News Letter

IBASM

Volume 5 #1 July 2002

Message from the President- Jim Mitchell

The Indiana branch had an exciting annual meeting at Clifty Falls State Park. Although attendance was down from last year's meeting, we enjoyed renewing old friendships and meeting new Hoosier microbiologists. In his ASM Foundation Lecture entitled "The Molecular Pathogenesis of Cryptosporidiosis", Dr. Douglas Clark from Johns Hopkins presented an excellent talk on the history of cryptosporidiosis and new developments in understanding this obligate intracellular protozoan parasite. Although the human disease was first recognized in 1976, it has been around for a long time and is very common

Continued on page 2

WHAT'S INSIDE...

Message from the President-Elect Jeanne Barnett

I am excited to serve IBASM as your new President-elect. For those of you who do not know me, I currently hold the position of Professor of Biology at University of Southern Indiana. Prior to starting at USI in 1989, I was a research scientist in the respiratory care division of Procter & Gamble. I received my PhD in virology from Duke University in 1983 and held post-doctoral positions at Vanderbilt University and USDA in East Lansing, MI. I have been active in IBASM since 1998.

Our 2003 meeting will be April 11-13, 2003, at Spring Mill State Park in Mitchell, IN. Information on the park and driving directions can be found at **http://www.state.in.us/dnr/parklake/parks/index.html**. I'll provide you with some preliminary information with more details to follow in the next newsletter. The registration will remain $25 for members and $5 for students. The cost of the meeting will be as follows:

Continued on page 3

The IBASM thanks the Indiana University School of Medicine-Fort Wayne for financially supporting the publication of this newsletter.

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

in developing countries. It is a self limited diarrheal illness in immune competent hosts but life-threatening in AIDS patients. Much of Dr. Clark's work on the disease has concerned the role of actin polymerization in establishing infection of intestinal cells, and he explained the current understanding of the mechanism and how it compares with diseases caused by such pathogens as *Listeria monocytogenes* and *Escherichia coli*. The second ASM Foundation Lecture entitled " Two-Component Signal Transduction Systems for Vancomycin Resistant Enterococci", was presented by Dr. Barry Wanner from Purdue University. Dr. Wanner provided information about the development of vancomycin resistance in enterococci, regulation of the resistance genes and their apparent origin from the Streptomycete that produces vancomycin. He next explained the difference between vancomycin resistance in *Staphylococcus aureus* and *Enterococcus*. Dr. Carl Bauer from Indiana University-Bloomington talked about the lateral transfer of genetic material in ancient organisms that led to the wonderful and highly efficient photosynthetic process that keeps this planet running. His seminar "The Microbial Origin and Evolution of Photosynthesis", provided great visuals to help us understand the functioning of photosystems I and II and how a variety of organisms contributed genes to what we know today as the cyanobacteria.

There were 7 posters presented at the meeting. The quality of the presentations was excellent and it was informative to see the different research areas. Wayne Blosser received the McClung award for first place graduate poster and Wes Marchione was awarded 2nd place in the graduate division. Both students are from Ball State University. Julia Fletcher from Saint Mary's College won 1st place undergraduate poster. Keynttisha Jefferson from Purdue University won 2nd place in the undergraduate category. Honorable mention was bestowed upon Huma Ansari (Burris High School, Muncie IN) as the first student from the high school division. We look forward to receiving more posters in the high school division at future meetings. Student members please consider presenting a poster at the next spring meeting which will be held April 11-13, 2003 at Spring Mill State Park. It is a great opportunity to meet professionals who can give you valuable ideas and provide leads and advice for future education and employment. Winners receive a plaque and monetary award when short papers are published in the IBASM newsletter.

In the Indiana Branch business meeting we elected one new officer. Jeanne Barnett from Univesity of Southern Indiana was elected as President-elect and will be actively fulfilling the duties of this office by the time you receive this newsletter. She will provide more details on local arrangements for the 2003 branch meeting in her article this issue. IBASM received a $3000 grant from ASM this year. These Regional Initiative Grants provided by the ASM and Foundation Lectureships are invaluable in maintaining the quality of our branch meetings. Any chance you get please thank these groups for their help.

There are three upcoming meetings you may be interested in attending. The annual meeting of the Society for Industrial Microbiology will be August 11-15 at Loews Philadelphia Hotel in Philadelphia, PA. The Southern Great Lakes section of the Society for Industrial Microbiology (SGL-SIM) will be meeting Saturday, October 19th at the University of Notre Dame. I especially encourage everyone to attend this annual event held each October in the Great Lakes region. At least 2 vans of students and faculty from Ball State University travel to this rendezvous each year and I would like to see more students and faculty from Indiana attend this very informative meeting. Although there are no student presentations, the conference provides an excellent avenue for students and Hoosier microbiologists to meet with industrial microbiologists from both the private and government sectors. This year's program has not yet been released, but is usually divided between industrial and environmental microbiology topics. If you are interested in more information you can email me or SGL-SIM president Chuck Kulpa (kulpa.1@nd.edu) or visit the web site +http://www.simhq.org/html/localsecs/sgreatlakes.html. The Seventh Conference on the Biotechnology of Microbial Products (BMP 2002) will be held October 27-30 at the Renaissance Ilikai Waikiki Hotel, Honolulu, Hawaii. BMP 2002 will focus on the discovery of metabolites, with symposia relating to genomes, novel sources of microbial metabolites, metabolic engineering and novel targets for discovery.

Looking forward to seeing you all at one of these meetings.....mark April 11-13, 2003 on your calendars now!

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

Double rooms (2 double beds)		**$69 per night**
Meals:	**Friday dinner**	**10.30**
	Saturday breakfast	**6.30**
	Saturday lunch	**6.75**
	Saturday dinner	**10.30**
	Sunday breakfast	**6.30**

We plan to provide some financial support for student presenters at the meeting, so encourage your students to present their research. We are also going to try to provide a roommate matching service. There will be a central address to request a roommate for the meeting. The details of service will be in the next newsletter. We hope this will allow more students to attend by providing a more economical stay.

Our ASM Foundation speaker for the 2003 meeting is Dr. Ian Lipkin. Dr. Lipkin is Professor of Epidemiology in the Mailman School of Public Health of Columbia University. Dr. Lipkin has received numerous awards and was involved in the identification of the West Nile Virus during the encephalitis outbreak in New York City in fall, 1999. He has also used molecular methods for identifying Borna disease virus. His presentation will be from 7:30 – 8:30 on Saturday, April 12. It promises to be an exciting and informative session.

Planning for the 2004 meeting is in the works. The preliminary plan is for April, 2004, in Indianapolis.

InforMax®

BALL STATE UNIVERSITY

In an effort to provide the state of Indiana's research community with world class bioinformatics software, InforMax and Ball State University (Dr. Carolyn Vann, Director of the Biotechnology Certificate Program) have entered into a collaboration to provide researchers within the state shared access to Vector NTI Suite at a substantial decrease in price ($1000 per computer as compared to $3500). Vector NTI is the company's award-winning desktop package of molecular biology data analysis and data management tools. The Suite features functionality that allows users to perform a very large number of informatics workflows in one integrated desktop environment. For example, it is possible to move from the assembly of chromatogram data, to edited and finished consensus sequences, to the recovery and multiple sequence analysis of related, BLAST-generated hits, all in a graphically-rich workspace that avoids the need to reformat data. Vector NTI Suite also includes sophisticated features for DNA sequence mapping, annotation and illustration, PCR primer design, protein and DNA sequence analysis, recombinant cloning strategy design, and many other common bioinformatics tasks. All of the sequence, oligo, BLAST hit, restriction enzyme, and citation data that might be used in these workflows can be stored in a user-configurable object-oriented database, which provides additional levels of functionality in terms of data management, integrity, and sharing.

For additional details about Vector NTI Suite functionality, access to a fully functional trial license, or information on how you can purchase the software, please contact Jeremy Broffman at 240-747-4105 and/or jebroffman@informaxinc.com.

Second Place Graduate Winner

Characterization of Pathogen Resistance Genes and Proteins in Orchids

by Wes Marchione and Carolyn Vann. Ball State University, Department of Biology, Muncie, IN 47306.

All organisms have evolved mechanisms to defend themselves against attacks by pathogens. Numerous current research projects by other investigators are addressing associated problems such as: 1) How does the host recognize the pathogen? 2) Following pathogen identification, how do signaling cascades elicit the appropriate responses which may include initiation of defense or repair systems or may induce programmed cell death of infected cells? 3) What are the identities of the genes/proteins acting within the signaling cascade? 4) What is the timing and duration of expression of the various components of the signal systems? The answers to these questions provide important information on host-pathogen interactions which can lead to the development of technologies to protect or alter hosts such they are resistant to specific pathogens or diseases.

Recently, many genes encoding disease-resistance factors (R genes) have been isolated from a variety of plants and some have been found to have regions of homology to highly conserved domains in resistance genes present in mammals or model organisms such as Drosophila (Wilson et al., 1997). Thus, the function of an unknown protein may be hypothesized by computer-assisted comparisons to sequence information available on better characterized genes/proteins (a bioinformatics approach). This approach involves identifying specific, conserved domains in the gene/protein and determining the function of these regions and of the entire protein based on comparisons to homologous gene/protein regions with known function.

Many animal signaling pathways have been fairly well-characterized but, little is known about the members or signaling pathways in plants. Five classes of R genes isolated from monocots and dicots, are now recognized based on common motifs: intracellular protein kinases; receptor-like protein kinases with an extracellular LRR domain; intracellular LRR proteins with a nucleotide binding site (NBS) and a leucine zipper (LZ) motif; intracellular NBS-LRR proteins with a region with homology to the Toll and interleukin-1 receptor (TIR) proteins; and LRR proteins that encode membrane-bound extracellular proteins (Martin, 1999). A majority of plant R-genes belong to the class of NBS-LRR, which consists of the LZ-NBS-LRR and TIR-NBS-LRR families (Meyers et al., 1999).

One of the NBS-LRR R gene families has members which contain an amino-terminal TIR domain with homology to both Toll and the *Drosophila* and interleukin-1 like proteins of mammals, shown to be involved in non-specific cellular immunity in animals (Young, 2000). The Toll region in *Drosophila* has been associated with two main functions, one being the establishment of dorsoventral polarity during embryogenesis and the other with resistance to fungal pathogens (Wilson et al., 1997). Plant TIR proteins are thought be involved in signal transduction and/or associated with pathogen recognition (Young, 2000). Highly conserved through evolution, Toll proteins have been found in plants previously but it is currently believed that they do not exist in monocots such as orchids.

The TIR-NBS-LRR subclass has not been seen in cereals although it is predominant (75% of all NBS-LRR genes) in *Arabidopsis* (Pan et al., 2000). It is believed that the TIR subclass evolved at least 200 million years ago (Bold, 1977), but after that time, these two main groups of R genes are believed to have undergone divergent evolution (Pan et al., 2000). We are characterizing 3 orchid clones which encode genes whose expression in the cell is induced following exposure of the cell to the tobacco mosaic virus (TMV-O). Each has a conserved region (encoded by 23 nucleotides) with exact homology to the N-terminal Toll/interleukin-1 receptor gene (TIR) domain in mammals. These clones also have some homology to the C-terminal leucine rich repeats (LRR) common among resistance genes.

Other conserved domains in R genes are the NBS and LRR regions. The NBS regions occur in diverse proteins that bind ATP or GTP, suggesting that nucleotide triphosphate binding is essential for activation of these proteins (Bent, 1996). LRRs consist of multiple repeats of about 24 amino acids that contain many leucine residues found to be conserved in structural domains, but highly variable in the functional domains that allow for pathogen recognition specificity (Bent, 1996).

To study R genes that are expressed when the *Sophrolaeliacattleya* Ginny Champion 'Firefly' orchid, tissue was infected with TMV-O, a subtraction library of cDNA clones was constructed using isolated mRNA before and after infection (Shuck, MS Thesis, 2000). From 200 clones collected, several were randomly selected and sent to be sequenced (Davis Sequencing, UC Davis, CA).

Clones 1, 2, and 24E were determined to have cDNA lengths of 385 bp, 655 bp and 1033 bp respectively and all contained a conserved region (encoded by 23 nucleotides) with exact homology to the N-terminal Toll/interleukin-1 receptor gene (TIR) domain in mammals. These clones were partially characterized using bioinformatics databases and analysis packages available on the Internet and molecular software (VectorNTI, Informax, Bethesda, MD). Several programs were used (e.g., ALIGNX, BIOPLOT, and ALIGNXBLOCKS) to search for homology between each clone and other sequences housed in sequence databases. We were specifically looking for other conserved domains such as NBSs and LRRs that are associated with pathogen resistance in other characterized plant genes. These clones also have some homology to the C-terminal leucine rich repeats (LRR) common among resistance genes. Only, clone 23E contained a domain that resembled a NBS region. However, when translating the clones into their different amino acid reading frames no significant homology hits were found to any known plant resistance genes.

Further characterization was performed on other conserved motifs associated with genes in mammalian and *Drosophila* resistance signaling pathways such as coil-coils, leucine zippers, death domains and other components downstream of the IL-1 receptor such as myeloid differentiation protein (MyD88) and IL-1R-associated kinase (IRAK), which are both involved in early signaling in the mammalian innate immune response (Aderem and Ulevitch, 2000). The clones' nucleic acid sequences were also aligned with MyD88 and IRAK using the AlignX program, however no conserved domains were determined. From nucleic acid searches, we determined clones 1 and 24E also contained some homology to MEK kinases and double-zinc-fingers which suggests the clones may function as adaptor proteins in the signaling pathway.

Since we have identified at least three genes that appear to contain TIR domains in orchids, we are anxious to fully characterize them using bioinformatics and molecular approaches which will provide data on the physical structures and specific functions of the genes/proteins. Most plant R genes are found in clusters comprised of very large multigene families (Pan et al., 2000).

Although R genes are structurally related with several distinctive domains in their amino-terminal regions, they are extremely divergent in their DNA sequence. Thus, this variability may be the cause of the low sequence homology of our cDNA clones to other characterized genes.Molecular characterizations have been initiated using the Stratagene Bacterial-two hybrid system vectors to observe protein-protein interactions. In addition, we are examining timing and patterns of expression of various mRNA and proteins.

References

Aderem, A., and R.J. Ulevitch. 2000. Toll-like receptors in the induction of the innate immune response. Nature. 406:782-787.

Bent, A.F. 1996. Plant Disease Resistance Genes: Function Meets Structure. The Plant Cell 8:1757-1771.

Bold, H.C. 1977. The plant kingdom. Prentice-Hall, Englewood Cliffs, NJ.

Martin, G.B. 1999. Functional analysis of plant disease resistance genes and their downstream effectors. Curr. Opin. Plant Biol. 2:273-279.

Meyers, B.C., A.W. Dickerman, R.W. Michelmore, S. Sivaramakrishnan, B.W. Sobral, N.D. Young. 1999. Plant disease resistance genes encode members of an ancient and diverse protein family within the nucleotide-binding superfamily. Plant J. 20(3):317-332.

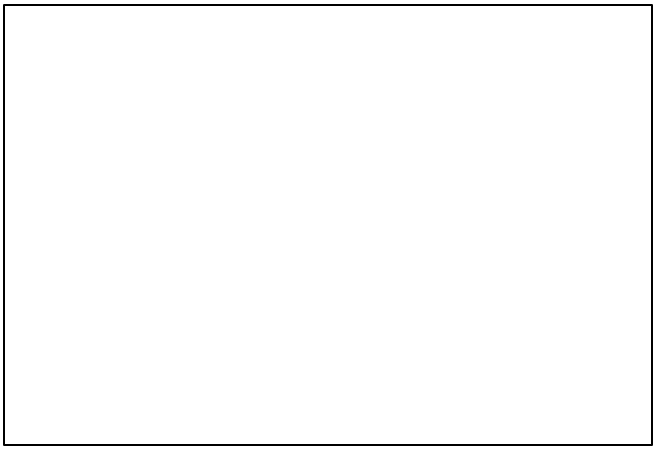
Pan, Q., J. Wendel, and R. Fluhr. 2000. Divergent evolution of plant NBS-LRR resistance gene homologues in dicot and cereal genomes. J. Mol. Evol. 50:203-213.

Shuck, H.A. 2000. Differently Expressed Genes of Sophrolaeliacattleya Ginny Champion 'Riverbend' in Response to the Odontoglossum Ringspot Virus. Master's thesis. Biology Department, Ball State University, Muncie, IN. Unpublished.

Young, N.D. 2000. The genetic architecture of resistance. Curr. Opin. Plant. Biol. 3:285-290.

Wilson, I., J. Vogel, S. Somerville. 1997. Signaling pathways: A common theme in plants and animals? Curr. Biol. 7:175-178.

Wes presenting his poster



First Place Undergraduate Winner

Nitric Oxide Production in Knockout Murine Macrophage Cell Lines Stimulated by LPS, Interferon and Flavone Acetic Acid

by Julia A. Fletcher and Kara Eberly, Biology Department, Saint Mary's College, Notre Dame, IN 46556.

Macrophages are involved in phagocytosis, production of cytokines, antigen-presentation to lymphocytes and killing bacteria and tumor cells. In order to perform any of these functions, macrophages must be activated. IFN-g and LPS are very effective in causing macrophages to become fully activated, and the synthesis of nitric oxide (NO) is an indicator of high-level activation (Bastian and Hibbs, 1994). The NO is then spontaneously converted to equimolar concentrations of nitrite and nitrate ions. Nitrite levels can then be determined using Greiss reagent in a colorimetric assay (Green et al., 1982).

Flavone acetic acid (FAA) has been studied as a potential anticancer drug (Wiltrout and Hornung, 1988) and shown to induce a variety of cytokine mRNAs (Futami et al. 1991). This study examined the effect of combinations of treatments by FAA, LPS, and IFN-g on the production of NO by murine macrophage cell lines with a variety of gene knockouts (KO) related to immune function.

Materials and Methods

The ANA-1, normal, and knock-out (GKO, TNF-R, TKO, iNOS, and NFkB) murine macrophage cells were obtained from Howard Young, Frederick Cancer Research Institute, National Cancer Institute, and grown in a humidified 5% CO_2 incubator at 37°C in DMEM high glucose supplemented with 10% heat inactivated fetal bovine serum, penicillin/streptomycin, and HEPES buffer.

Cells were stimulated with FAA (250mg/mL); IFN-g (Genzyme) at 100U/mL; FAA and IFN-g; and E. coli LPS 0111:B4 (Sigma) at 100ng; and LPS and IFN-g for two days at a cell density of 10^6/mL in 96 well microtiter plates???All determinations were done in triplicate and repeated at least three times. Media was then assayed for nitrite content using Greiss reagent and reading absorbance at 550 nm (Green et al., 1982).

Results and Discussion

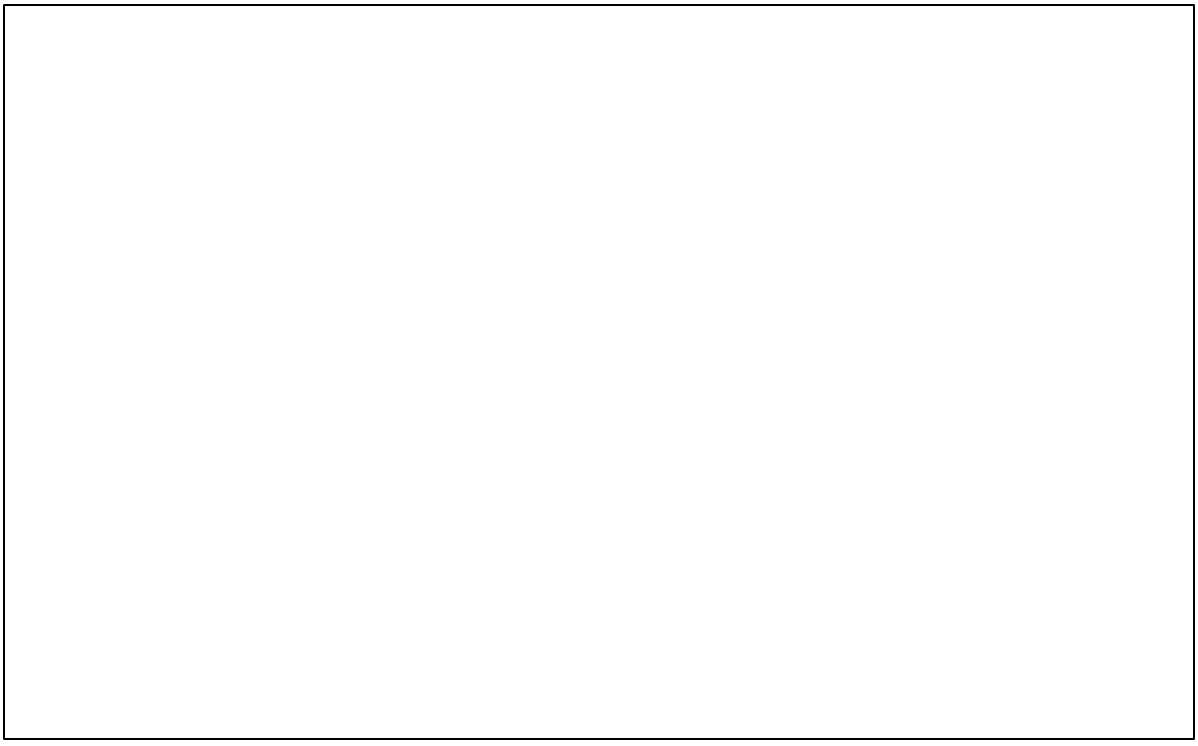
Three normal murine macrophage cell lines showed considerable variation in the magnitude of response to stimulation. ANA-1 cells produced the largest amount of NO of any of the normal cells and the greatest response to LPS and IFN-g (Fig 1.). Stimulating doses of each agent were intentionally low to increase sensitivity to differences and interactions. FAA alone did not induce NO, but did enhance IFN-g-dependent induction in ANA-1. The importance of IFN-g in activation of macrophages is well established and a possible interpretation is that IFN-g induces the expression of the IRF-1 transcription factor, which turns on the iNOS gene. Therefore, GKO, the KO like lacking the ability to produce IFN-g but possessing the IFN-g receptor, should respond normally to IFN-g or LPS plus IFN-g, but it might not be able to respond to LPS. In this study, LPS induced NO production by the GKO line, perhaps through the induction of IFN-b (Jacobs and Ignarro, 2001). IFN-b induces the transcription factor IRF-1, which binds to DNA and stimulates transcription of the iNOS gene. This contention is indirectly supported by FAA data. FAA induced the same response pattern in ANA-1 and GKO (no response to FAA alone but enhanced the response to IFN-g), and FAA has been reported to induce IFN-b (Futami *et al*. 1991).

It has been previously reported that TNF is required to induce iNOS (Chan et al., 2001; Bellocq et al., 1998). The TKO line is not capable of producing TNF-a, TNF-b or its receptors. Therefore, if

TNF is required, TKO should not produce NO. In this study, TKO produced NO in response to IFN-g stimulation. The TNF-R KO line can produce TNF-a and TNF-b but does not have the appropriate receptor to respond these products. TNF-R did produced NO in response to LPS and IFN-g showing that TNF is not necessary for the induction of iNOS or the production of NO.

Previous studies showed that the NFkB pathway enhanced IFN-g stimulated cells to produce NO (Chan et al., 2001). Two subunits, p50 and p65, were required for maximum iNOS induction by LPS and IFN-g, and the inhibition of the NFkB pathway would inhibit iNOS, thus inhibiting the production of NO. In this study, the cell line lacking the p50 subunit of the NFkB gene produced just slightly less NO than ANA-1 under the same stimulation, which establishes that the p50 subunit is not necessary for iNOS production. Ching et al (1999) proposed that NFkB plays an important role in FAA cytokine induction. FAA enhanced NO production induced by IFN-g in ANA-1 normal cells and IFN-g KO line (GKO). However, it had no effect on IFN-g induced production in the NFkB KO, which supports the contention that NFkB plays a significant role in the action of FAA (Ching et al., 1999). The observation that FAA inhibited NO production by TKO and TNF-R may also support this contention, since both lack the ability to respond to TNF, and TNF acts through a NFkB pathway (Bellocq et al., 1998).

In conclusion, this study showed that stimulation by LPS and IFN-g upregulates the production of NO. The two stimuli combined were the best for all cell lines except iNOS, which lacks the inducible nitric oxide synthase. Although TNF-a and IFN-g produced by the macrophage may enhance NO production (Bekker et al., 2001; Guillemard et al., 1999), they are not necessary. Furthermore, the p50 subunit of NFkB is not essential for functional NO production as previously reported (Chan et al., 2001). FAA alone did not induce NO at levels reliably detected by this assay, but it did enhance IFN-g-dependent induction in ANA-1 and GKO lines. It had no effect on the NFkB p50 line and inhibited the IFN-g response in TKO and TNF-R.



Selected References

Bastian, N.R. and J.B. Hibbs. 1994. Assembly and regulation of NADPH oxidase and nitric oxide synthase. Current Opinion on Immunology 6: 131-139.

Bekker, L.G., S. Freeman, P.J. Murray, B. Ryffel, and G. Kaplan. 2001. TNF-a controls intracellular mycobacterial growth by both inducible nitric oxide synthase-dependent and inducible nitric oxide synthase-independent pathways. The Journal of Immunology 166: 6728-6734.

Bellocq, A., S. Suberville, C. Philippe, F. Bertrand, J. Perez, B. Fouqueray, G. Cherqui, and L. Baud. 1998. Low environmental pH is responsible for the induction of nitric-oxide synthase in macrophages. The Journal of Biological Chemistry 273: 5086-5092.

Chan, E.D., K.R. Morris, J.T. Belisle, P. Hill, L.K. Remigio, P.J. Brennan, and D.W.H. Riches. 2001. Induction of inducible nitric oxide synthase-NO by lipoarabinomannan of Mycobacterium tuberculosis is mediated by NEK1-ERK, MKK7-JNK, and NF-kB signaling pathways. Infection and Immunity 69: 2001-2010.

Ching, L.M., H.A. Young, K. Eberly, and C.R. Yu. 1999. Induction of STAT and NF?B activation by the antitumor agents 5,6-dimethylxanthenone-4-acetic acid and flavone acetic acid in a murine macrophage cell line. Biochemical Pharmacology 58: 1173-1181.

Futami, H., L.A. Eader, K.L. Komschlies, R. Bull, M.E. Gruys, J.R. Ortaldo, H.A. Young, and R.H. Wiltrout. 1991. Flavone Acetic Acid Directly Induces Expression of Cytokine Genes in Mouse Splenic Leukocytes but not in Human Peripheral Blood Leukocytes. Cancer Research 51: 6596-6602.

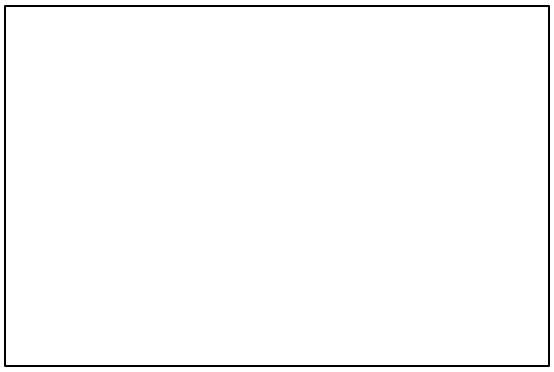
Green, L.C., D.A. Wagner, J. Glogowski, P.L. Skipper, J.S. Wishnok, and S.R. Tannenbaum. 1982. Analysis of nitrate, nitrite, and [15N] nitrite in biological fluids. Analytical Biochemistry 126: 131-138.

Guillemard, E., B. Varano, F. Belardelli, A.M. Quero, and S. Gessani. 1999. Inhibitory activity of constitutive nitric oxide on the expression of alpha/beta interferon genes in murine peritoneal macrophages. Journal of Virology 73: 7328-7333.

Jacobs, A.T. and L.J. Ignarro. 2001. Lipopolysaccharide-induced expression of interferon-b mediates the timing of inducible nitric-oxide synthase induction in RAW 264.7 macrophages. The Journal of Biological Chemistry 276: 47950-47957.

Webb, J.L., M.W. Harvey, D.W. Holden, and T.J. Evans. 2001. Macrophage nitric oxide synthase associates with cortical actin but is not recruited to phagosomes. Infection and Immunity 69: 6391-6400.

Wiltrout, R.H., and R.L. Hornung. 1988. Natural products as antitumor agents: direct versus indirect mechanisms of activity of flavonoids. Journal of the National Cancer Institute 80: 220-222.



Julia's poster

Second Place Undergraduate Winner

H-NS is required for the Derepression of Methionine under Stress Conditions

by Keynttisha Jefferson and Dr Mark Levinthal, Dept. of Biological Sciences, Purdue University, West Lafayette, IN

H-NS is a small chromatin-associated protein found in enterobacteria. The major role of H-NS is to regulate the expression of a large number of genes, mostly by negatively affecting transcription. Many of the H-NS-regulated genes are regulated by environmental signals, and expression of most of these genes is positively regulated by specific transcription factors. Therefore, one of the purposes of H-NS could be to repress expression of some genes under conditions characteristic of a non-intestinal environment, but allow expression of specific genes in response to certain stimuli in the intestinal environment. We compared the growth of a strain of E. coli with a transposon insertion in the promoter region of h-ns with its isogenic part. We measured the activity of the metA promoter for each strain using a metA::lacZ fusion. The fusion caused methionine auxotrophy. When grown in the presence of all 20 amino acids the â-galactosidase activity, and therefore metA transcription, is the same in the h-ns mutant its isogenic parent.

When methionine is limited by substituting D-methionine, the level of expression for the isogenic parent is much higher than the mutant. The mutant strain was unable to derepress completely under the condition of methionine limitation. When a downshift experiment is performed on the isogenic parent and the mutant strain where was first grown in rich media and then diluted into limited media the derepression phenomena is observed. The h-ns mutant was unable to derepress as well as the isogenic parent strain. This derepression phenomenon is not observed when the mutant strain was grown first under methionine-limited conditions and then diluted into fresh methionine limited media. Both the mutant and isogenic parent strain had the about same metA transcriptional activity. We conclude that h-ns is required for metA derepression and inorder to see the effect of these mutations on metA transcription it is necessary to perform a down shift.

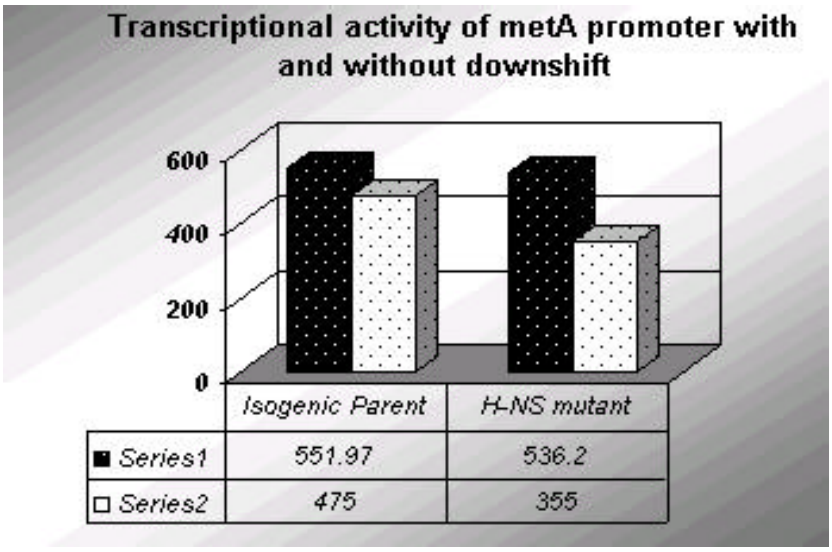
Materials and Methods

? -Galactosidase Assay
? -Galactosidase Assay were preformed as described by Miller and altered by Giacomini et al.

Results

The results of the downshift are shown in figure 1. Notice that the control metA transcriptional activity of the mutant strains are essentially the same as their isogenic parent strain. The h-ns mutant only had 71% of the derepression of metA that of its parent strain.

Figure 1



Discussion

The h-ns mutant strain only derepressed 71 % of that of the parent strain. This derepression phenomenon is not however observed when the strain was grown first under methionine-limited conditions and then diluted into fresh methionine limited media. The h-ns mutant strain showed almost equal amounts of metA derepression under these conditions.

These results suggest that the h-ns mutant does not respond as well to starvation for methionine as the parent strain. Derepression is stunted in the h-ns mutant. We conclude that h-ns is required for metA derepression and inorder to see the effect of these mutations on metA transcription it is necessary to perform a down shift.

Prof. Jagger presenting the award to Keynttisha

Employment Opportunities

MICROBIOLOGIST
ASSISTANT/ASSOCIATE PROFESSOR
DEPARTMENT OF BIOLOGY
BALL STATE UNIVERSITY
MUNCIE, INDIANA

Tenure-track position (years toward tenure considered) available August 17, 2003. Responsibilities: teaching introductory microbiology, medical microbiology, introductory biology and biotechnology courses; conducting and promoting student involvement in research specialty in support of biology major/microbiology option; providing service to the academic community. The person chosen should have a commitment to excellence in teaching and competency in current approaches in microbiology. Minimum qualifications: earned doctorate in a biological science by August 17, 2003; effective written and oral communication skills. Preferred qualifications: demonstrated teaching ability, publications and/or evidence of other scholarly activity. Send letter of application, curriculum vitae, documentation of scholarly activity and teaching ability, copies of transcripts, and three letters of reference to: Chair, Microbiology Search and Selection Committee, Department of Biology, Ball State University, Muncie, IN 47306. Review of applications will begin October 4, 2002.

Ball State University is an equal opportunity, affirmative action employer and is strongly and actively committed to diversity within its community.

IMMUNOLOGIST
ASSISTANT/ASSOCIATE PROFESSOR
DEPARTMENT OF BIOLOGY
BALL STATE UNIVERSITY
MUNCIE, INDIANA

Tenure-track position (years toward tenure considered) available August 17, 2003. Responsibilities: teaching immunology, introductory biology and biotechnology courses; conducting and promoting student involvement in research specialty in support of biology major/microbiology option; providing service to the academic community. The person chosen should have a commitment to excellence in teaching and competency in current approaches in immunology. Minimum qualifications: earned doctorate in a biological science by August 17, 2003; effective written and oral communication skills. Preferred qualifications: demonstrated teaching ability, publications and/or evidence of other scholarly activity. Send letter of application, curriculum vitae, documentation of scholarly activity and teaching ability, copies of transcripts, and three letters of reference to: Chair, Immunology Search and Selection Committee, Department of Biology, Ball State University, Muncie, IN 47306. Review of applications will begin January 3, 2003.

Ball State University is an equal opportunity, affirmative action employer and is strongly and actively committed to diversity within its community.

Photos from the Spring 2002 IBASM Meeting

Prof. Carolyn Vann presenting the award for Teaching Excellence to Dr. Carl Bauer

ASM Foundation Lecturers

Dr. Barry Wanner

Dr. Douglas Clark

Prof. Eberly with student presenters

High school presenter, Huma Ansari

Poster Presentations

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Gene Protects Baker's Yeast from Freezing

Researchers from Spain have identified a gene that helps baker's yeast survive freezing temperatures. They report their results in the June 2002 issue of the journal Applied and Environmental Microbiology.

In the study, the scientists identified a number of genes in the yeast Saccharomyces cerevisiae, commonly known as baker's yeast, which appeared to be turned on when the yeast was exposed to sub-freezing temperatures. Further investigation revealed that one of the genes, ERG10, helped protect the yeast from freezing.

Commercial bread dough is often stored frozen, which can kill yeast cells and severely reduce the dough's ability to rise after thawing. "Consequently, the improvement of the freeze tolerance in baker's yeast is of significant commercial importance," say the researchers, adding that their findings could "open up the possibility of design strategies to improve the freeze tolerance of baker's yeast."

(S. Rodriguez-Vargas, F. Estruch and F. Randez Gil. 2002. Gene expression analysis of cold and freeze stress in baker's yeast. Applied and Environmental Microbiology, 68: 3024-3030.)

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Measuring Contamination in Meat with Infrared Light

A real-time method that rapidly tests meat for spoilage uses a spectroscope to measure compounds produced by microorganisms on the meat. Researchers from the University of Wales present this new methodology in the June 2002 issue of the journal Applied and Environmental Microbiology.

The process, called fourier transform infrared spectroscopy (FT-IR), uses infrared light waves to identify specific organic compounds within the meat. The technique measures the absorption of various wavelengths of infrared light by the meat. The pattern of absorption creates a unique fingerprint for each compound.

In the study the researchers tested the ability of FT-IR to detect the biochemical byproducts of microbial contamination in chicken breasts left at room temperature to spoil. Every hour, FT-IR measurements were taken directly from the meat surface and microbial counts were taken by standard culture methods. Estimates made from FT-IR in less than 60 seconds were similar to the final counts made by culturing.

"Using FT-IR, we were able to acquire a metabolic snapshot and quantify, non-invasively, the microbial loads of food samples accurately and rapidly (within 60 seconds) directly from the sample surface," say the researchers.

(D.I. Ellis, D. Broadhurst, D.B. Kell, J.J. Rowland and R. Goodacre. 2002. Rapid and quantitative detection of the microbial spoilage of meat by fourier transform infrared spectroscopy and machine learning. Applied and Environmental Microbiology, 68: 2822-2828.)

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Oral Vaccine Boosts Existing Tuberculosis Vaccine

An experimental oral vaccine appears to boost the existing tuberculosis vaccine in mice, say researchers from Statens Serum Institute in Copenhagen, Denmark. Their findings appear in the June 2002 issue of the journal Infection and Immunity.

The current BCG vaccine for tuberculosis remains a mainstay of control programs world wide, but while it continues to be effective protecting children from systemic tuberculosis, it no longer appears to be effective in protecting many adults from the pulmonary version of the disease. The researchers have developed an oral vaccine that by itself does not elicit immunity but does boost immunity to protective levels in adult mice that had already been given the BCG vaccine.

"Despite decades of effort and enormous expenditure, tuberculosis remains of the world's most devastating diseases," say the researchers. "The approach outlined in this report offers the possibility of a simply administered oral booster vaccine specifically targeted to the prevention of adult pulmonary tuberculosis."

(T.M. Doherty, A. Weinrich Olsen, L. van Pinxteren and P. Anderson. 2002. Oral vaccination with subunit vaccines protects animals against aerosol infection with Mycobacterium tuberculosis. Infection and Immunity, 70: 3111-3121.)

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Not All UV Light Effective in Treating Drinking Water

Researchers from the University of Waterloo in Ontario, Canada have determined that some types of ultra violet light are not effective in killing harmful bacteria in drinking water. Their results appear in the July 2002 issue of Applied and Environmental Microbiology.

In the study the researchers tested the ability of *Escherichia coli* to repair DNA damage caused by varying levels of UV radiation from low and medium pressure lamps. They found that while *E.coli* was able to fully repair damage done to its DNA after being subjected to low-pressure UV rays, they remain unclear as to the effects of medium-pressure rays.

"The results of this study show that polychromatic medium-pressure UV radiation may offer an advantage over monochromatic low-pressure UV radiation in lower-dose water treatment applications," claim the researchers. "It is recommended that further studies be carried out with medium-pressure UV to determine which wavelengths cause additional damage and where the damage is induced."

(J. L. Zimmer and R. M. Slawson. 2002. Potential repair of *Escherichia coli* DNA following exposure to UV radiation from both medium- and low-pressure UV sources used in drinking water treatment. Applied and Environmental Microbiology, 68: 3293-3299.)

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Bacteria Could Be New Meningococcal Vaccine

A harmless bacteria can elicit an immune response that protects against a common cause of bacterial meningitis and could serve as a potential vaccine against the disease, say researchers from the United Kingdom. Their findings appear in the July 2002 issue of the journal Infection and Immunity.

Neisseria lactamica is a bacterium that does not cause human disease, but closely resembles *Neisseria meningitidis*, one of the causes of bacteria meningitis. Previous studies have suggested that the development of natural immunity to menigococcal disease results from colonization of the nasal cavities by harmless Neisseria bacteria, particularly *N. lactamica.*

"The present study explores experimentally the hypothesis that immunization with N. lactamica can mimic infection by and enhance natural immunity to the meningococcus," say the researchers. In the study mice that were immunized with killed N. lactamica bacteria were protected from lethal infection by a number of strains of menigococcal bacteria. "The results confirm the potential of N. lactamica to form the basis of a vaccine against meningococcal disease."

(K.J. Oliver, K.M. Reddin, P. Bracegirdle, M.J. Hudson, R. Borrow, I.M. Feavers, A. Robinson, K. Cartwright and A.R. Gorringe. 2002. *Neisseria lactamica* protects against experimental meningococcal infection. Infection and Immunity, 70: 3621-3626.)

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Immune Cells in Breast Milk Protect Infants from HIV

Scientists have found immune cells in the milk of HIV-infected mothers that target and kill the virus. This finding, which could help explain the low transmission rate from mother to child via breastfeeding despite high levels of the virus in mother's milk, appears in the August 2002 issue of the Journal of Virology.

Researchers from the University of Alabama at Birmingham, the Zambia Exclusive Breast-Feeding Study, Boston University and Columbia University tested breast milk cells (BMC) from HIV-infected women in the United States and Africa for their ability to identify and respond to components of the AIDS virus. While BMC's from all of the HIV-infected women reacted to the HIV proteins, there was no reaction from the cells from the uninfected women.

Further tests revealed that the responses were due to the presence of immune cells known as CD8+ T cells, the same immune cells that play a critical role in controlling HIV levels in the blood.

"These studies provide evidence that maternally derived T cells make their way into the infant's circulation and potentially protect the infant via adoptive transfer of maternal T cells," say the researchers. "In addition to the protection these cells may afford the neonate, we speculate that they may also be acting locally to reduce the viral load in breast milk, lowering the viral burden, and potentially decreasing transmission to newborns from their HIV-infected mothers."

(S. Sabbaj, B.H. Edwards, M.K. Ghosh, K. Semrau, S. Cheelo, D.M. Thea, L. Kuhn, G. D. Ritter, M.J. Mulligan, P.A. Goepfer, and G.M. Aldrovandi. 2002. Human immunodeficiency virus-specific CD8+ T cells in human breast milk. Journal of Virology, 76: 7365-7373.)

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NOTE:

We are looking for a new Educational Representative for this year as Kathleen Jagger is leaving Indiana. Please contact Jim Mitchell at jkmitchell@bsu.edu if you are interested in the position.

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