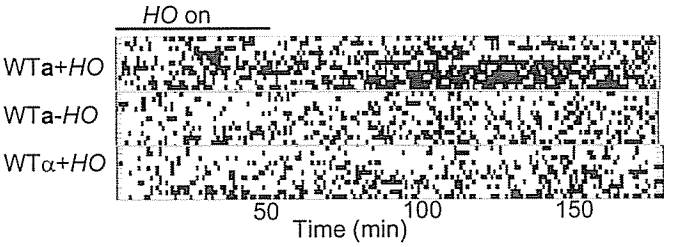

Figure 5. Individual cell traces 11 cells under each condition on the y axis sorted least associated, top, to most associated, bottom. Matrix is black if the loci associated at that instant, and white if the loci was not associated. Events in the wild type *MATa* cut with the HO nuclease at the top correspond to recombination.

A genetic screen to identify genes involved in donor selection- I have developed a system of serial mating and judicious marker selection using ordered arrays of yeast mutants to replace the donor loci with fluorescence based reporters *hmr::CFP* and *hml::YFP*. Upon switching, the yeast cell fluoresces as the fluorescent reporter replaces the mating information at *MAT*. The outcome of the selection can then be observed in each mating type. In the wild type case *MATa* will become yellow by selecting *hml::YFP,* and *MATα* will become cyan by selecting *hmr::CFP*. Using this approach, I have generated such strains with every viable yeast mutant. Thus, I can examine the genetic requirements for this fascinating phenotype.

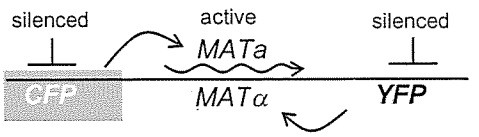

Figure 6. Fluorescence-based assay of donor selection. Bias in selection of loci in a cell dependent manner can be observed by replacement of donors with fluorescent markers.

The results of my research provide a new viewpoint into how the cell repairs double stranded DNA breaks. The dynamic nature was not appreciated previously, and is consistent with the analysis of recombination in large populations of cells at steady state (Holmes and Haber, 1999). Thus, my research experience has provided me with the skills to help me examine DNA strand exchange processes from a biochemical to a physical and genetic point of view.

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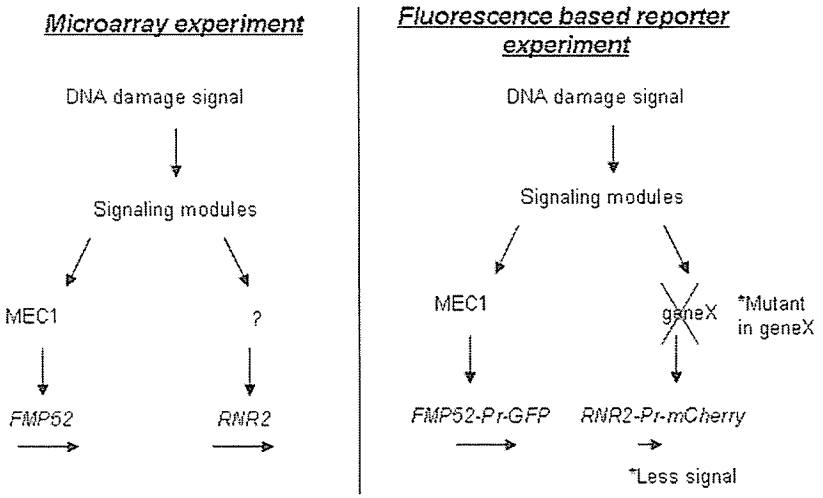
A Reverse Genetic Systems Biology Approach to Identify DNA Damage Signaling Components

The study of biology has undergone a post-genomic transformation based on the ability to use computers, robots and high throughput experimental techniques to gather and analyze large amounts of data. Thus, the study of biological problems now includes not only hypothesis-driven science, but also discoveries of interesting systems that originate from the examination of large data sets.

Specific Aims

1) Construct protein coded fluorescence based reporters of promoters that are specific and independently regulated in the DNA damage response.
2) Cross the reporters into an ordered array of the viable yeast deletion set using established SGA methods.
3) Develop methodology for the high throughput analysis of reporters in yeast using a confocal microarray format.

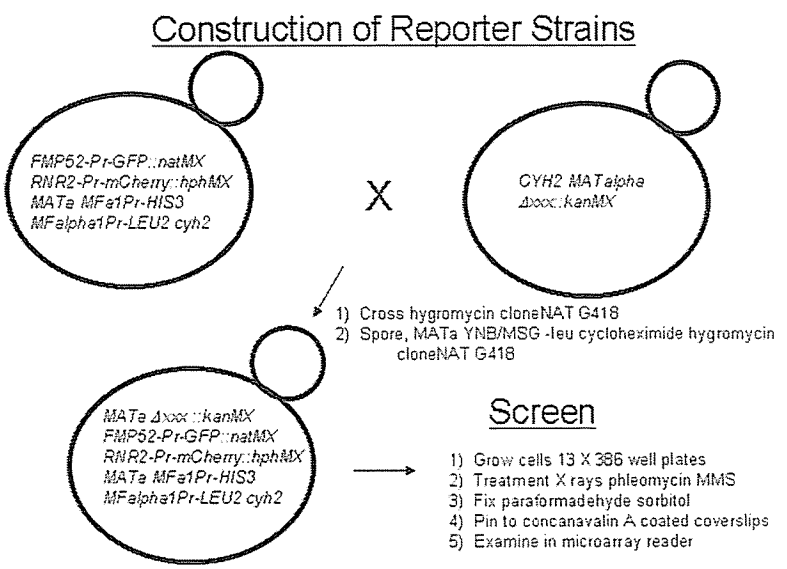
The aim of my research is to create a general method by which yeast deletions can be utilized to identify unknown components of cellular signaling modules that have been identified in microarray experiments. cDNA microarray databases give a footprint of outputs derived from a signaling network's integration of complex inputs. While systematic and directed modification of signaling apparatus by a candidate approach can be useful in the identification of new components of the signaling network, there are still many systems where signaling components affecting the microarray output are unknown, and whose identification and characterization is required to understand how the system functions. For instance, DNA damage elicits a response that is dependent on known signal transduction modules in yeast, like *MEC1*, and some outputs that are independent of *MEC1* and unknown. By introducing all viable deletion mutants into a strain that contains florescence based reporters of central outputs of the response, I will identify genes that modify the reporters signal and thus will be required for the attenuation of the response. Further, I propose novel high-throughput methodology to execute the proposed work.



Construct florescence based reporters of the DNA damage response- I will demonstrate the usefulness of this system in illuminating signaling components involved in the DNA damage-induced response by pairing the fluorescence based reporters with interesting promoters. Gasch and coworkers identified that *FMP52* and *RNR2* mRNAs were induced by DNA damage but largely unaffected by other stresses (Gasch et al., 2001). Specifically the *FMP52* mRNA was induced in a *MEC1* dependent manner and *RNR2* was partially *MEC1* independent. Therefore, I will construct *FMP52*-Pr-GFP::natMX and *RNR2*-Pr-mCherry::hphMX transcriptional fusions using PCR-mediated integration (Longtine et al., 1998).

Serially cross reporters into mutant strain backgrounds- These reporters can be crossed to every mutant in the ordered deletion set array by positive selection of the antibiotic resistance markers using synthetic genetic array (SGA) methods (Davierwala et al., 2005). Upon sporulation and selection I will have over four thousand strains, each with a particular mutation, *Δxxx::kanMX*, and both reporters that can be queried to find out if the mutant is required for the output of the DNA damage signal. These strains can then be exposed to a range of DNA damaging conditions and analyzed by microscopy or FACS to obtain a metric of the promoter output for each mutant strain background. Interesting mutants, *Δxxx::kanMX*, that show diminished signal of the reporters relative to the wild type strain will be selected and further examined. Northern blot analysis to measure the mRNA output of the *FMP52* and *RNR2* genes in the wild type strain versus the mutant, *Δxxx::kanMX*, will be performed to confirm the validity of this technique.

Confocal optimized fluorescence based reporters- I will develop a method for high throughput reporter analysis through the construction of fluorescence reporters and optimization of their usefulness. The outputs of the regulatory network will be coupled to a reporter system which uses GFP and mCherry (Shaner et al., 2004). These fluorescent proteins have the advantage of being much brighter and overlap with common lasers used in FACS and confocal microarray readout (Thorn, 2005). I must first construct high output strains to facilitate the development of this methodology. Therefore, I have constructed strains that have overexpressed these reporters by placement on a high copy plasmid or by induction of a *GAL1-10* promoter. I will use these strains to optimize conditions such that the microarray reader gives a significant signal. I have successfully quantified GFP and mCherry by FACS. After a significant signal is detected, I can optimize the system to use these constructs as reporters for gene output in the entire viable yeast mutant array.



Analysis of fluorescence reporters by confocal microarray technology- The efficient analysis of the strains can be performed using existing technology and novel techniques that I propose here. After the cells have been given the insult of DNA damage by X-rays, the radiomimetic drug phleomycin, or the alkylating agent MMS, they will be fixed and pinned robotically to the microarray slide or examined by high throughput automated FACS. In the former method, the cells can be attached to a microarray coverslip that is concanavalin A coated after being fixed using paraformaldehyde and sorbitol. Scanning of the coverslip with microarray technology or analysis by automated FACS will quantitate the gene's output. From this, I can identify genes to DNA damage specific function based on their effect on the reporter output in a condition relative to the control in every mutant background. Since the interesting mutants are diminished in activation versus the wild type, I can assign these as essential for the activation of that gene in a DNA damage dependent manner. One added advantage to this approach is the use of a protein-based readout that will diminish artifacts based on translation and mRNA turnover. It may be possible to detect overexpression of the promoter in some mutants that will indicate the gene is required for tempering the output of the signal. This technology can be modified to find genes that are master regulators of other important phenotypes in any system where reporters can be introduced, mutants quickly generated and microarray data is available to identify key promoters.

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Teaching Statement

Many aspects of the instruction process are beyond the scope of this document; however I wish to mention a few important concepts that will give you insight into my teaching style and philosophy. First, the assessment of the students is necessary to identify strong and weak points in the class so these can be addressed in the lesson plan. Starting the course with an atmosphere of rigor insures that the student is committed. This assessment can be balanced with flexibility later in the course. The teaching media should be variable, since it is not always appropriate to teach using one type of media. While a focus on memorizing facts and a classical approach may be necessary in some courses, a close examination of the thought processes leading to discovery or breakthrough is often more meaningful. Below are expanded examples of the methods that can be applied in the classroom.

The primary assessment is vital to addressing certain needs of the student and to capitalize on the strong points of students' prior knowledge. A primary assessment can be initiated by giving an entry exam or by an oral question and answer discussion. To keep the class interest it may be necessary to give remedial training to those in need. The advanced students can be given extra material. The curriculum most helpful would include a breadth of material that can be applied simultaneously to a variable student group. A standard that is at or above the mean of the class is appropriate since the advanced student should not be left idle while the less prepared students are playing catch up. It is important to set a pace that will allow a thorough analysis of the course material for all.

Another advantage to giving an exam early in the course is to set a standard of rigorous study. It also provides students a chance to drop the course if they discover it is not appropriate for their present goals. A rigorous beginning can be balanced on the back end of the course as students can be given leeway to drop an exam or do special projects for extra credit. The ability to get a course off to a good start can make the difference in the attentiveness, work ethic and attitude of the student and therefore should be a high priority.

The teaching media and the approach to the material are important facets of the teaching program. These deserve attention and are an asset to the teacher in portraying the curriculum. Enhanced computer visual aids can often overcome conceptual problems students may have in subjects like cell biology and chemistry. However, it is not a good idea to have the same media throughout the course of the lectures. Repetitive media format causes the student to become dependent on a particular media type and could lead to the development of bad study habits. PowerPoint, for example, is easy to prepare and disseminate but can lead to over reliance on clear visual representation of the material presented. It frequently handicaps one who is giving a chalkboard lecture, as the student may not take in the material as well. The use of chalkboard lectures, photocopied overheads, PowerPoint, TV and movies engages the student on multiple levels. Furthermore, the ability to explain complicated topics becomes more acceptable.

I believe a well rounded approach that presents coursework in a classical lecture style is necessary for sound teaching but it can become monotonous. It is important to examine facts and history. This examination in light of the concepts that led to the discovery or historical event can enhance the student's ability to understand the substance of the coursework. However, it is better to give the student the tools to examine additional facts in the library when needed. Further, the engagement of the student in the act of discovery helps to nurture the scientific method in the students' problem solving repertoire. It is most important to nurture critical ability so that the student can be autodidactic in the subject after the course had ended. My teaching methods have been successful and would be an asset to your department. I look forward to discussing them with you.