News Letter

# IBASM

Volume 6, #1 July 2003

## Message from the President- Jim Mitchell

Outstanding-Outstanding-Outstanding! Ranked in order, these are the 3 words I heard at Spring Mill Inn among participants describing the Spring IBASM meeting! Over 100 microbiologists, molecular biologists and immunologists attended this event which nearly filled Lakeview Hall and all of the available rooms at the Inn. Lodging accommodations and meals were great and Spring Mill Inn staff were wonderful to work with. You could not ask for a better weekend for wildflower forays and hiking during the Saturday afternoon break. Virginia Bluebell, Dutchman breeches, Nodding Trillium, Twin Leaf, Spring Beauty and Rue Anemone were a few of the wildflowers I recognized blooming along Trails 4 & 7.

**Continued on page 2**

**WHAT'S INSIDE...**



## Message from the President-Elect Jeanne Barnett

The April 11-13, 2003, IBASM meeting was a resounding success. Thanks to all of those who came and participated. We had approximately 100 registrants – a record I believe. I can say it was one of the best meetings we have had since my participation in IBASM. Not only was the weather beautiful and the park inviting, It was stimulating to meet new microbiologists and hear about their work. The invited speakers, Dr. Stan Spinola and Dr. Ian Lipkin, were great. Jim Mitchell reviewed Dr. Stan Spinola's presentation in his article in this newsletter. Dr. Ian Lipkin, our Saturday evening speaker, was outstanding. Dr. Lipkin is involved with Pandora's Box, developing diagnostic tests for those infectious diseases that are associated with sudden outbreaks or chronic conditions. He is interested in developing models of the disease and new methods for diagnosis. Dr. Lipkin discussed the current SARS (sudden acute respiratory syndrome) outbreak. A new coronavirus has been identified as the etiologic agent. An animal reservoir is suspected but not confirmed for SARS. West Nile Virus was also discussed.

**Continued on page 4**

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

I timed it perfectly to arrive at Pioneer village as they opened up the aqueduct to power the Grist Mill. I heard that the cave tours were also a big success. It was especially nice to hear that the guide still holds the record for most consecutive emergences from the cave without getting lost!

The Friday evening speaker Dr. Stan Spinola discussed infection of human volunteers with Haemophilus ducreyi. This bacterium is the etiologic agent for chancroid, a sexually transmitted genital ulcer disease that facilitates the transmission (4X) and acquisition (3-23X) of HIV-1. Dr. Spinola described that infections are usually localized in the genital area and wounds may be required for infection with a transmission rate as high as 70%. Stan presented details of an epidermis assay he developed which causes minimal risk to the test patient and is cured with antibiotic therapy. The rationale behind this experimentation was due to a lack of human specimens to study this disease, since humans are the only reservoir, and there is limited information on pathogenesis. To understand the kinetics of localization, Stan examined infected sites on volunteers between 0-48 hr after inoculation and at the clinical end point. Immediately after inoculation, bacteria were found predominantly in the dermis but also in the epidermis. Few bacteria were detectable at 24 hr; however, by 48 hr bacteria were readily seen in the pustule and dermis. His findings demonstrated that H. ducreyi was associated with PMN leukocytes and macrophages in the pustule and at its base, but was not associated with T cells, Langerhans' cells, or fibroblasts. H. ducreyi co-localized with collagen and fibrin but not laminin or fibronectin. Association with phagocytes, collagen, and fibrin was seen as early as 48 hr and persisted at the pustular stage of disease. Data collected identifies collagen and fibrin as potentially important targets of adherence in vivo and strongly suggest that H. ducreyi remains extracellular throughout infection and survives by resisting phagocytic killing in vivo. The results also indicate no difference in male vs. female papule formation rate but odds of a male progressing to pustule were 2.5 fold > than a female. Jeanne will give you details of the Saturday evening seminar presented by the ASM Foundation speaker Dr. Ian Lipkin.

There were a total of 52 posters presented at the meeting. No, this is not a typo, we had 52 poster presentations which is the largest turnout I have seen in my 11 years as a member of IBASM! The quality of the student presentations was awesome and it was very informative for me to see the range of different research areas. Lee Swem (Indiana University Bloomington) received the McClung award for 1st place graduate poster. Lina Li and Miriam Martin were awarded 2nd place in the graduate division. Both are also from IU Bloomington. Valerie Horobik from Hanover College won 1st place and Trent Miller from Indiana University Kokomo won 2nd place in the undergraduate category. All of us who viewed the poster session look forward to a similar number of participants next year, and I hope to possibly see students compete in the high school division. The socializing which occurred during the judging segment was almost deafening at times, but a great opportunity for students to visit with each other and to interact with professionals who provided valuable ideas and advice for future education and employment. All winners receive a complimentary ASM membership, a certificate and a monetary gift when a short paper is published in the IBASM newsletter.

We discussed several issues at the Indiana branch business meeting held 5pm on Saturday. We chose May 30th to meet and plan details of the 2004 IBASM meeting. Although we will have met prior to publication of this newsletter, I wanted to announce that any full member is welcome to attend this meeting. One item on the agenda will be to consider additional divisions in student competition (e.g. 1st year graduate student vs. exiting graduate student). Next Spring rendezvous (April 16-17, IU School of Dentistry, Indianapolis) will be my last meeting as President. Jeanne Barnett will take over the reins beginning in Spring 2005. This means that we must vote for a new President-Elect next year. If any full member is interested in this position or has someone they wish to nominate for the 2004 ballot please email me. We now have in possession for branch use a portable 4X6 ft professional poster board and laser pointer. Please contact me if you wish to use either of these items for microbiology outreach. The Regional Initiative Grant Proposal was submitted last November. I asked for $2810 to cover publication costs of the newsletter, student award stipends, speaker honoraria, and outreach supplies. There is no word as yet from ASM headquarters how much funding will be awarded.

**Continued on the next page**

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

There are several upcoming meetings you may be interested in attending. The Mycological Society of America (MSA) will hold its annual meeting July 26-31 in Asilomar, CA. The American Phytopathological Society (APS) meeting will be held August 9-13 in Charlotte, NC. The Society for Industrial Microbiology (SIM) will hold its annual meeting August 10-14 in Minneapolis, MN. The 43rd ICAAC (Interscience Conference on Antimicrobial Agents & Chemotherapy) will meet September 14-17 in Chicago. The Southern Great Lakes section of the Society for Industrial Microbiology (SGL-SIM) will be meeting Saturday, October 11th at Loyola University. 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 conference each year and I would like to see more members from Indiana attend this very informative meeting. Although there are no student presentations, it will provide an excellent avenue for students and Hoosier microbiologists to meet with industrial microbiologists from both the private and government sectors. This years' 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 website **http://www.simhq.org/html/localsecs/sgreatlakes.html.** The 5th meeting of RAFT (Recent Advances in Fermentation Technology) will be held November 9-12 in St. Petersburg, FL.

I recently conducted an online search for websites providing information on medical mycology including: image banks, antifungal agents, MIC databases, and educational tools/lectures. The best one I found was Doctor Fungus (http://www.doctorfungus.org/index.htm) which contains information on all of the above. Other useful sites include: Bad Bug Book: Foodborne Pathogenic Microorganisms and Natural Toxins Handbook (http://vm.cfsan.fda.gov/~mow/chap40.html), Mycology Image Gallery (http://microbiology.mtsinai.on.ca/mig/index.shtml), Airborne Fungal Spore Types (http://pollenuk.worc.ac.uk/Aero/FUNGI/types.htm), Medical Mycology-Univ. Wisconsin (http://www.medmicro.wisc.edu/Resources/ImageLib/Mycology/index.html), and Mycology Online (http://www.mycology.adelaide.edu.au/). If any of you know of additional useful medical mycology sites or any new microbiology sites you would like to share with others please email these to me to include in upcoming news-letters.

Looking forward to seeing you at one of these meetings.....mark April 16-17, 2004 on your calender now!

**Special Thanks to All Judges!**

On behalf of all of the students in the poster competition I would like to express sincere appreciation to all of the members who volunteered their time to judge at the meeting. Students were evaluated in 4 different categories: scientific thought, creativity, thoroughness and presentation (abstract, oral and poster). This was no easy task! Next time you see any of these persons please thank them for sweating through a very difficult challenge:

**Graduate Team #1** = Jim Olesen (BSU), Jeff Hughes (HC), and Yves Brun (IUB)
**Graduate Team #2** = Diana Catt (IUPUI), Kathleen Dannelly (ISU), and Kara Eberly (SMC).
**Graduate Team #3** = Christian Chauret (IUK), Arthur Koch (IUB), and Carl Bauer (IUB).
**Undergraduate Team #1** = Mark Levinthal (PU), Clay Fuqua (IUB), and David Kehoe (IUB).
**Undergraduate Team #2** = Jim Mitchell (BSU), Richard Hardy (IUB), and Srisuda (Ho) Dhamwichukorn (UND).

**McClung First Place Graduate Winner**

# A Redox Active Cysteine Affects the Autophosphorylation Ability of RegB

Lee R. Swem and Carl E. Bauer Indiana University, Bloomington, IN, 47405

*Rhodobacter capsulatus* is a purple non-sulfur photosynthetic bacterium that demonstrates substantial metabolic diversity through its ability to grow under a vast array of conditions. In an aerobic environment, *R. capsulatus* uses $O_2$ as a terminal electron acceptor ultimately reducing oxygen to water. However, *R. capsulatus* can also respire under anaerobic conditions, using DMSO as a terminal electron acceptor. If neither $O_2$ nor DMSO is present, *R. capsulatus* can synthesize a photosystem that harvests light energy, which is converted to chemical energy. It was previously demonstrated that the necessary protein components for each growth condition are under the global regulation of the two-component signal transduction system, RegB/RegA. More specifically, it has been shown that the structural components of the entire photosystem, nitrogenase, hydrogenase, and carbon fixation pathway are all under the transcriptional control of the RegB/RegA two component signal transduction system. RegB, a histidine sensor kinase, is capable of sensing cellular oxidation state and autophosphorylating itself in response to decreased oxygen tensions. Once phosphorylated, RegB is capable of transferring the phosphate from its conserved histidine residue to an aspartate residue on the cognate response regulator, RegA. It is proposed that upon phosphorylation, RegA is capable of binding DNA and activating transcription.

To determine if RegB/RegA also controls the transcriptional expression of electron transport chain components, *lacZ* promoter fusions were constructed to cytochromes; $c_2$, $c_y$, $bc_1$, *ccoN* and *cydAB*. The *lacZ* reporter fusions were then introduced into wild-type and a *regA* mutant strain of *R. capsulatus* and assayed under aerobic, semi-aerobic and photosynthetic growth conditions. The ?-galactosidase expression levels were significantly different in the *regA* mutant strain versus wild-type for all of the *lacZ* fusions assayed. To determine if RegA was directly affecting the transcription of these operons, DNase I protection assays were performed using purified RegA and probes corresponding to the promoter regions of each cytochrome and terminal oxidase mentioned above. RegA protection assays revealed that RegA binds to the promoters and directly affects the transcriptional levels of all components in the respiratory chain. These findings qualified RegB/RegA as a global regulatory circuit, which provides transcriptional modulation to multiple operons based on cellular redox state.

After determining the global nature of the RegB/RegA regulon, it became extremely important to determine the redox sensing mechanism within the sensor kinase, RegB. As mentioned previously, RegB is a histidine sensor kinase that only autophosphorylates when environmental oxygen becomes limited. It was not yet fully understood if RegB sensed $O_2$ directly or simply monitored the redox equilibrium within the cell. RegB is a classic histidine sensor kinase, which contains six transmembrane spanning domains at the N-terminus followed by the dimerization domain containing the H-box and finally the ATP binding domain at the C-terminus.

To understand the nature of this sensing mechanism, a truncated form of RegB was constructed that lacks transmembrane spanning domains. This truncated form of RegB was overexpressed and purified from *E. coli* and will be referred to as RegB". The truncated form of the protein (RegB") was still active based on autophosphorylation assays and responded slightly to redox. Specifically, RegB" would phosphorylate to a higher level upon treatment with dithiothreitol (DTT), which is a strong reducing agent. This is consistent with a higher level of RegB phosphorylation *in-vivo* when

environmental conditions become reducing. It was hypothesized that a redox active metal may allow RegB" to sense redox. To determine if RegB" was binding a metal ion, the protein was first treated with EDTA (a general metal chelator) and then again assayed for autophosphorylation under oxidizing and reducing conditions. Surprisingly, RegB" no longer exhibited a phosphorylation difference between oxidized and reduced samples, indicating that a metal ion plays a crucial role in the redox sensing capability. EDTA treated RegB" was then reconstituted with various divalent cations to determine which metal was allowing RegB to sense redox. RegB was incubated with a molar equivalent of divalent metal and then dialyzed for two days to remove excess unbound metal before being assayed for autophosphorylation activity. Unexpectedly, any divalent metal ion allowed RegB" to again sense reducing conditions and phosphorylate only when treated with the reducing agent DTT. To determine if RegB" was truly binding a metal, copper treated RegB" was examined using electro paramagnetic resonance (EPR) because of the characteristic spectral information associated with copper. EPR was performed on copper loaded RegB", which exhibited a nitrogen and oxygen coordinated $Cu^{2+}$ EPR spectrum. After reducing the copper treated RegB" with DTT the EPR spectrum was lost, which is consistent with the fact that $Cu^{1+}$ does not exhibit an EPR signal. From this, it was concluded that the metal ion actually binds to RegB" instead of having only a transient affect on the kinase.

To determine the redox midpoint potential of RegB", a redox gradient was established using ratios of oxidized to reduced DTT. The gradient ranged from -200 mV to -400mV in 10 mV increments. After incubating RegB" at each redox potential for 18 hours, an autophosphorylation assay was performed. The percent phosphorylation was then plotted against ambient potential, which yielded a sigmoidal curve best fit by a two electron Nernst equation. The midpoint potential of the curve was then determined to be -300 mV. Interestingly, the data best fit a two electron line equation, which makes it impossible for the copper ion to be the redox sensing component. Copper is a divalent cation, which can only accept one electron to become fully reduced. The midpoint data indicated that the redox active component within RegB" actually accepts two electrons to become fully reduced. Since, RegB" did not exhibit spectral characteristics associated with another chromophore that could accept two electrons, it was hypothesized that RegB" may contain a redox active disulfide bond, which when formed would turn off the kinase and when reduced would allow RegB" to autophosphorylate. Each sulfur atom within a disulfide bonds accepts one electron to become reduced, therefore a single disulfide bond would need two electrons to become fully reduced. The amino acid composition of RegB" revealed that only one cysteine residue was present in the truncated form of the protein. The cysteine residue was mutated to an alanine and the redox sensing capability of the kinase was lost even if the copper ion was still present within RegB". In this model, the metal ion only plays a structural role, allowing the disulfide bond to form under oxidizing conditions, ultimately turning off the kinase. If the metal ion is not present, the disulfide bond cannot form and the kinase is on under both oxidizing and reducing conditions.

To see if this cysteine residue was crucial for redox sensing *in-vivo*, the cysteine to alanine mutant form of RegB was placed in the chromosome of *R. capsulatus* replacing the wild type copy of RegB. The RegB mutant strain exhibited a much darker phenotype than wild type under aerobic conditions, indicating that RegB was autophosphorylating under aerobic conditions and passing this phosphate to RegA, which was then activating the bacterial photosystem when oxygen is present. This solidified the notion that the cysteine residue was critical for the redox sensing mechanism of RegB within *R. capsulatus*. The exact state of the cysteine *in-vivo* has not yet been determined, however *in-vitro* data indicates that the cysteine residue participates in a disulfide bond when oxidized

Swem's paper (coninued from page 5)

and is a free sulfhydryl when reduced. *In-vivo* however, the cysteine residue may just become oxidized to a thiolate derivative under aerobic conditions such as sulfenic acid, which could affect the kinase ability of RegB. Further research will be required to understand the exact role this cysteine plays *in-vivo*.

**Lee Swem, the first place graduate winner**

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

There are 2 lineages of the virus with lineage 1 spreading more rapidly. This lineage appears to replicate more efficiently, so it out competes lineage 2. The NS-1 protein of this virus can move from cell to cell and cause bystander effect on uninfected cells. About 1% of individuals infected with West Nile Virus will have serious disease. A special area of interest for Dr. Lipkin is neuropsychiatric disease. He has studied Borna disease virus and has developed a rat animal model. With adult exposure, the rat shows chronic disease that appears to be immune mediated. There is a reduction in dopamine receptors and resulting disease similar to obsessive-compulsive disorder. In neonatal exposure, the animal develops a disease similar to schizophrenia. There is cytokine dysregulation with an increase in Fas with resulting apoptosis. An increase in serotonin appears and is similar to what is seen autism. A model of generic gestational insult in humans has been developed that highlighted 2 windows of time when exposure to infectious agents may result in chronic neuropsychiatric disorders. Those times are 21-24 days and just prior to 30 weeks of gestation. The infectious insult may include rubella and influenza. PANDAS (pediatric autoimmune neuropsychiatric disorders) has been associated with *Streptococcus* infectious. New procedures for diagnosing infection have been developed and include mass tag PCR and subtraction methods. Dr. Lipkin's talk was both interesting and timely.

We are in the beginning stages of planning the meeting for next year. The meeting will be Friday evening through Saturday afternoon, April 16-17, 2004. The meeting will be in Indianapolis at the Indiana University School of Dentistry. The University Place Conference Center and Hotel has been reserved for lodging. The cost for the lodging is $115 for a single and $130 for a double. University Place is within walking distance of the Dental School. We hope the central location will be good for all of you. The Executive committee is meeting the end of May to plan the program, so stay tuned. Please put the date on your calendar and plan to attend. Let's continue the good participation and great interactions.

**Second Place Graduate Winner**

# Characterization of a Complex Response Regulator Involved in Complementary Chromatic Adaptation in *Fremyella diplosiphon*

Lina Li and David Kehoe, Indiana University, Department of Biology, Bloomington, IN, 47405

Microorganisms must be able to sense and rapidly respond to environmental changes. Photosynthetic microbes, in particular, must be capable of closely monitoring and adapting to changing light conditions to maintain photosynthetic efficiency. In certain cyanobacteria, cells change color with environmental light quality: when growing under red light (RL), cells appear to be green; while growing under green light (GL), cells appear to be red. This process is termed complementary chromatic adaptation (CCA). Upon shift to new light qualities, the photosynthetic efficiency has been shown to drop significantly (Campbell, 1996). However, this efficiency drop is transient, since it is gradually eliminated as the cells undergo CCA.

We know that CCA is a result of alteration in light harvesting structures, the phycobilisomes (PBS) (Bogorad, 1975, Grossman, *et al*, 1986). PBS are peripheral membrane structures in prokaryotic cyanobacteria and eukaryotic algae that efficiently harvest light energy and direct it to photosynthetic reaction centers embedded in the thylakoid membranes. These massive complexes are organized into two distinct regions, the inner core and the outer rods. Both regions contain pigmented phycobiliprotein, which involved in light harvesting, and non-pigmented linker polypeptides, which facilitate phycobiliproteins assembly and stabilize PBS structures. During CCA, the composition of rods of the PBS is altered in respond to changes in the wavelength of prevalent light, while the cores, composed of allophycocyanin (AP, maximum absorbance ~650 nm) do not change. In RL, accumulation of the blue pigment phycocyanin (PC, maximum absorbance ~565 nm), but not the red pigment phycoerythrin (PE), to a high level in the rods makes cells appear blue-green; in GL, PE is present at a high level and the PC level is decreased dramatically, so cells appear red. The accumulation of RL-absorbing PC in RL and of GL-absorbing PE in GL allows the cells to optimize their use of the predominant wavelength of light in their environment and maintain their high photosynthetic efficiency. This reversible photocontrol of PC and PE level has been shown to be primarily a consequence of transcriptional control of *cpcB2A2* (encoding PC and its linker) and *cpeBA* operons (encoding PE) (Oelmüller, et al, 1988a, and 1988b); the transcript abundance of *cpeCDE*, the operon encoding PE linkers, has also been shown to be regulated by light conditions (Federspiel, *et al*, 1990 and 1992).

In *Fremyella diplosiphon*, the cyanobacteria we study, three key regulatory proteins have been isolated that control these three operons' light responsiveness: a sensor, RcaE, a single domain response regulator, RcaF and a complex response regulator, RcaC. RcaC has two receiver domains, a putative DNA-binding domain and a histidine-containing phosphotransfer domain (HPt). Two conserved aspartate residues at position 51 (designated as D1) and D576 (designated as D2) together with a histidine at position 316 are thought to be the site of phosphorylation within RcaC. Several lines of evidence have been used to develop a model for the early steps in CCA (Figure 1). In this model, when cells are grown under RL, RcaE acts as a kinase, autophosphorylates at a histidine residue, then transfers

the phosphoryl group to RcaF, which is then transferred to RcaC. When cells are grown under GL, RcaE acts as a phosphatase, and RcaF and RcaC are dephosphorylated. The phosphorylation of RcaC is believed to change its DNA binding affinity and thus regulate downstream gene transcription.

The regulation of CCA appears to be complex, because CCA is not regulated through just a single sensory system. Kahn *et al*. (1997) noted residual light responsive accumulation of PE in a mutant containing a lesion in *rcaC*. More recently, detailed analysis of *cpeBA*, *cpeCDE*, and *cpcB2A2* light regulated transcript accumulation patterns in the *rcaE* null mutant demonstrate that these cells retain residual light responsiveness of both *cpeBA* and *cpeCDE* (Sieb *et al*, 2002). However, *cpcB2A2* seems to be solely regulated by the RcaE pathway, since the transcript abundance from this operon remains the same regardless of the light condition in an *rcaE* null mutant. Whether these two pathways act independently or share some common components remains to be determined.

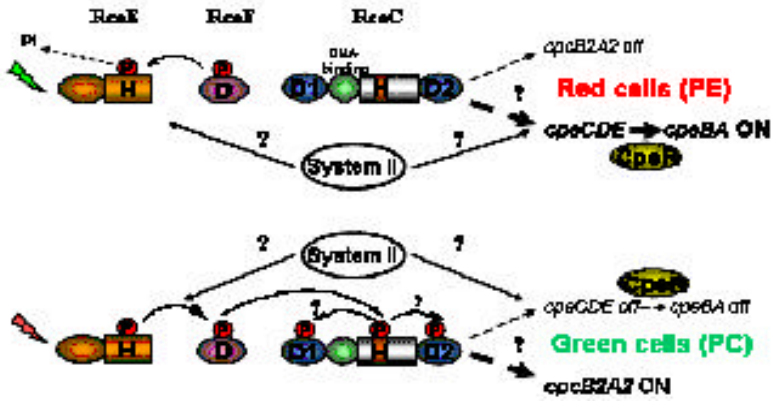


**Figure1:** Model for CCA signal transduction in *F. diplosiphon*. The three components thus far described in this pathway are the putative sensor RcaE and response regulator RcaF and RcaC. The residues of these components that may be phosphorylated are H of RcaE, D of RcaF, and H, D1 and D2 of RcaC.

My research addresses the following questions:

- **Are the conserved aspartate and histidine residues in RcaC essential in controlling CCA?**
- **Does RcaC play a role in signaling through the second pathway?**

**I. Are the conserved aspartate and histidine residues in RcaC essential in controlling CCA?** The putative phosphorylation sites in RcaC were identified through sequence similarity. To test *in vivo* the roles of these conserved H and D residues within RcaC, site-directed mutated *rcaC* genes were reintroduced into an *rcaC* null mutant, CR2. D1 and D2 residues were replaced with either glutamate (E) or asparagine (N). It has been shown in another response regulator, NtrC, that D to E changes mimic the phosphorylated state of the response regulator, while the D to N changes mimic the protein in its dephosphorylated form (Klose *et al*, 1993).

Whole cell scans of the transformed lines were used to estimate the ability of mutated forms of RcaC to regulate CCA properly. We found that when D2 was replaced with either E or N residue, and D1 remained un-

changed, CCA was relatively normal, while D1 mutations severely reduced the amount of CCA when D2 was intact. Thus the phosphorylation sate of D1 plays a major role in controlling CCA. The D1 and D2 double mutations showed that the "phosphorylation state" of D1 residue overrides the effect of the "phosphorylation state" of D2 residue. The double mutant E1N2 (D1 replaced with E, and D2 replaced with N) mimiced the phosphorylated state of RcaC, while N1E2 mutant (D1 replaced with N, and D2 replaced with E) mimiced the dephosphorylated state of RcaC. Interestingly, even in the mutants containing changes at both D1 and D2, some light responsiveness appeared to remain. This may be activity that operates through a recently described second signaling pathway (Seib *et al*, 2002). Similarly, the conserved histidine within HPt domain of *rcaC* was mutated to either glutamate (E) or glutamine (Q) and reintroduce into CR2, then each analyzed for its effect on CCA. Whole cell absorption scans of cells containing these mutant forms of RcaC showed that almost all CCA was lost, and the cells constitutively accumulated high levels of PE but not PC. Thus this H is extremely important for controlling CCA but at least one additional system is operating to allow some residual CCA. We also conducted northern analysis to monitor RNA levels of the three main operons known to be regulated by CCA, *cpeBA*, *cpeCDE*, and *cpeB2A2* in these histidine mutants. Reflecting the scan data, all three operons retain some residual responsiveness. We are currently testing whether this residual responsiveness of *cpcB2A2* operon in particular is operating through the two D residues by making combinations of D1, D2 and H site directed mutants and testing these for whether light responsiveness remains.

II. Does RcaC play a role in the second pathway? We also isolated RNA from CR2 mutant cells grown in GL and RL. The expression of *cpeBA* and *cpeCDE* showed the same levels of responsiveness as in an *rcaE* (encoding a CCA photoreceptor) mutant, indicating that RcaC is not critical for the functioning of the second pathway.

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# Photos from the Spring 2003 IBASM Meeting

From L to R: Dr. Kara Eberly (Alternate Councilor & Educational Representative, IBASM), Dr. Ian Lipkin (ASM Foundation Speaker at the April 2003 IBASM meeting), and Dr. Jim Mitchell (IBASM President)


Dr. Stanley Spinola,the local sepaker at the 2003 IBASM meeting

Lina-Li (L) and Miriam Martin (R) who received the 2nd place graduate awards

**Spring 2003 meeting attendees listening to Dr. Lipkin's talk**


**IBASM Branch poster**

**Poster presentations**

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## HARMLESS BACTERIA MAY CONTRIBUTE TO PRODUCTION OF DEADLY TOXIN

The virulent *E. coli* O157:H7 bacteria could be enlisting the help of formerly harmless intestinal bacteria to cause some lethal side effects say researchers from the University of Cincinnati and Cincinnati Children's Hospital Medical Center. Their findings appear in the June 2003 issue of the journal Infection and Immunity.

*E. coli* O157:H7 bacteria are a common cause of gastrointestinal disease which in some cases can lead to hemolytic uremic syndrome (HUS), which results in acute kidney failure in children. Antibiotic treatment of the infection can trigger progression to HUS in some cases but not in others. The reason for this is not known, but the production of shiga toxin by the bacteria is believed to be involved.

In the study, the researchers found that *E. coli* O157:H7 bacteria, when killed by antibiotics, not only released shiga toxin, but also a bacteriophage, a virus that infects other bacteria, that contained the genetic code for production of the toxin. The phage would then infect the harmless *E. coli* bacteria that normally inhabit the intestines, causing them to produce more shiga toxin and more phage, which would infect more bacteria and continue the cycle.

(S. D. Gamage, J. E. Strasser, C. L. Chalk, A. A. Weiss. 2003. Nonpathogenic Escherichia coli can contribute to the production of Shiga toxin. Infection and Immunity, 71. 6: 3107-3115.)

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## HERPESVIRUS MAY CAUSE LUNG DISEASE

A herpesvirus may contribute to the development of a fatal lung disease say researchers from Vanderbilt University, Meharry Medical College, Duke University Medical Center, National Institutes of Health, and Emory University School of Medicine. Their findings appear in the June 2003 issue of the Journal of Clinical Microbiology. Idiopathic Pulmonary Fibrosis (IPF) is a disease of inflammation that results in scarring, or fibrosis, of the lungs. In time, this fibrosis can build up to the point where the lungs are unable to provide oxygen to the tissues of the body. In studies of patients with IPF, the average survival rate has been found to be 4 to 6 years after diagnosis. The cause of IPF
is currently unknown.

In the study, lung specimens from patients with IPF were tested against samples with other diseases for signs of infection with the herpesvirus. Researchers detected up to four different strains of the herpesvirus in thirty-two out of thirty-three subjects with IPF. Of the twenty-five controls, only nine showed positive signs of the virus.
"We have detected the DNA of four herpesviruses in the lungs of all but 1 of 33 unselected patients with familial or sporadic IPF, a frequency much greater than that for the lungs of the controls with other diseases," say the researchers. "Establishment of chronic pulmonary herpesvirus infection as the cause of IPF will require detection of a herpesvirus in the lungs before or at the beginning of the time of appearance of clinical manifestations in a large sample of untreated patients early in the course of their disease and demonstration that eradication of the viral infections stops the progression of lung fibrosis."

(Y. W. Tang, J. E. Johnson, P. J. Browning, R. A. Cruz-Gervis, A. Davis, B. S. Graham, K. L. Brigham, J. A. Oates Jr., J. E. Loyd, A. A. Stecenko. 2003. Herpesvirus DNA is consistently detected in lungs of patients with Idiopathic Pulmonary Fibrosis. Journal of Clinical Microbiology, 41. 6: 2633-2640.)

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Look for more award papers in the next issue!

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