CBI STUDENT SABBATICAL PROPOSAL

08/05/11

Derek M. Lyons, Chemical Biology Program

Patrick J. O’Brien, Department of Biological Chemistry

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University of Michigan

10 weeks

August/September 2011

I will complement my *in vitro* characterization of Base Excision DNA Repair (BER) mediated frameshift mutations using *S. Cerevisiae* to observe the affect of imbalanced DNA repair pathways on frameshift mutation rates *in vivo*. A reporter gene has been engineered into yeast that allows auxotrophic selection of a frameshift mutation. Heterologous expression of human BER enzymes in combination with DNA damaging agents are expected to increase the rate of frameshift mutagenesis, creating a signature of bias toward -1 frameshift events.
Alteration of Frameshift Mutation Rate Resulting from Imbalanced DNA Repair

Background and Significance:

Genome instability is a hallmark of cancer cells, providing an increased rate of mutations that facilitate the global changes in gene expression that drive carcinogenesis\(^1,2\). Accurate DNA repair provides a crucial defense against genome instability and is often misregulated in cancer cells\(^3\). For example, downregulation of the Mismatch DNA Repair (MMR) pathway is clinically identifiable by an increased rate of frameshift mutations\(^4\). A frameshift mutation occurs by the loss or gain of one or more basepairs within a gene, often knocking out gene function by interrupting the codon sequence\(^5,6\). MMR prevents frameshift mutations by intercepting a bulged, unpaired nucleotide product of a polymerase slipping error\(^7\). Intriguingly, some cancers display an increased frequency of frameshift mutations in the absence of MMR dysfunction\(^8\).

We have demonstrated the ability of enzymes in the Base Excision DNA Repair (BER) pathway to recognize and improperly process the bulged nucleotide resulting from polymerase slipping, which could compete with MMR for proper repair of the bulged intermediate \textit{in vivo}. Several cancers upregulate glycosylases, the enzyme responsible for initiating BER, causing an imbalance of repair pathways and increasing the ability of BER to compete against MMR\(^9\). Upregulation of eukaryotic and bacterial glycosylases in living cells correlated with significant increases in frameshift mutation rates\(^11,12\), however, it is still
unclear what mechanism the glycosylase is using to produce frameshift mutations.

The glycosylase provides specificity for BER by recognizing and excising a wide array of damage nucleotides. The human cell has 11 known glycosylases, each providing specificity for a subset or type of damage. Human alkyladenine-DNA glycosylase (hAAG), responsible for recognizing and excising alkylative and oxidative damage to purines, has been reported to bind with high affinity to an undamaged, bulged purine\textsuperscript{13,14}. This model would passively block repair by MMR and lead to a frameshift mutation upon the next round of replication\textsuperscript{14}.

**Preliminary Studies:**

We utilized a combination of biochemical assays to demonstrate the ability of BER pathway to actively process a bulged nucleotide into a deletion. Screening undamaged and damaged bulged nucleotides in HeLa whole cell extracts displayed robust excision of damaged nucleotides in a bulge. Undamaged nucleotides in a bulge were unprocessed. Incubation of an undamaged adenine bulge with the alkylating agent chloroacetaldehyde, showed that an undamaged bulge is 18-fold more susceptible to incurring damage than a canonical basepair.

Recent reports demonstrated that several of the human glycosylases possess the ability to excise a damaged nucleotide in a bulge\textsuperscript{9,15,16}. Using recombinant human enzymes, we reconstituted BER in vitro and showed processing of a damaged bulge produced the expected intermediates and a deletion product. Incubating a damaged bulge containing oligonucleotide in
several different cancer cell line extracts displayed the ability to delete a damaged bulge, implicating a damaged bulge as a substrate for production of BER mediated frameshift mutations and providing an explanation for a bias toward -1 frameshift mutations\(^9\).

\textit{In vitro} biochemical reconstitution permitted observation and control of several steps of BER processing a damaged bulge into a deletion\(^9\). Expanding on \textit{in vitro} work, we propose to perturb the balance of repair pathways in living cells and quantitate the affect of imbalanced DNA repair pathways on the frequency of frameshift mutagenesis in combination with DNA damaging agents.

**Aim 1: Quantifying the effect of glycosylase upregulation and DNA damage on frameshift frequency.** Measuring affects of combining imbalanced DNA repair and DNA damaging agents on frameshift mutation frequency in \textit{S. Cerevisiae}.

1.1 A frameshift mutation reporter gene has been engineered into \textit{S. Cerevisiae}\(^17\). Strain E133 contains a 14-basepair poly-A tract insert in the Lys2 gene that inactivates the Lys2 gene by being out of frame. Repetitive, homopolymeric tracts increase the frequency of polymerase slipping events and are a hot spot for frameshift mutations. A -1 frameshift within the poly-A tract will produce a reversion to an active Lys2 gene product and provides growth on media lacking lysine. Strain E134 contains a 12-base insertion that a +1 frameshift will revert back into frame.

Overexpression of galactose-inducible hAAG for 16 passages in E133 and E134 strains provided a 20-fold increase in -1 frameshift mutations and a 5-fold increase in +1 frameshift mutations\(^12,14\). I will confirm this report, providing a
baseline affect of hAAG overexpression on frameshift rates that can be compared to cells grown with a combination of hAAG overexpression and DNA damaging agent. Chloroacetaldehyde produces an hAAG specific lesion, selectively damaging bulged adenines⁹.

Yeast will be propagated under four separate conditions to quantitate the frameshift mutation rate. Overexpression of hAAG in combination with chloroacetaldehyde is expected to significantly increase the rate of -1 frameshift mutations and proportionally decrease +1 frameshift mutations in comparison to hAAG overexpression or chloroacetaldehyde conditions alone. An empty expression vector, lacking hAAG, will provide a baseline for frameshift mutation rate. Frameshift mutation rates will be measured by counting the number of colonies that grow on media lacking lysine, due an appropriate frameshift mutation putting the Lys2 gene in frame, divided by the number of viable colonies that grow on media supplemented with lysine. Therefore, the predicted increase in -1 frameshift mutation rates will increase survivability of strain E133 in auxotrophic conditions.

1.2 My unpublished data has utilized in vitro biochemical assays to characterize the bulge excision activity of hNth1 (Endonuclease III homolog 1), a human glycosylase that recognizes and excises oxidatively damaged pyrimidines¹⁸. After producing a plasmid for Nth1 overexpression in S. Cerevisiae, I will overexpress Nth1 in SJR1243 and SJR1244 strains, each containing poly-C tracts to observe the affect of Nth1 on the rate of frameshift mutations and observe any bias toward -1 frameshift mutations. Analogous to
1.1, overexpressing hNth1 in combination with hydrogen peroxide is expected to increase the bias toward -1 frameshift mutations.

1.3 Several active site mutations in hAAG are known to decrease selection against excision of undamaged nucleotides. N169S mutation increases the rate of undamaged guanine excision *in vitro* by 50-fold\(^{19,20}\). Overexpression of N169S hAAG in SJR1243 and SJR1244 strains is expected to increase the frequency of -1 frameshift mutations in G/C tracts in comparison to E133/E134 strains containing A/T tracts.

**AIM 2: Deep sequencing yeast genome for frameshift mutation spectrum.**

2.1 Recent advances in DNA sequencing technology allow rapid and cost-effective sequencing of the entire yeast genome using paired-end sequencing to facilitate high levels of genome coverage using short genome fragments\(^{21,22,23}\). Lacking the need of a specific reporter gene, the genome sequence can be mined in its entirety for frameshift mutations. Overexpression of hAAG is predicted to reveal hot spots of frameshift mutations, potentially endowed from tertiary structures or DNA sequence context. For example, the known hypermutability of homopolymeric runs will serve as a useful control for our ability to detect hot spots of frameshift mutations.

Propagating E133/E134 yeast strains for up to 100 passages, up to twelve parallel colonies will be chosen and individually sequenced on a single Illumina lane, utilizing software to reconstruct the genome\(^{24}\). Overexpression of hAAG is expected to cause a significant increase in frameshift mutations, with a particular bias toward -1 frameshift mutations or deletion of a basepair within a gene.
Additionally, growth under DNA damaging conditions is predicted to enhance the bias for -1 frameshift mutations.

References:

Derek Lyons
Patrick O’Brien Laboratory
Chemical Biology Program
Department of Biological Chemistry
University of Michigan

Re: CBI Student Sabbatical

Dear Derek:

I am writing to indicate my willingness to act as mentor for the sabbatical portion of your training program in Chemical Biology. I am very familiar with the project through many discussions with Dr. O’Brien and am eager to see the work get under way. I have many different backgrounds that make me an ideal co-mentor for this project directed at exploring the frameshift mutagenic potential of BER enzymes in conjunction with DNA damage. First, I have a long standing interesting and expertise in DNA repair. Second, my lab has traditionally been a yeast lab and we are fully equipped and knowledgeable with the handling of yeast for your genetic experiments. Finally, I have more recently developed extensive expertise in high throughput sequencing as applied to mutation detection in many organisms, including having developed software platforms for analyzing the data. Of particular interest to your work, we have applied these tools to the identification of yeast mutations in other experiments, as we recently published in Genetics. I am very confident that these tools will be of great use to exploring the genome-wide mutagenic potential of BER enzymes. Moreover, I know that you will have a great exposure in my group to very modern computational assessment methods of such data. In a short sabbatical you may not become completely expert in their use, but at a minimum you will become familiar with their execution and the methods used. I think such experience will be invaluable to anyone’s future career in modern science.

I look forward to working with you.

Sincerely,

Thomas E. Wilson

July 26, 2011
Dr. Anna Mapp, Interim Director
Department of Chemistry
University of Michigan
Chemistry/Biology Interface Training Program

August 2, 2011

RE: Letter of Support for Derek Lyons

I am writing to express my enthusiastic support for Derek Lyons’ proposed research sabbatical in the lab of Dr. Tom Wilson in the Department of Pathology at the University of Michigan Medical School. Dr. Wilson will be an excellent mentor for this project and he provides expertise in computational biology and yeast genetics that I would not be able to provide on my own. Derek proposes to perform genetic experiments and to apply modern DNA sequencing technology to test several hypotheses that have come out of his thesis work studying human DNA repair pathways. One of the key findings that Derek has made is that human base excision repair pathways can cause genetic instability if there is an imbalance between repair pathways. In collaboration with the Wilson lab Derek will be able to test some of these hypotheses in cells. Beyond the scientific objectives, this experience will broaden Derek’s exposure to new experimental techniques and new perspectives that will be invaluable for his training as a future leader at the interface between biology and chemistry. I look forward to hearing about the results from this exciting project.

Sincerely,

Patrick J. O’Brien, PhD

Sincerely,

Patrick J. O’Brien, Ph.D.
Assistant Professor of Biological Chemistry