SITE-SPECIFIC SCISSION OF *E. COLI* RIBOSOMAL RNA BY AMINOPHENANTROLINE LINKED TO THE 3' END OF TRANSFER RNA [MAS]

N.V. Jammi and W.E. Hill
Division of Biological Sciences, University of Montana, Missoula, MT

In order to study the interaction between the 3'- end of tRNA and rRNA, aminophenanthroline was linked to the adenine nucleotide at the 3' end of E.coli transfer RNA^{Phe}. This was done via a phosphoramidate linkage. The "modified" tRNA was then bound to 70S ribosomes under conditions described by Moazed and Noller (Cell, vol 47, 985-994,1986). Under reducing conditions and in the presence of Cu^{2+} the phenanthroline caused site-specific cleavage(s) of the 23S RNA. The positions of these sites were determined using primer extension analyses. The cleavages were found to be within a 10 Å "sweep" of the modified nucleotide. The data presented indicate that chemical nucleases, such as phenanthroline can be used to study biomolecular interactions and determine rRNA neighborhoods of ligands bound to the ribosome.

GAMMA/DELTA T CELLS DEFINED BY A NEW LINEAGE SPECIFIC MONOCLONAL ANTIBODY (mAb) [MAS]

Ward Jones, Mark Jutila, and Bruce Walcheck
Veterinary Molecular Biology, Montana State University, Bozeman, MT

In the mid-1980s a new T cell subset was described based upon a unique T cell receptor (TCR). The new TCR, termed gamma/delta, is expressed on an unusual population of T cells. In humans, gamma/delta T cells comprise 2-5 percent of circulating lymphocytes. However, in bovine, gamma/delta T cells can comprise up to 80 percent of the lymphocyte population. Gamma/delta T cell function is not fully understood. They appear to be one of the more ancient immune cell types and possess some myeloid-like characteristics. These T cells are found in non-lymphatic tissues such as the intestinal mucosa and skin. Due to their unusual location, it is thought that gamma/delta T cells may play a role in the "first line of defense". We demonstrated, by FACS analysis and polyacrylamide gel electrophoresis, a lineage specific 220kD epitope on gamma/delta T cells that is distinct from other known cell surface markers. Upon mitogen stimulation, this marker regulates differently than other known cell surface markers on gamma/delta T cells. Finally, *in vivo* and *in vitro* data suggest that virtually all circulating gamma/delta T cells express the 220kD antigen while tissue gamma/delta T cells appear to down regulate the expression of this marker.

Recombinant Vaccines to Prevent Gonorrhea: Maltose Binding Protein/Gonococcal Antigenic Peptide Protein [MAS]

Douglas C. Marchion
University of Montana, Missoula, MT

Gonorrhea, with up to a million new cases each year, is one of the most reported communicable diseases in the United States. Of particular concern is an increasing incidence of gonorrhea infection among inner city youths. Vaccine trials using major outer membrane components of *Neisseria gonorrhoeae* have proven ineffective due to the immunogenic dominance of variable sequences in comparison to the weak immunogenicity of conserved sequences. We propose to use conserved immunorecessive sequences in a recombinant vaccine. To test that an immunorecessive invariant peptide will elicit protective antibodies, a fusion protein was constructed using the pMAL-CR1 Vector System, which fuses maltose binding protein of *Escherichia coli* with a conserved surface exposed antigenic peptide of the multiple transferable resistance 44,000 dalton protein (MtrC) of *Neisseria gonorrhoeae.* This system, which efficiently produces fusion proteins that can be purified in a single step, provides the opportunity to generate antibodies to specific, conserved, immunorecessive outer membrane peptides. Antibodies to these peptides will be used in assays to determine their ability to bind native protein and to kill gonococci. Ultimately, peptides identified in these studies will be used for immunoprophylaxis against *Neisseria gonorrhoeae.*

Partial biochemical and functional characterization of gamma delta T cell receptor antibiotics [MAS]

E. Wilson, M. Jutila, G. Perry, G. Watts, and B. Walcheck
Veterinary Molecular Biology, Montana State University, Bozeman, MT

The functional role of gamma delta T cells in infectious disease is enigmatic. Gamma delta T cells have been shown to be important in experimental listeriosis, leishmaniasis and malaria; gamma delta T cells have also been shown to be toxic for cells infected with herpes simplex virus and tumor cells. In new born ruminants, gamma delta cells comprise up to 70 percent of total of lymphocytes, indicating that these cells are vital to the ruminant immune system; although actual function of the gamma delta T cell in new born ruminants remains unclear. We have generated antibodies with specificity for the bovine gamma delta T cell receptor (TCR), both pan TCR and subset TCR. Specificity of these antibodies was determined by flow cytometric and biochemical analysis, the ability of these antibodies to simulate T cell proliferation was determined, as well as immuno-histologic staining to determine the tissue specificity of various TCR subsets. These antibodies may serve as useful probes for analyzing the functional importance of gamma delta T cells in newborn ruminants.

Studies of Lysosomal Membrane Protein Functions Using Transgenic Mice [MAS]

S. Wimer-Mackin, B.L Granger
Veterinary Molecular Biology, Montana State University, Bozeman, MT
and J. D. Coffin
McLaughlin Research Institute for Biomedical Sciences, Great Falls, MT

Transgenic mice have been employed to study the function of a number of proteins by either expressing a protein in excess of normal (gain of function) or eliminating expression (loss of function). The functions of the lysosomal membrane glycoprotein lgp-B are not known, although this protein is both abundant and highly conserved in mammals. We have generated mice transgenic for the hamster lgp-B cDNA and are examining the mice for expression of this hamster protein. In addition, mice without a functional lgp-B gene (knockouts) are being developed for loss of function studies.

Introduction of Dam DNA methyltransferase into *Drosophila* for *in vivo* chromatin mapping [MAS]

D. R. Wines, P. B. Dalbert, D. V. Cark, and S. Henikoff
Biological and Physical Sciences Dept.
Montana State University - Billings, Billings, MT

We have introduced the *Escherichia coli* dam gene for DNA methytransferase into *Drosophila* to determine if patterns of methylation can be used to map chromosomal structure *in vivo* in *Drosophila*. This approach has been successful in yeast. The dam methylase gene was cloned into a pUAST vector, which is designed to direct GAL4 dependent transcription of inserted genes in transgenic flies, and introduced into *Drosophila* by P-element mediated transformation. We obtained ten individual lines containing insertions of the dam methylase construct. The extent of methylation was checked directly by cleaving *Drosophila* genomic DNA with enzymes which have differential sensitivity to methylation. The extent of methylation was estimated in EtBr stained gels by comparison to digests of *in vitro* methylated *Drosophila* genomic DNA and of genomic DNA from flies which do not contain a methylase construct. The methylase is active *in vivo* in four out of five lines tested so far. We have examined the extent of methylation at three housekeeping genes, one at a euchromatic locus (Prat), and two at heterochromatic loci *(light and rolled)* on Southern Blots. The *Prat* locus shows high levels of methylation in all active methylase lines. The heterochromatic loci also show high levels of methlylation in lines with high methylase activity. However, in lines which have low methylase activity, there appears to be selective decrease in methylation at heterochromatic loci. These results suggest that the dam methylase system may be a powerful *in vivo* tool to map chromosomal structure.