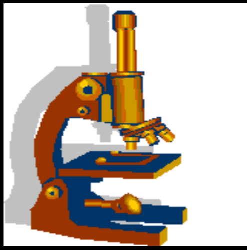

IBASM

Volume 7, #3 July 2005

News Letter

Message from the President

Jeanne Barnett

What a great meeting in 2005! Dominique will give you a few details about the attendance. The research presentations were excellent. My thanks goes to those giving oral presentations. The research was interesting and you kept us on schedule. We will continue with oral presentations by students for the 2006 meeting.

For those who did not attend the meeting, I am going to give a quick overview of the events. Friday evening began with the student presentations. These were followed by our Waksman Foundation speaker, Dr. Ralph Tanner. Dr. Tanner gave an intriguing talk on research done with his students examining the bacteria in the Canadian River and other aquatic sties near Norman, OK. The organisms were identified, when possible, and their antibiotic resistance determined. Even in pristine areas, bluegill were colonized by ciprofloxacin-resistant *Enterococcus*. There was an increase in multiple drug resistance in other organisms found. This raises questions about induction and occurrence of drug resistance in organisms found in various environments. Our

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Message from the President-Elect

Dominique Galli

I want to thank all of you who attended this year's annual meeting at Brown County State Park. We were very pleased with the outstanding quality of the oral and poster presentations given by our students. A special thanks goes to all the judges for their hard work and to Mark Levinthal, who was in charge of the bar. We had 98 attendees (25 full members and 71 student members) and I hope to see you all again next year when we meet at the Canyon Inn at McCormick's Creek State Park on April 21-23, 2006. We are currently considering

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J. Barnett's message (continued from page 1)

organisms found in various environments. Our thanks to Ralph for an intriguing and timely presentation.

Saturday morning was filled with poster presentations. Thirty-one abstracts were submitted. Again, the quality of the research was impressive. All the presenters are to be commended.

After a beautiful afternoon, provided by Mother Nature, we were treated to 2 excellent presentations. The first was by the Teaching Award winner, Dr. Nancy Behforouz. Nancy received the award from IBASM for her years of outstanding teaching and service at Ball State University. Nancy's presentation was a sobering update on HIV/AIDS in Africa. This was a reminder of the increasing number of affected individuals and what can be done to address the problems.

Our final speaker was the current ASM president, Dr. Jim Tiedje. Jim gave an elegant presentation on the relatedness and environmental impact of microorganisms. In addition, we had the opportunity to discuss the relationship between the National ASM and the Branches. This was a very productive and informative discussion. It was truly an honor to have Dr. Tiedje join us.

News from the National ASM includes the opportunity for you to renew your Branch membership when you renew National membership. Christian Chauret will give you more information on that in this newsletter.

IBASM received a $2000 grant from Region III. This grant will allow us to continue to support student presenters at the Branch meeting. In 2004, IBASM had the largest membership/ attendance in the Region. Let's keep up this progress.

Region III is looking for a new Regional Branch Coordinator. Dr. John Stolz is the current coordinator. If you are interested in volunteering for the position, please end your name to John at

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This is an excellent opportunity to become involved in ASM.

The planning committee is contemplating a one-day meeting in the fall, 2006. We are looking at one of the participating campuses (likely University of Southern Indiana) for the day. If you are interested in this type of meeting, please let me know so we can decide whether to continue to plan. Send your indication of interest to me via e-mail at Barnett@usi.edu.

It's time to mark you calendars for 2006. The planning committee is busy putting together the meeting at McCormick's Creek State Park. The dates are April 21 – 23, 2006. We have 3 excellent speakers arranged for the meeting. Dr. William Summers, Yale University, will be our

J. Barnett's message (continued from page 2)

Waksman speaker. Dr. Yves Brun, Indiana University and the IBASM Academic Research Award winner for 2005, will present his research. The third speaker is Dr. Stanley Maloy, the current president of ASM. This promises to be an exciting meeting. Mark the dates on your calendar and look for more details in coming newsletters. I hope to see all of you there.

D. Galli's message (continued from page 1)

some changes to the registration process in 2006, including a switch to electronic submission of registrations. In addition, we will give you two options when making your room reservations. You can either book your room with us, which will exempt you from paying taxes but will require payment of lodging in advance, or you can book your room directly with the Canyon Inn. More details will follow in our next newsletter.

Call for Nominations

President-Elect 2006-2008

We are asking for you help to nominate candidates for the position of President-Elect for the next two-year term starting on July 1, 2006. Responsibilities include the arrangement of the 2007 and 2008 annual meetings. Elections will be held at the annual meeting in 2006. Self-nominations are encouraged. Potential candidates have to be full members. Please send your recommendations to Jeanne Barnett at Barnett@usi.edu.

Academic Awards 2006

We would like to invite you to nominate candidates for the Academic Scientific Achievement Award and the Academic Teaching Award. See http://users.ipfw.edu/merkel/conbylaws.html for selection criteria. The award will be presented at the annual meeting in 2006. Please send your nominations to Carl Bauer at cbauer@bio.indiana.edu.

Message from the Secretary-Treasurer
Christian Chauret

Changes are on the way for membership dues! A few months ago, IBASM received a letter from National ASM. In part, the letter stated:

> "National ASM has been looking for ways to increase membership in the Branches. At its last meeting in March, Council Policy Committee approved an initiative that was proposed by the Branch Organization Committee. The proposal was to have National collect Branch dues at the same time people rejoin National on-line. When a member goes to the National website to join or renew, one of the dialog boxes would say, "Do you want to join a Branch?" If the answer is affirmative, there would be a link to a page listing the Branches and showing their geographic boundaries. The person clicks on the Branch they wish to join and they are displayed information about the amount of the dues for that Branch. They can then add that amount to their payment to National. "

The IBASM officers have decided to join this initiative, which means that as of October 1, 2005, full IBASM members will be able to pay their IBASM dues through ASM if they do it online. You will still have the option to pay your dues directly to IBASM as in the past if you wish to do so, and we will continue to publish a membership form in our newsletter. This change does NOT affect student dues. Students will continue to pay their dues directly to IBASM.

The IBASM officers have also approved the creation of a new student travel award to the General ASM Meeting. This award will partially subsidize the cost of traveling to the General Meeting for one student. This competitive award will be awarded at the branch meeting to a student who will present at the General Meeting. More details will be available prior to the branch meeting, but the format of this competition will be similar to the current student competition.

Finally, ASM is sponsoring a CONTEST to design a new logo for ASM branches and to promote the branches. The logo will be used by ASM for various purposes. Logo entries can be designed by any individual but must be submitted by the Branch. All entries will be judged by the Branch Organization Committee. The logo artist and Branch will receive awards of $1000 and $500, respectively. One of the following formats must be used: .AI, .EPS, .TIF, .JPG, or .PSD. If you are interested, please contact Christian Chauret (cchauret@iuk.edu) for more details.

McClung First Place Graduate (Ph.D.) Winner

Adaptive Mutation Occurs at Various Locations on the Episome in *E. coli* Strain FC40

Jeffrey D. Stumpf, William A. Rosche, and Patricia L. Foster
Department of Biology, Indiana University, Bloomington, IN

Mutation rates in bacteria can be affected by stressful events, such as exposure to UV or chemical mutagens. However, the effect of mutation rates during starvation is controversial. The paradigm for studying mutagenesis in stationary phase has been *E. coli* strain FC40. This Lac$^-$ strain reverts to Lac$^+$ at an abnormally high rate during lactose selection, a phenomenon known as adaptive mutation (Cairns and Foster, 1991). Under these same conditions there is no increase in chromosomal mutations in Lac$^-$ population; therefore, these mutations appear to be directed to *lac*. This observation led to intense study of the characteristics of adaptive mutation. FC40 is deleted for *lac* on the chromosome and contains a +1 frameshift in the *lac* operon on the F' episome (Müller-Hill *et al.*, 1964). Unlike mutations that occur during the growth of the culture, adaptive mutations are almost exclusively due to a -1 frameshift mutation (Foster and Trimarchi, 1994;Rosenberg *et al.*, 1994), which is primarily caused by the error-prone DNA polymerase IV (Pol IV) (Foster, 2000;McKenzie *et al.*, 2001). The rate of adaptive mutation is increased 100-fold when *lac* is on the episome (Foster and Trimarchi, 1995;Radicella *et al.*, 1995), and these mutations require recombination functions (RecA, RecBCD) (Cairns and Foster, 1991;Harris *et al.*, 1994) and a nick at the origin of transfer (Foster and Trimarchi, 1995;Galitski and Roth, 1995;Radicella *et al.*, 1995;Rodriguez *et al.*, 2002;Foster, 1997). One explanation for these results is described by the following recombination-dependent mutagenesis model (Foster, 2004). The persistent nick on the episome that occurs even during stationary phase (Frost and Manchak, 1998) leads to a double-strand break after episome replication; the double-strand break is repaired by homologous recombination and subsequent DNA replication by the available DNA polymerase. Pol IV may be more available than other polymerases because it is upregulated three-fold during stationary phase (Layton and Foster, 2003). The recombination-dependent mutagenesis model predicts that during starvation the mutation rate at various locations on the episome should increase regardless of their proximity to *lac*.

There are three nutritional requirements for adaptive mutation: the *lac* allele must be leaky (the allele in FC40 produces about 1% of wildtype â-galactosidase) (Cairns and Foster, 1991; (Andersson *et al.*, 1998), lactose must be present in the medium (Cairns and Foster, 1991), and galactose metabolizing enzymes must be functional (Stumpf and Foster, unpub.). These observations led to the following amplification-dependent mutagenesis model (Roth and Andersson, 2004). During the growth of the culture, there is a subpopulation of cells that contain a duplication of the *lac* allele. Since the *lac* mutation is leaky, a strain with duplications in the mutant *lac* allele will express more â-galactosidase, allowing for slow growth and selection for amplification. As *lac* copy number increases, the cells are able to metabolize enough lactose to form microcolonies that contain more mutational targets. Even though there is an appearance of an increased mutation rate during starvation, the amplification-dependent mutagenesis model describes how the increase in Lac$^+$ colonies can occur without an increase in mutation rate during starvation. This model requires that adaptive mutations are limited to the amplified regions (10-40 kb centered at *lac*) (Andersson *et al.*, 1998) and that *lac* copy number must increase in the Lac$^-$ population during incubation in minimal lactose media.

Stumpf's paper (continued from page 4)

Second-site mutations were shown to occur on the episome in Lac$^-$ populations during incubation in lactose (Foster, 1997). Using a +1 frameshift mutation in the *tetA* gene that encodes tetracycline resistance (Tc^R), Foster, 1997 demonstrated that Tc^R mutants arose in Lac$^-$ populations during lactose selection. Furthermore, these mutations had the same genetic requirements as Lac$^+$ mutations. However, the *tetA* gene is only 4.7 kb away from *lac* (Hendrickson *et al.*, 2002), allowing the possibility that coamplification of *tetA* with *lac* caused the increase in the Tc^R.

Even though *tetA* confers tetracycline resistance, overexpressing wild-type *tetA* causes tetracycline sensitivity (Eckert and Beck, 1989;Godoy and Fox, 2000). This unusual phenotype was exploited to test whether *tetA* is being amplified (Hendrickson, *et al.*, 2002). Using strains with transposons 4.7 kb, 20 kb, and greater than 30 kb from *lac*, Hendrickson *et al.*, 2002 presented evidence that in the presence of tetracycline, adaptive mutation was low in the strain that had the transposon 4.7 kb from *lac* but higher in strains that had the transposon 20 kb and greater than 30 kb away from *lac*. They concluded that when *tetA* is only 4.7 kb from lac, wild-type TetA is overexpressed in potential Lac$^+$ revertants due to coamplification of *tetA* and *lac*, causing Lac$^+$ mutants to die. Our lab reproduced these results but also assayed adaptive mutation in these strains in the absence of tetracycline. All strains with transposons in the episome showed the same decrease in adaptive mutation when tetracycline was present, indicating that this decrease is independent of the proximity of *tetA* to *lac*. Tetracycline only decreases adaptive mutation when *tetA* is on the episome; there is no decrease in adaptive mutation when *tetA* is on the chromosome. We hypothesize that the effect if tetracycline on adaptive mutation is caused by increased *tetA* copy number as a result of being on the episome, not as a result of amplification with *lac*.

To test directly whether second mutations occur at various places on the episome, +1 frameshift mutations were created in the *tetA* gene placed at various distances from *lac* on the episome. We found that Tc^R mutants arose in a Lac$^-$ population under lactose selection, regardless of the proximity of the mutational target to *lac*, suggesting that an increased mutation rate on the episome accounts for adaptive mutation. Since Tc^R mutations were found over 30 kb away from *lac* (further than the largest known *lac* amplification), we conclude that amplification of the *lac* allele cannot account for all adaptive mutations.

Finally the amplification-dependent mutagenesis model requires that *lac* copy number must increase when Lac$^-$ cells are incubated with lactose media. We performed Southern blot analysis using a probe specific for *lac* and a second probe specific for *dnaQ* as a control for normal chromosomal gene copy number. For the first four days of incubation in lactose minimal medium, there was no increase in the amount of *lac* DNA relative to *dnaQ*. Since it takes two days for a Lac$^+$ colony to form, this result indicates that Lac$^+$ colonies that arise during the first six days of lactose incubation did not come from cells with a detectable increase in *lac*. On the fifth day, the amount of *lac* DNA doubled, indicating that some Lac$^+$ colonies arising seven days after incubation in lactose may result from an amplification of the *lac* allele. Other groups have reported evidence that some Lac$^+$ colonies arising seven days or more after plating result from amplification of *lac* allele (Powell S.C. and Wartell R.M., 2001;Hastings *et al.*, 2004). However, since many Lac$^+$ colonies arise in the first six days of incubation in minimal lactose medium, Lac$^+$ mutations must occur independently of amplification of the *lac* allele.

Stumpf's paper (continued from page 5)

In conclusion, we have generated evidence that mutations occur in a non-dividing population of Lac$^-$ cells under non-lethal selection at several sites on the episome. We conclude that the episome undergoes an increased mutation rate during nutrient limitation, and that this mutagenesis is dependent on recombination functions and Pol IV DNA synthesis. These observations have interesting implications for bacterial evolution. It has been estimated that 20% of natural isolates contain F conjugal plasmids (Boyd *et al.*, 1996). F plasmids can recombine with the chromosome and might facilitate horizontal transfer between *E. coli* and *Salmonella* strains. We hypothesize that a population of *E. coli* could have extra copies of genes on the F plasmid that would be exposed to a high mutation rate during starvation, allowing the possibility of gain-of-function mutations without the risk of lethal mutations (since there is a functional copy on the chromosome). The mutant allele on the episome could recombine into the chromosome or be transferred to another recipient cell, thus providing a powerful evolutionary mechanism.

References

Andersson, D.I., Slechta, E.S., and Roth, J.R. (1998) Evidence that gene amplification underlies adaptive mutability of the bacterial *lac* operon. *Science* **282**: 1133-1135.

Boyd, E.F., Hill, C.W., Rich, S.M., and Hartl, D.L. (1996) Mosaic structure of plasmids from natural populations of *Escherichia coli*. *Genetics* **143**: 1091-1100.

Cairns, J., and Foster, P.L. (1991) Adaptive reversion of a frameshift mutation in *Escherichia coli*. *Genetics* **128**: 695-701.

Eckert, B., and Beck, C.F. (1989) Overproduction of transposon Tn10-encoded tetracycline resistance protein results in cell death and loss of membrane potential. *J Bacteriol* **171**: 3557-3559.

Foster, P.L. (2004) Adaptive Mutation in *Escherichia coli*. *J Bacteriol* **186**: 4846-4852.

Foster, P.L. (1997) Nonadaptive mutations occur on the F' episome during adaptive mutation conditions in *Escherichia coli*. *J Bacteriol* **179**: 1550-1554.

Foster, P.L. (2000) Adaptive mutation in *Escherichia coli*. *Cold Spring Harbor Symp Quant Biol* **65**: 21-29.

Foster, P.L., and Trimarchi, J.M. (1995) Adaptive reversion of an episomal frameshift mutation in *Escherichia coli* requires conjugal functions but not actual conjugation. *Proc Natl Acad Sci USA* **92**: 5487-5490.

Foster, P.L., and Trimarchi, J.M. (1994) Adaptive reversion of a frameshift mutation in *Escherichia coli* by simple base deletions in homopolymeric runs. *Science* **265**: 407-409.

Frost, L.S., and Manchak, J. (1998) F$^-$ phenocopies: characterization of expression of the F transfer region in stationary phase. *Microbiology* **144**: 2579-2587.

Galitski, T., and Roth, J.R. (1995) Evidence that F plasmid transfer replication underlies apparent adaptive mutation. *Science* **268**: 421-423.

Godoy, V.G., and Fox, M.S. (2000) Transposon stability and a role for conjugational transfer in adaptive mutablility. *Proc Natl Acad Sci USA* **97**: 7393-7398.

Stumpf''s paper (continued from page 6)

Hastings, P.J., Slack, A., Petrosino, J.F., and Rosenberg, S.M. (2004) Adaptive Amplification and Point Mutation Are Independent Mechanisms: Evidence for Various Stress-Inducible Mutation Mechanisms. *PLoS Biol* **2**: e399.

Hendrickson, H., Slechta, E.S., Bergthorsson, U., Andersson, D.I., and Roth, J.R. (2002) Amplification-mutagenesis: evidence that "directed" adaptive mutation and general hypermutability result from growth with a selected gene amplification. *Proc Natl Acad Sci USA* **99**: 2164-2169.

Layton, J.C., and Foster, P.L. (2003) Error-prone DNA polymerase IV is controlled by the stress-response sigma factor, RpoS, in *Escherichia coli*. *Molec Microbiol* **50**: 549-561.

McKenzie, G.J., Lee, P.L., Lombardo, M.-J., Hastings, P.J., and Rosenberg, S.M. (2001) SOS Mutator DNA polymerase IV functions in adaptive mutation and not adaptive amplification. *Molecular Cell* **7**: 571-579.

Müller-Hill, B., Rickenberg, H.V., and Wallenfels, K. (1964) Specificity of the induction of the enzymes of the lac operon in *Escherichia coli*. *J Mol Biol* **10**: 303-318.

Powell, S.C., and Wartell, R.M. (2001) Different characteristics distinguish early versus late arising adaptive mutations in *Escherichia coli* FC40. *Mutat Res* **473**: 219-228.

Radicella, J.P., Park, P.U., and Fox, M.S. (1995) Adaptive mutation in *Escherichia coli*: A role for conjugation. *Science* **268**: 418-420.

Rodriguez, C., Tompkin, J., Hazel, J., and Foster, P.L. (2002) Induction of a DNA nickase in the presence of its target site stimulates adaptive mutation in *Escherichia coli*. *J Bacteriol* **184**: 5599-5608.

Rosenberg, S.M., Longerich, S., Gee, P., and Harris, R.S. (1994) Adaptive mutation by deletions in small mononucleotide repeats. *Science* **265**: 405-407.

Roth, J.R., and Andersson, D.I. (2004) Adaptive mutation: how growth under selection stimulates Lac(+) reversion by increasing target copy number. *J Bacteriol* **186**: 4855-4860.

Jeanne presenting 1st place McClung award to Jeffrey Stumpf
(Ph.D. Category)

First Place Undergraduate Winner

Examination of Reduced Numbers of T Lymphocytes in Mice Expressing a Continuously Activated Stat6 Protein

Nicole Stephenson, and Heather Bruns
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Introduction

Cytokines are important regulators of immune responses. Interleukin (IL)-4 is a cytokine that is produced by several cells including T cells, mast cells, NK cells and basophils. Not only is it secreted by multiple cell types, it also influences the functions of several different cell types. IL-4 is both a positive and negative regulator of lymphocyte (B and T cells) function. Importantly, it significantly influences T cell differentiation, thus influencing the type of immune reactions that occur. T cells that encounter antigen and become activated in the presence of IL-4 acquire a Th2 phenotype, characterized by the production of high levels of IL-4 and an ability to regulate allergic reactions and fight parasitic infections. IL-4 is also known to be a protective factor for naïve T cells (those that have not previously encountered antigen and become activated).

IL-4 exerts its effects on cells by activating multiple signaling pathways. One pathway of particular importance in lymphocytes is the JAK (Janus Kinase)-STAT (Signal Transducer and Activator of Transcription) pathway. Phosphorylation of Stat6 molecules following IL-4 binding of its receptor activates STAT6, allowing it to form homodimers which then enter the nucleus and bind IL-4-responsive gene promoters. Transgenic mice, expressing a mutant Stat6 (in B and T lymphocytes) that is continuously phosphorylated and thus, continuously activated even in the absence of IL-4, have been used to examine IL-4-stimulated functions that Stat6 alone is sufficient to mediate. However, these transgenic mice have altered lymphocyte populations, such that T cell populations are reduced, while B cell populations are increased (Bruns et al, The Journal of Immunology, 2003). This was surprising, since IL-4 is known to be protective for both B and T cells. Interestingly, T cells from these mice appear to be continually activated.

Recently, it has been debated how IL-4 plays a role in activation-induced cell death (AICD) of T cells. AICD results from repeated stimulation of the T-cell receptor with antigens, thus activating a T cell multiple times. In a paper by Zhang et al. (The Journal of Immunology 107: 3495-3503), it was determined that AICD of wild-type T lymphocytes was increased following antigen stimulation and in the presence of IL-4 and IL-2. These findings are surprising since IL-4 is generally known as an anti-apoptotic factor. However, these findings may provide an explanation for the reduced number of T lymphocytes found in Stat6VT transgenic mice. Our hypothesis is that the presence of high levels of IL-4 in the transgenic mice, due to the T cells having a Th2 phenotype, may be result in the decreased T cell numbers that are observed due to IL-4's enhancement of AICD. To investigate this hypothesis we set-up experiments following methods described in the Zhang et al. paper to examine the amounts of apoptosis that activated T cells from wild type mice are undergoing in the presence and absence of IL-4.

Stephenson's paper (continued from page 8)

Methods and Results

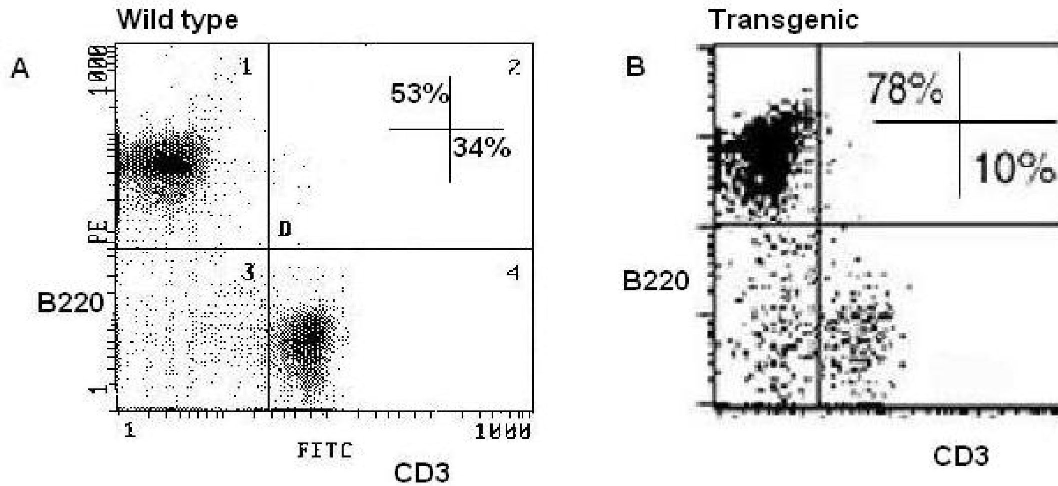

Figure 1. T cell numbers are reduced in transgenic Stat6VT mice. Published data (Bruns et al) demonstrating that T cell numbers are reduced in Stat6VT transgenic mice compared with wild-type mice. Splenocytes from wild-type (A) or Stat6VT transgenic mice (B) were stained with FITC-CD3 or PE-B220 antibodies and analyzed by flow cytometery. Percentages of B220+ and CD3+ cells are indicated in the upper right quadrant.

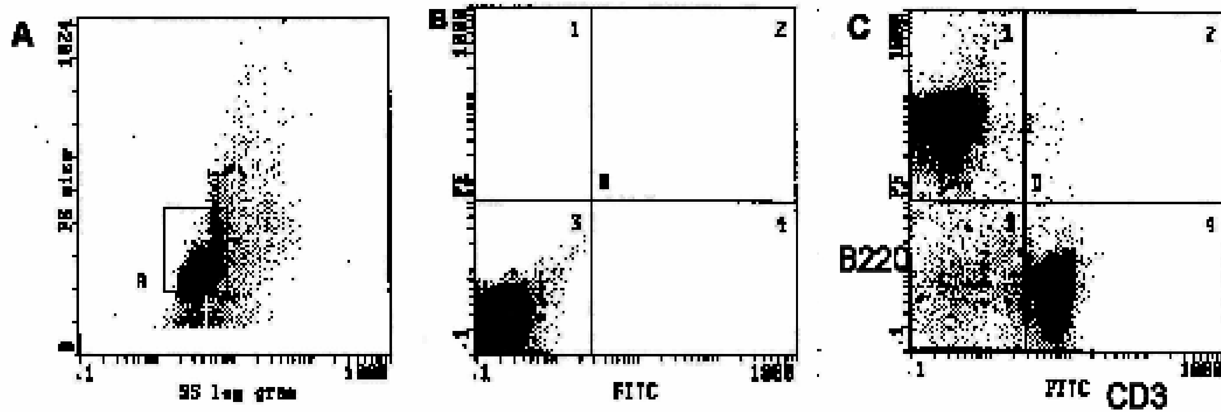

Figure 2. Setting up controls for flow cytometer. Flow cytometric analysis of unstimulated, freshly isolated wild-type splenocytes. Panel A is a dot plot demonstrating size verses granularity for each cell passing through the flow cytometer. Each dot represents a cell and its position is based on its size and granularity. The square gate represents the region where most lymphocytes are found based on their size and granularity and this gate will determine the populations examined on the following plots. Panel B shows the location of unstained cells (negative control) on a FITC verses PE dot plot. FITC and PE are fluorochromes bound to antibodies of interest (FITC-CD3 and PE-B220). The flow cytometer detects the light emission from excited fluorochromes, thus detecting if the antibody that the fluorochrome is bound to is present on the surface of the cell, indicating that the protein the antibody recognizes is present on the surface of the cell. Flow cytometric analysis of stained splenocytes is shown in panel C. To identify specific cell populations present in the total splenocyte population, we used the FITC-CD3 and PE-B220 antibodies to identify T and B cells, respectively.

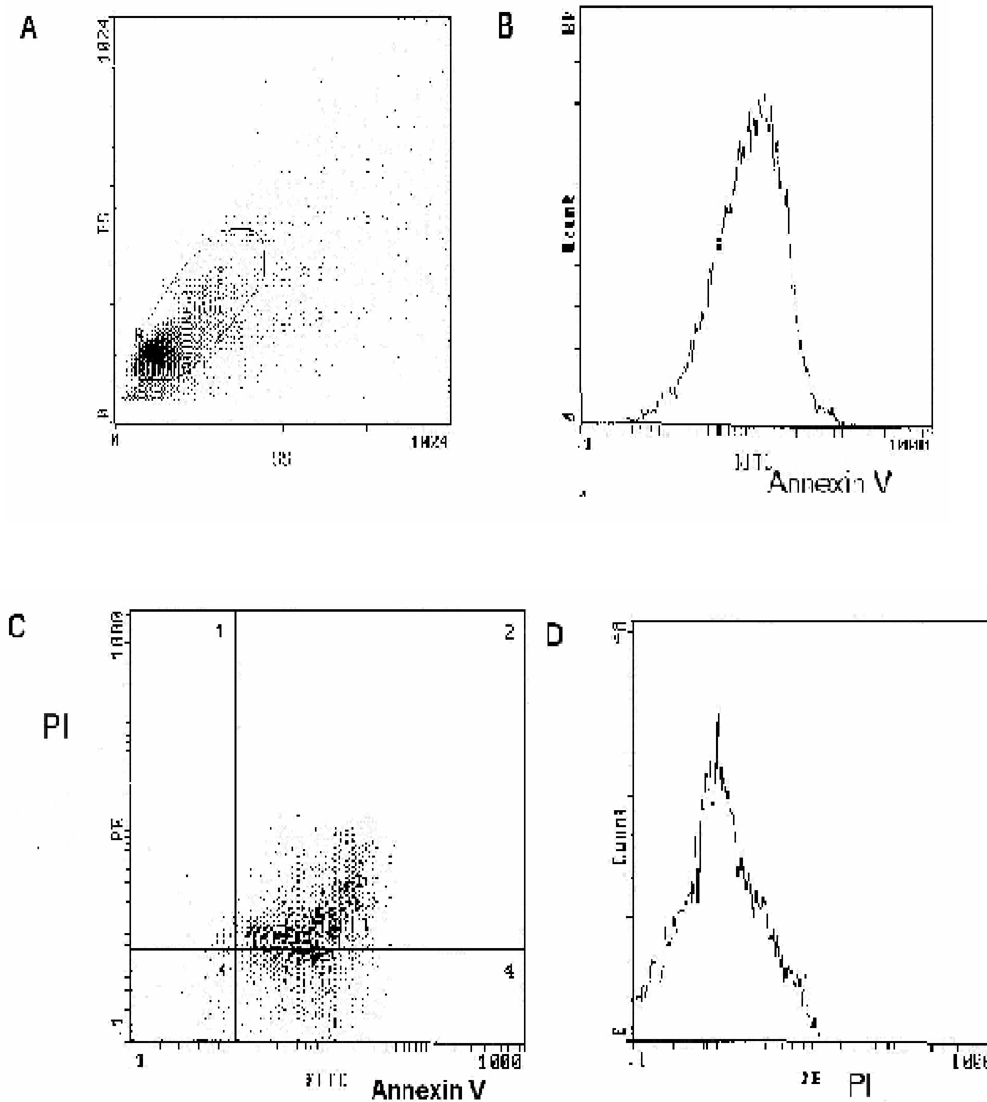

Figure 3. Assay to assess amount of apoptotic cell death occuring in a specific cell population. Following in vitro stimulation/activation of T cells, they were analyzed by flow cytometry to determine amounts of apoptosis that were occurring. The lymphocyte population was identified via size and granularity (A). During apoptosis, the phospholipids on the cell membrane translocate, exposing phosphatidylserine to the external cellular environment. Annexin V has a high affinity for phosphatidylserine and will bind to it. Propidium iodide (PI) is a dye taken up by cells whose membranes are breaking down. Cells that are Annexin V negative and PI negative are viable. Annexin V positive and PI negative indicates cells undergoing early apoptosis. Cells that are only PI positive are dying a necrotic death, and cells which are both Annexin V and PI positive are in late apoptosis, necrotic, or already dead. Lymphocytes were stained with FITC-Annexin V and PI, which reads in the same channel as the PE fluorochrome (C). Histograms demonstrate the relative levels of Annexin V (B) and PI (D) staining present.

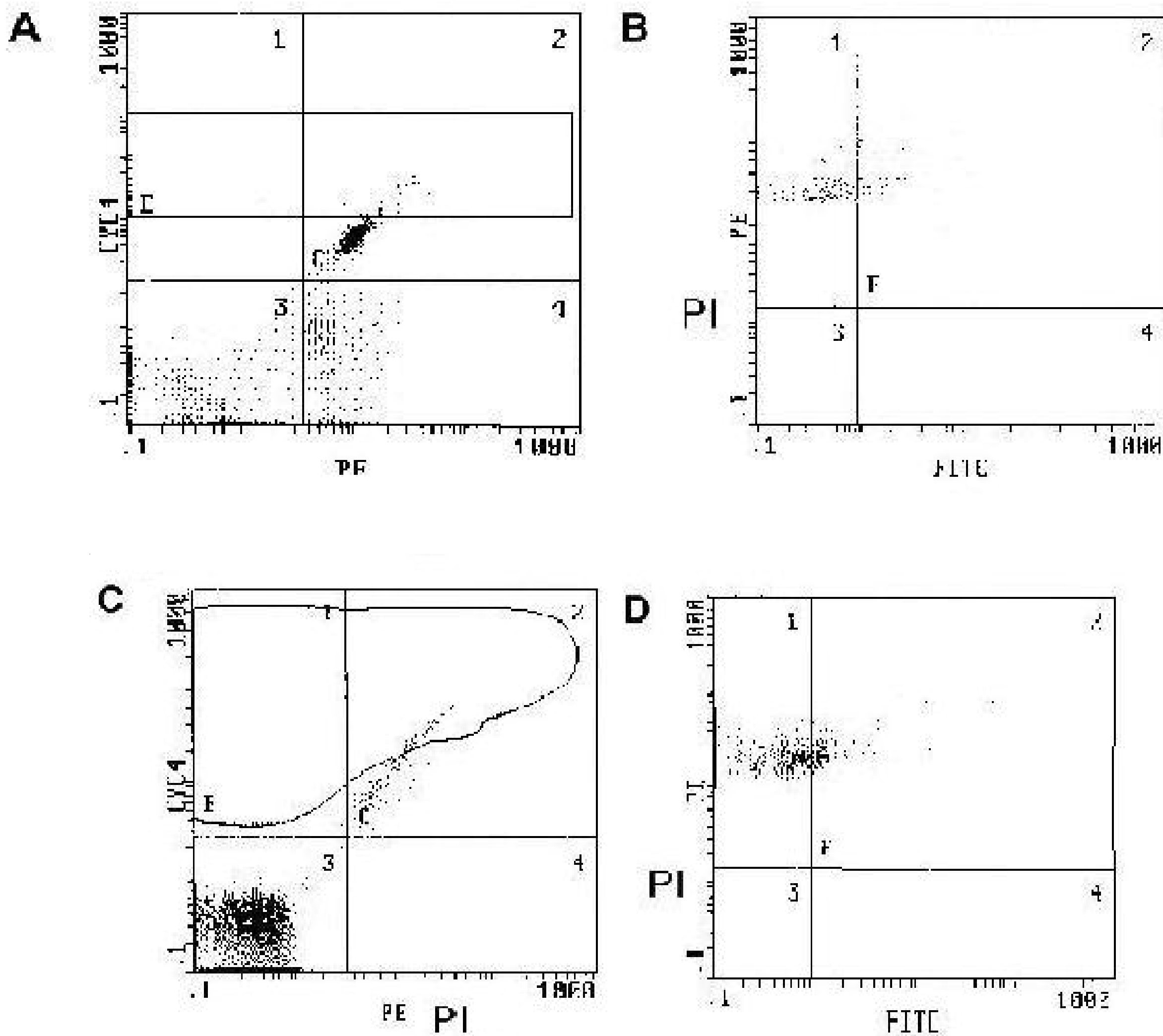

Figure 4. Troubleshooting the fluorochrome wavelengths bleeding into multiple channels. To assess the amount of apoptosis occurring just in the T cell population, we stained lymphocytes with FITC-Annexin V, PI, and Cychrome CD3 following in vitro stimulation. We discovered that the light emission from PI and the Cychrome fluorochrome bleed into the same channels. Trying to create different gates (A and C) in order to screen out cells that are artifacts due to the channel bleeding was ineffective, as cells appeared in the dot plots representing only the cells from the gates (B and D).

Stephenson's paper (continued from page 11)

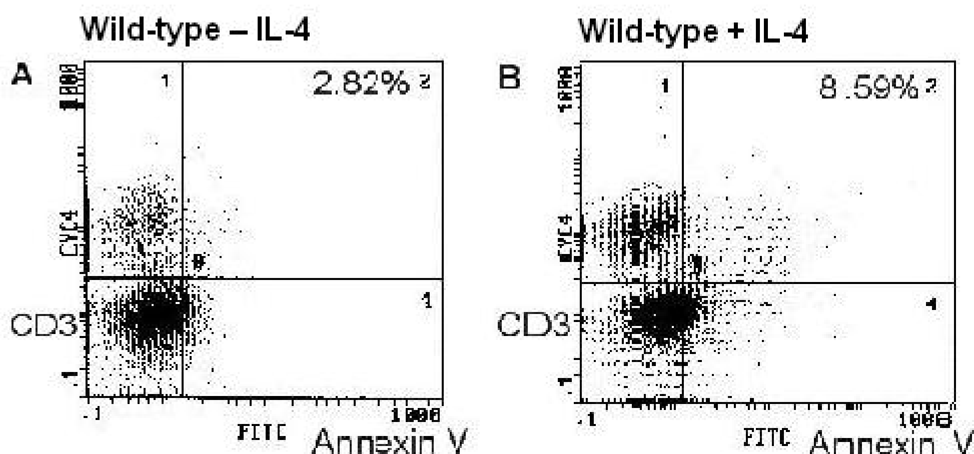

Figure 5. Activated T cells from wild type mice stimulated with IL-4 have increased amounts of apoptosis. Assessment of T cells undergoing apoptosis following in vitro stimulation, staining with only Cychrome-CD3 and FITC Annexin V. Graph A shows a population of cells that have been re-stimulated with IL-2 and anti-CD28 in the absence of IL-4 stimulation. The cells in quadrant 2 are CD3 positive and annexin V positive cells. This tells us that they are T-cells undergoing apoptosis and out of the population of cells analyzed, 2.82% of the cells are T-cells undergoing apoptosis. Graph B shows a population of cells that have been re-stimulated with IL-2, anti-CD28, in the presence of IL-4. The cells in quadrant 2 show that 8.59% of the cells analyzed are T-cells undergoing apoptosis. This is an increase from the negative control cells in graph A.

Discussion

The procedure for staining cell surface proteins and analysis by flow cytometry has been optimized and a template/protocol to use on the flow cytometer has been generated as shown in Figure 2. In vitro stimulation and activation of T lymphocytes appears to be occurring as expected due to the increased size of the T cells and the increased numbers of cells that become Annexin V and PI positive as assessed by flow cytometery in Figure 3. Optimization of Annevin V, PI, and Cychrome CD3 staining needs to be completed, as we discovered that the light emission from the PI stain bleeds into the channel that reads the fluorochrome light emission of cychrome. Figure 4 shows our attempts to troubleshoot this problem. To correct for this problem, only FITC-Annexin V and Cychrome CD3 are used together, without PI. This allows us to assess (by Annexin V staining only) the amount of apoptosis occurring only in the CD3+ T cells. Using this technique, we were able to assess that there were increased amounts of apoptotic T cells when activated in the presence of IL-4 as seen in Figure 5.

Future Directions

The same protocol will be repeated, using wildtype and Stat6VT transgenic mice, to compare the amounts of apoptosis occurring in T cells from these mice and determine if the ability of IL-4 to enhance AICD is the mechanism behind reduced T cell numbers in the Stat6VT transgenic mice.

Second Place Graduate (Ph.D.) Winner

Error-Prone DNA Polymerase IV (DinB) and the Stress-Response of *E. coli*

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E. coli's DNA Polymerase IV (Pol IV, encoded by the *dinB* gene) is one of a family of error-prone DNA polymerases known as the Y family of polymerases (Yeiser *et al.*, 2002). These polymerases are found in all three domains of life and can replicate past DNA damage with some loss of replicative fidelity. However, Pol IV's ability to replicate damaged DNA is very dependent upon the type of lesion (Lenne-Samuel *et al.*, 2000;Wagner *et al.*, 1999).

When a population of non-growing cells is placed under non-lethal selection, mutations accumulate to alleviate the selective pressure, a phenomenon called adaptive mutation (Cairns and Foster, 1991). This phenomenon is used to study Pol IV because Pol IV is responsible for 50-80% of these adaptive mutations (Foster, 2004). Most laboratory studies of adaptive mutation have utilized a strain called FC40 in which the *lac* operon is deleted on the chromosome, but the strain has a frameshifted *lac* operon on the episome that can be readily reverted to *lac*$^{+}$ when lactose is the only carbon source (Cairns and Foster, 1991). Unlike spontaneous, growth dependent mutations, adaptive mutation requires recombination functions for double strand break repair (*recA* and *rec BCD*) and branch migration (*ruvAB* and *ruvC*) (Cairns and Foster, 1991;Foster *et al.*, 1996;Foster, 1993;Harris *et al.*, 1996). It is interesting to note that a mutation in *recG*, a helicase that plays a role in recombination and DNA repair, increases the rate of adaptive mutation about 100-fold (Harris *et al.*, 1996;Foster *et al.*, 1996). For a high rate of adaptive mutation, the *lac* allele must be on the F' episome and conjugal functions must be expressed (Foster and Trimarchi, 1995;Galitski and Roth, 1995).

Pol IV is induced as part of the SOS response (Kenyon and Walker, 1980). During the SOS response, RecA is activated by binding to single stranded DNA. Accumulation of activated RecA leads to LexA self-cleavage, inducing SOS genes that are otherwise repressed by LexA. The *dinB* gene is repressed by LexA and is part of a pathway for SOS-induced mutagenesis (Kim *et al.*, 1997).

Mauch's paper (continued from page 14)

There are two other polymerases in *E. coli* that are induced by the SOS response, Pol II and Pol V (Kenyon and Walker, 1980). Both of these polymerases are present in low numbers in uninduced cells. These cells have approximately 50 Pol II molecules and 15 Pol V molecules. On the other hand, there are 250-1000 molecules of Pol IV present in uninduced cells. Upon SOS induction, there are approximately 210-350 molecules of Pol II, 200 molecules of Pol V, and 2500 molecules of Pol IV (Kim *et al.*, 2001). Pol IV's expression at high levels even in uninduced cells suggests that it must be kept under strict control to avoid the accumulation of deleterious mutations.

To determine how this control is accomplished, the Foster laboratory conducted a screen for mutants that affect the level or activity of Pol IV. A mini Tn10Cm insertion in *rpoS* reduced adaptive mutation 10-fold in FC40 and 20-30 fold in FC526, an isogenic *recG*$^-$ strain. Viability was not decreased enough in the *rpoS*$^-$ strains to account for the lower mutation rate (Layton and Foster, 2003). This prompted further study into how RpoS affects adaptive mutation and Pol IV.

A sigma factor is a subunit of RNA polymerase that recognizes promoters and directs transcription of specific genes. The primary sigma factor in *E. coli* is sigma-70, which directs transcription of housekeeping genes and genes expressed during exponential phase. *E. coli* has five other sigma factors, each of which is involved in responses to different stressors (Loewen *et al.*, 1998). The gene *rpoS* encodes a sigma factor that helps to regulate gene expression during stationary phase and starvation. To date, there is no agreed upon consensus sequence for the RpoS binding site at promoters. This is in part because often RpoS recognizes the same sequence as sigma-70. (Hengge-Aronis, 2002). It has been suggested that when RpoS and sigma-70 recognize the same promoter, RpoS will bind to the promoter preferentially in stationary phase because of a change in salt concentration, superhelicity of the DNA, or perhaps accumulation of ppGpp or other factors (Colland *et al.*, 2000;Hengge-Aronis, 2002;Lacour and Landini, 2004;Kusano *et al.*, 1996). RpoS is responsible, either directly or indirectly, for the positive regulation of over 100 genes in stationary phase. Even more genes are negatively regulated at this time in an RpoS dependent manner (Patten *et al.*, 2004;Vijayakumar *et al.*, 2004;Weber *et al.*, 2005)

As previously stated, in an *rpoS*$^-$ culture, the rate of adaptive mutation is decreased 10-fold. Using immunoblotting, Layton and Foster (2003) showed that in stationary phase cells the amount of Pol IV protein in FC40 without RpoS is decreased 3-5 fold. They also showed that Pol IV transcript is decreased 3-fold in this strain in stationary phase cultures. To address if stationary phase induction of Pol IV occurs in an RpoS dependent manner and to determine when in the growth curve Pol IV's induction occurs, Layton and Foster used immunoblotting of samples taken from various points along the growth curve. Their data clearly illustrates that Pol IV induction is RpoS dependent. Layton and Foster went on to show that regulation of Pol IV by RpoS occurs in addition to SOS regulation rather than the *rpoS* mutant simply causing induction of the SOS response.

This research has lead to the question of whether RpoS controls *dinB* directly by binding to the promoter of *dinB* and directing transcription or whether there is indirect control via a transcription regulator cascade. Currently, there are two pieces of evidence that regulation could be indirect. The first is that only one gene, *sbmC*, has been shown to be directly coregulated by RpoS and LexA. The second piece of evidence is the timing. RpoS is induced early in stationary phase while Pol IV is induced late in stationary phase. This delayed induction could be the result of a transcription factor cascade, or it could be the result of the need for cofactors, such as ppGpp, to accumulate. The latter option does not make a compelling argument for either direct or indirect regulation (Layton and Foster, 2003;Hengge-Aronis, 2002). The evidence for direct regulation lies in the proposed promoter for *dinB.* This promoter contains no strong –35 region which may be indicative that RpoS could outcompete sigma-70 during stationary phase (Layton and Foster, 2003;Hengge-Aronis, 2002).

Pol IV's control by RpoS could benefit the cells in several ways. First, the cells may be able to survive damage that occurs as a result of stressful conditions. Second, high levels of Pol IV may result in advantageous mutations arising in a population. This is particularly true of mutations that accumulate on an episome because there is little to no risk of deleterious mutations occurring in the genomic DNA. In natural environments, bacteria are routinely exposed to nutrient limitation and stress(Hengge-Aronis, 2000). Bacteria that are mutants in any one of the SOS induced polymerases show decreased fitness for the long-term survival of *E. coli* (Yeiser *et al.*, 2002).

Mauch's's paper (continued from page 16)

Bjedov et. al. (2003) state that when natural populations cannot adapt to stressful conditions, the mutation rate must increase (Bjedov *et al.*, 2003). This makes studying the mechanisms of stationary phase regulation of Pol IV very interesting as it provides a link between stressful environmental situations and genetic change. This link has broad implications for evolution.

References

Bjedov,I., Tenaillon,O., Gerard,B., Souza,V., Denamur,E., Radman,M. *et al.* (2003) Stress-Induced Mutagenesis in Bacteria. *Science* **300**: 1404-1409.

Cairns,J., and Foster,P.L. (1991) Adaptive reversion of a frameshift mutation in *Escherichia coli. Genetics* **128**: 695-701.

Colland,F., Barth,M., Hengge-Aronis,R., and Kolb,A. (2000) sigma factor selectivity of *Escherichia coli* RNA polymerase: role for CRP, IHF and lrp transcription factors. *EMBO J* **19**: 3028-3037.

Foster,P.L. (1993) Adaptive mutation: the uses of adversity. *Annu Rev Microbiol* **47**: 467-504.

Foster,P.L. (2004) Adaptive mutation in *Escherichia coli. J Bacteriol* **186**: 4846-4852.

Foster,P.L., and Trimarchi,J.M. (1995) Adaptive reversion of an episomal frameshift mutation in *Escherichia coli* requires conjugal functions but not actual conjugation. *Proc Natl Acad Sci U S A* **92**: 5487-5490.

Foster,P.L., Trimarchi,J.M., and Maurer,R.A. (1996) Two enzymes, both of which process recombination intermediates, have opposite effects on adaptive mutation in Escherichia coli. *Genetics* **142**: 25-37.

Galitski,T., and Roth,J.R. (1995) Evidence that F plasmid transfer replication underlies apparent adaptive mutation. *Science* **268**: 421-423.

Harris,R.S., Ross,K.J., and Rosenberg,S.M. (1996) Opposing roles of the Holliday Junction processing systems of *Escherichia coli* in Recombination-Dependent Adaptive Mutation. *Genetics* **142**: 681-691.

Hengge-Aronis,R. (2002) Stationary phase gene regulation: what makes an *Escherichia coli* promoter sigmaS-selective? *Curr Opin Microbiol* **5**: 591-595.

Hengge-Aronis,R. (2000) The General Stress Response in *Escherichia coli*. In Bacterial Stress Responses. Storz,G., and Hengge-Aronis,R. (eds). Washington D.C.: ASM Press, pp. 161-178.

Kenyon,C.J., and Walker,G.C. (1980) DNA-damaging agents stimulate gene expression at specific loci in *Escherichia coli. Proc Natl Acad Sci U S A* **77**: 2819-2823.

Mauch's paper (continued from page 17)

Kim,S.R., Matsui,K., Yamada,M., Gruz,P., and Nohmi,T. (2001) Roles of chromosomal and episomal dinB genes encoding DNA pol IV in targeted and untargeted mutagenesis in Escherichia coli. *Mol Genet Genomics* **266**: 207-215.

Kim,S.R., Maenhaut-Michel,G., Yamada,M., Yamamoto,Y., Matsui,K., Sofuni,T. *et al.* (1997) Multiple pathways for SOS-induced mutagenesis in Escherichia coli: An overexpression of dinB/dinP results in strongly enhancing mutagenesis in the absence of any exogenous treatment to damageáDNA. *PNAS* **94**: 13792-13797.

Kusano,S., Ding,Q., Fujita,N., and Ishihama,A. (1996) Promoter Selectivity of Escherichia coli RNA Polymerase E[IMAGE][IMAGE] and E[IMAGE][IMAGE] Holoenzymes. *J Biol Chem* **271**: 1998-2004.

Lacour,S., and Landini,P. (2004) SigmaS-dependent gene expression at the onset of stationary phase in Escherichia coli: function of sigmaS-dependent genes and identification of their promoter sequences. *J Bacteriol* **186**: 7186-7195.

Layton,J.C., and Foster,P.L. (2003) Error-prone DNA polymerase IV is controlled by the stress-response sigma factor, RpoS, in Escherichia coli. *Mol Microbiol* **50**: 549-561.

Lenne-Samuel,N., Janel-Bintz,R., Kolbanovskiy,A., Geacintov,N.E., and Fuchs,R.P. (2000) The processing of a Benzo(a)pyrene adduct into a frameshift or a base substitution mutation requires a different set of genes in Escherichia coli. *Mol Microbiol* **38**: 299-307.

Loewen,P.C., Hu,B., Strutinsky,J., and Sparling,R. (1998) Regulation in the rpoS regulon of Escherichia coli. *Can J Microbiol* **44**: 707-717.

Patten,C.L., Kirchhof,M.G., Schertzberg,M.R., Morton,R.A., and Schellhorn,H.E. (2004) Microarray analysis of RpoS-mediated gene expression in Escherichia coli K-12. *Mol Genet Genomics* **272**: 580-591.

Vijayakumar,S.R., Kirchhof,M.G., Patten,C.L., and Schellhorn,H.E. (2004) RpoS-regulated genes of Escherichia coli identified by random lacZ fusion mutagenesis. *J Bacteriol* **186**: 8499-8507.

Wagner,J., Gruz,P., Kim,S.R., Yamada,M., Matsui,K., Fuchs,R.P., and Nohmi,T. (1999) The dinB gene encodes a novel E. coli DNA polymerase, DNA pol IV, involved in mutagenesis. *Mol Cell* **4**: 281-286.

Weber,H., Polen,T., Heuveling,J., Wendisch,V.F., and Hengge,R. (2005) Genome-Wide Analysis of the General Stress Response Network in Escherichia coli: {sigma}S-Dependent Genes, Promoters, and Sigma Factor Selectivity. *J Bacteriol* **187**: 1591-1603.

Yeiser,B., Pepper,E.D., Goodman,M.F., and Finkel,S.E. (2002) SOS-induced DNA polymerases enhance long-term survival and evolutionary fitness. *Proc Natl Acad Sci U S A* **99**: 8737-8741

Selected Highlights from the Journals of the ASM, June 2005 (from ASM Tipsheet)

Newly Identified Inhibitor of Anthrax Toxin May Contribute to Safer Vaccine and Offer Postexposure Therapy

A newly identified inhibitor of the anthrax toxin may be used to develop a safer and more effective vaccine and act as a therapeutic agent after exposure say researchers from Massachusetts and Germany. Their findings appear in the June 2005 issue of the journal *Infection and Immunity*.Anthrax is a highly contagious and toxic disease that results from infection with the bacterium *Bacillus anthracis*. If not caught immediately, those infected may die within a matter of days. Anthrax poses a deadly threat as a potential biological weapon placing added emphasis on the need for a safe and effective vaccine. The vaccine currently available doesn't protect against the bacilli and may be hazardous to its host when used immediately after exposure.

In the study researchers infected two groups of mice with anthrax and immunized one group with a dominant-negative inhibitor (DNI) and the other with a protective antigen (PA) currently used in the anthrax vaccine. They monitored the mice for several weeks and found that DNI alone produced higher immune responses than PA. Due to DNI's ability to inhibit the anthrax toxin, researchers also believe that DNI-based vaccines may increase immunity and provide therapeutic activity when administered postexposure.

"The strong immunogenicity and retained antigenicity of DNI suggest that DNI is a promising and potentially safer candidate for use in an anthrax vaccine than PA," say the researchers. "Moreover, in the event of anthrax infection, the administration of DNI can serve not only as an antitoxic therapy as an immediate response but also as a prophylactic vaccine to prevent late-onset or future anthrax infection.

(**B.A. Aulinger, M.H. Roehrl, J.J. Mekalanos, R.J. Collier, J.Y. Wang**. 2005. Combining anthrax vaccine and therapy: a dominant-negative inhibitor of anthrax toxin is also a potent and safe immunogen for vaccines. Infection and Immunity, 73.6: 3408-3414.)

Old Drug May Offer New Hope in Treating SARS

Cinanserin, a drug that underwent preliminary clinical testing on humans in the 1960's, may inhibit the SARS virus say researchers from Europe and China. Their findings appear in the June 2005 issue of the *Journal of Virology*.

Severe acute respiratory syndrome (SARS) emerged as a highly infectious respiratory disease in 2002 and reached epidemic levels within six months. An estimated 8,096 cases were reported resulting in 774 deaths throughout 29 countries. Although the causative agent was quickly identified as a new strain of the coronavirus, an effective method of treatment has yet to be determined.

In the study researchers scanned a database of 8,000 existing drugs for those that would likely bind to the 3C-like proteinase of SARS. Cinanserin, a well-characterized serotonin antagonist, scored high in the screening and was selected for further experimentation. The antiviral activity of cinanserin was evaluated in tissue samples containing the SARS virus and revealed a strong inhibition of coronavirus replication at nontoxic drug concentrations.

"These findings demonstrate that the old drug cinanserin is an inhibitor of SARS-CoV replication, acting most likely via inhibition of the 3CL proteinase," say the researchers.

(**L. Chen, C. Gui, X. Luo, Q. Yang, S. Gunther, E. Scandella, C. Drosten, D. Bai, X. He, B. Ludewig, J. Chen, H. Luo, Y. Yang, Y. Yang, J. Zou, V. Thiel, K. Chen, J. Shen, X. Shen, H. Jiang.** 2005. Cinanserin is an inhibitor of the 3C-like proteinase of severe acute respiratory syndrome coronavirus and strongly reduces virus replication in vitro. Journal of Virology, 79. 11: 7095-7103.)

Selected Highlights from the Journals of the ASM, July 2005 (from ASM Tipsheet)

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Visible Light May Kill Bacteria That Infects the Human Stomach

Treatment with visible light may kill the bacteria that commonly cause ulcers in humans say researchers from Massachusetts and Minnesota. Their findings appear in the July 2005 issue of the journal *Antimicrobial Agents and Chemotherapy*.

Helicobacter pylori, often referred to as the world's "commonest infectious agent", colonizes in the stomach causing chronic gastritis, gastric ulcers, and was recently implicated in the development of gastric cancer. Infecting more than fifty percent of the world's population and up to ninety percent of the population in some countries, the bacterium can persist, once acquired, sometimes for life. A twenty percent failure rate in antibiotic therapy reinforces the need for alternative treatment methods.

In the study various strains of *H. pylori* were cultured and found to produce porphyrins, naturally occurring compounds, which can cause photosensitivity. When the cultures were exposed to visible broadband light, results showed both virulent and drug-resistant strains of *H. pylori* were killed. Blue/violet light was found to be the most effective and the research indicates that photodynamic therapy, a combination of medication and radiation would be the method of treatment.

"We have shown that all tested strains of *H. pylori* naturally accumulate a mixture of PPIX and CP (porphyrins) that can sensitize the bacteria to killing by visible light, particularly blue light," say the researchers. "This finding suggests that a novel phototherapy approach may be applied in the human stomach to eliminate *H. pylori* infection."

(**M.R. Hamblin, J. Viveiros, C. Yang, A. Ahmadi, R.A. Ganz, M.J. Tolkoff**. 2005. *Helicobacter pylori* accumulates photoactive porphyrins and is killed by visible light. Antimicrobial Agents and Chemotherapy, 49. 7: 2822-2827.)

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Mediterranean Fruit Fly May Transmit Human Pathogens to Fruit

The Mediterranean fruit fly has the capability to contaminate commercial and wild fruits with bacteria harmful to humans say researchers from Israel. They report their findings in the July 2005 issue of the journal *Applied and Environmental Microbiology*.

The Mediterranean fruit fly is considered to be a major menace to the commercial fruit industry worldwide. They feed on animal feces for protein in order to produce eggs, which they then lay in fruit by puncturing the skin and injecting them. Outbreaks of food-borne diseases associated with fresh produce consumption are rapidly increasing, reinforcing the need to identify the source of contamination.

In the study flies were fed feces contaminated with *Escherichia coli* and caged with intact apples. After limited exposure researchers found the apples to be contaminated with *E. coli* and rinsing them with tap water did not rid them of the bacteria. The flies studied harbored *E. coli* in their systems up to seven days after infection.

"These findings highlight the potential of the fly to carry human pathogens and to serve as a vector for transmission of food-borne diseases," say the researchers.

(**S. Sela, D. Nestel, R. Pinto, E. Nemny-Lavy, M. Bar-Joseph.** 2005. Mediterranean fruit fly as a potential vector of bacterial pathogens. Applied and Environmental Microbiology, 71. 7: 4052-4056.)

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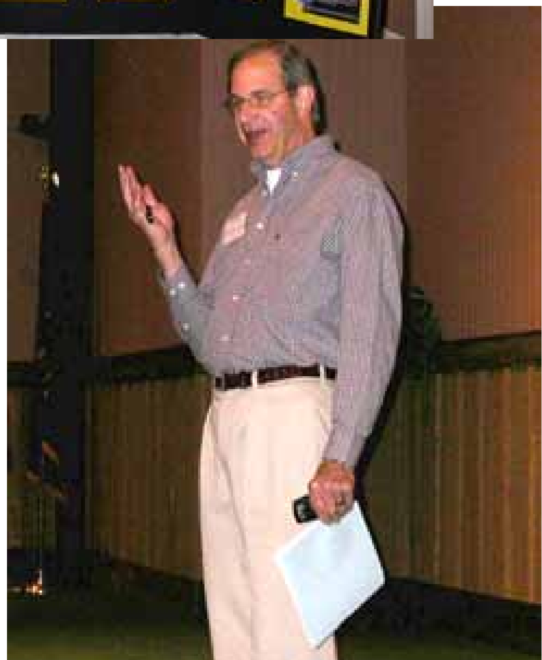
Indiana Branch poster board display



Dr. Jim Tiedje, ASM President and ASM Foundation Speaker

More Photos...

(L) Dominique with a
student at the
registration table

(R) Jeanne presenting
Dr. Nancy Behforouz with the
IBASM
Teaching Award

Jeanne congratulating
Nicole Stephenson,
the1st place undergraduate winner

Kimberly Mauch, the second place
winner in the Ph.D.
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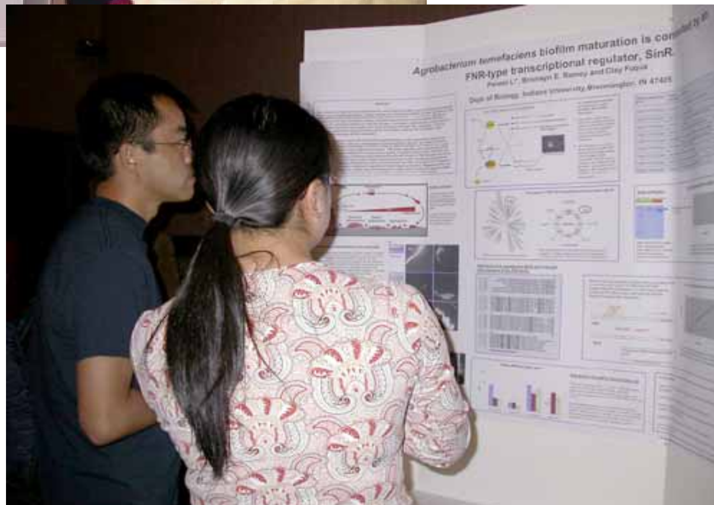
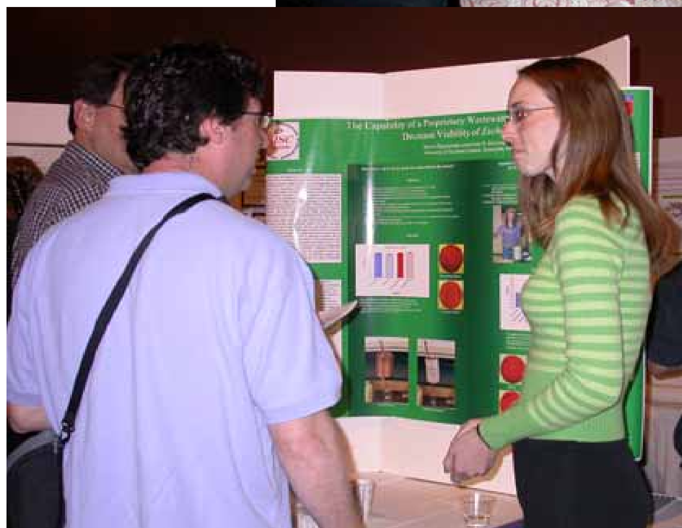
Poster Presentations & Judging





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