Colored transparent surface rendering of the dimeric amino-terminal domain of the hepatitis C virus NS5A protein overlaid on a background of hepatitis C virus–infected cells stained to detect NS5A using a monoclonal antibody. Work and image done in the laboratory of Tim Tellinghuisen, Ph.D., Assistant Professor, Department of Infectology, Scripps Research, Florida.
Sukhvir Mahal, Ph.D.
Staff Scientist
Department of Infectology
Chairman’s Overview

Researchers in the Department of Infectology currently focus on 3 areas: prion diseases, hepatitis C, and leishmaniasis. Two groups, those of Corinne Lasmézas and my own, study the molecular biology of prions and the pathogenesis of the disease. A further prion group was established when Joaquin Castilla joined us as an assistant professor in September.

Prion diseases are of interest not only because of the emergence of bovine spongiform encephalopathy (mad cow disease) in the United Kingdom and chronic wasting disease in the United States but also because of the unusual properties of the infectious agent, which consists mainly or entirely of a protein, PrP^Sc, a conformer of the normal host protein PrP^C. Interestingly, many strains of prions are associated with the same protein sequence, raising the question as to how strain-specific properties are encoded. An important resource we have established is a semiautomated, cell-based assay for prions (scrapie cell assay), which replaces in most instances the slow, expensive, and less accurate mouse-based bioassay. The scrapie cell assay is being used to analyze the properties of prions and prion propagation, both in cell culture and in cell-free systems.

Sukhvir Mahal has isolated cell lines that will replicate some prion strains but not other strains and has
extended the scrapie cell assay to differentiate between prion strains. A major effort at elucidating the mechanism by which cells distinguish between different prion strains involves, in addition to Dr. Mahal and Chris Baker, Shawn Browning and Prem Subramaniam, Dr. Lasmézas and her group are collaborating on this effort but also are investigating the therapeutic possibilities of using antibodies to PrP and PrP-binding aptamers to cure prion diseases.

A further research effort is directed toward screening for drugs against leishmaniasis, specifically against J-binding protein, a putative target, and involves Scripps scientists in both California and Florida. Dr. Subramaniam, in collaboration with D.P. Millar, Department of Molecular Biology, and P. Wentworth, Department of Chemistry, has developed a high-throughput assay for the binding of an oligonucleotide containing the modified DNA base J. In collaborative studies with P.R. Griffin and his colleagues, Department of Biochemistry, hydrogen-deuterium exchange was used to identify residues involved in the binding of the oligonucleotide to J-binding protein.

A third field of endeavor is hepatitis C. Donny Strosberg and members of his group are studying interactions between hepatitis C virus proteins to find drug candidates that disrupt such interactions. Tim Tellinghuisen, who joined the department as an assistant professor in November 2005, is elucidating the role of the hepatitis C virus protein NS5A in viral replication. As further space becomes available, the studies on hepatitis C virus will be extended.

A histopathology service is being set up by Nicole Salès.

INVESTIGATORS’ REPORTS

Generation and Transmission of Prions

C. Weissmann, C.A. Baker, S.P. Mahal, S. Browning, C. Demczyk, A. Sherman

The “protein only” hypothesis proposes that prions, the agents that cause transmissible spongiform encephalopathies, consist mainly or entirely of PrPSc, an abnormal conformer of a normal host protein, PrPC, and that the agents propagate by a PrPSc-catalyzed conversion of PrPC. A specific mechanism is suggested by the “seeding hypothesis.” Intriguingly, distinct prion strains, which generate different disease phenotypes, may be associated with the same PrP sequence, suggesting that the phenotypes are encoded by different PrP conformations. We are elucidating the mechanism of prion replication, the structural basis of strain specificity, and the mechanism of strain recognition by cells.

We have improved the sensitivity and accuracy of the cell-based prion assay (scrapie cell assay), which can now be carried out in 10 days and allows the simultaneous, semiautomated processing of hundreds of samples per week. This assay largely obviates the mouse bioassay, which requires large numbers of animals and many months to complete. We have isolated cell lines susceptible to infection by certain prion strains but not by other strains and have established a cell panel (strain discrimination panel) that allows us to distinguish various prion strains, a task that otherwise requires many mouse bioassays and might take years to complete.

We have also isolated neuroblastoma sublines that are susceptible to the RML prion strain, the 22L strain, both strains, or neither strain. This finding suggests that PrP conformation alone most likely is insufficient to allow discrimination by cells and that prions contain other components that codetermine strain specificity. We are purifying prions to identify such components.

In other research, using the scrapie cell assay, we studied the kinetics of infectivity amplification in a cell-free system containing PrPC and primed with prion-infected brain homogenate. Using the strain discrimination panel, we are determining whether prions are replicated in a strain-specific manner in the cell-free system.

Even after repeated cloning, cell populations persistently infected with prions are heterogeneous, composed
of infected and uninfected cells. We have identified a nondividing subpopulation that is the most prolific in producing and secreting prions and a dividing subpopulation that generates low levels of prions. Our hypothesis is that the doubling rate of dividing cells is greater than the doubling rate of the prions within the cells, so that infectivity is diluted out of the cells. This process would ultimately result in curing of the cells if the cells were not continuously reinfected by prions secreted by the nondividing population. The hypothesis assumes that the dividing population continuously throws off nondividing producer cells and predicts that sequestration of secreted prions will cause “curing” of the cell culture; this prediction has been confirmed in preliminary experiments. The switch between productive and nonproductive states is an epigenetic phenomenon that may also underlie susceptibility to infection by prion strains.

PUBLICATIONS

J-Binding Protein of Leishmania as a Potential Drug Target
C. Weissmann, P. Subramaniam, P. Wentworth, D.P. Millar
R. Sabatini, P.R. Griffin, P. Borst
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The DNA of Leishmania organisms contains a modified base, J (β-D-glucosyl-hydroxymethyluracil), that does not occur in higher eukaryotes. Leishmania species contain a protein, J-binding protein (JBP), that binds J-containing DNA sequences and is involved in the conversion of thymine to J. Deletion of JBP is lethal for the organisms. Therefore, most likely compounds that interfere with the binding of JBP to J are detrimental to growth or survival of Leishmania organisms. Because J and JBP do not occur in the host, such a compound might lead to a therapeutic drug for treatment of leishmaniasis.

In collaboration with P.R. Griffin, Department of Biochemistry, using hydrogen-deuterium exchange technology, we compared JBP with the JBP-oligonucleotide complex. We identified 4 regions in JBP that had significant reduction in hydrogen-deuterium exchange upon binding of the oligonucleotide and that may define a binding site.

We will search for compounds that interfere with the binding of J to JBP, seek proof of principle in the mouse leishmaniasis model, and, if we are successful, endeavor to develop a therapeutic drug. The fluorescence polarization assay for the binding of a J-containing, fluorescently labeled oligonucleotide to recombinant JBP is adequate for high-throughput screening. A trial run with 360 randomly selected compounds yielded 1 significant hit. The compound, reactive blue 2, a polysulfonated anthraquinone dye, is not an attractive candidate for a lead compound. We have also set up a Leishmania growth assay and used it to screen the 360 compounds for ones that inhibit growth of the organism. We found 2 inhibitors, ketoconazole and ribavirin, the first of which is a recognized second-line treatment for leishmaniasis. This finding validates the screening approach.

Pathogenesis of Transmissible Spongiform Encephalopathies
C.I. Lasmézas, N. Salès, P. Saá Prieto, M. Zhou, M. Lefebvre-Roque, A. Sturny, G. Sferrazza

Prions, the infectious agents responsible for prion diseases, are thought to consist mainly or entirely of an abnormally folded isoform, PrPSc, of the prion protein PrP. Prions are thought to replicate by an autocatalytic process of template-induced conformational change.

Most approaches for treatment of prion diseases have focused on PrP and are difficult to implement because PrP is a normal host protein. We are exploring the possibility of using PrP-specific antibodies to impede prion replication in the brain. Our findings are revealing the limitations of such an approach.

Using osmotic pumps, we delivered antibodies against PrP into the brains of prion-infected mice. We
found that PrP-specific Fab’2 fragments were more widely distributed in the brain than were immunoglobulins. The potential efficacy of such a treatment was offset by the toxicity of the antibodies, as indicated by glial reactions and neuronal apoptosis. Overall, these results call for caution in the use of therapies based on antibodies to PrP that target the CNS. In order to determine new targets of intervention, our current goals are to better understand the replication of prions and the mechanisms of the neurotoxic effects and to identify factors other than PrP that are involved in these processes.

Although the central role of PrP in the pathogenesis of transmissible spongiform encephalopathies is firmly established, one or more other molecules might play an important role, particularly in regard to the different kinetics of replication and different distributions in the body and brain of different prion stains. In collaboration with C. Weissmann, Department of Infectology, we are searching for such putative factors.

As part of our effort to devise approaches for treatment of prion diseases, we are studying the route of entry of prions after oral infection of mice. It is known that prions accumulate in follicular dendritic cells and macrophages of the germinal centers of the intestinal Peyer’s patches. However, the mechanism of transport from the gut lumen to the lamina propria and the cells that take up prions and transport the proteins to the lymphoid follicles are not known. Our immunohistochemical studies revealed accumulation of PrP in 2 distinct cell populations of the mucosa and in nerve endings. In further studies, we hope to identify the relevant cells and determine their role during the early phases of oral infection.

We are also studying an RNA aptamer that binds specifically to PrPSc. We plan to use this aptamer as a tool to prevent PrPSc from acting as a template for the conversion of PrP into PrPSc and to detect PrPSc.

Aptamers are small nucleic acids (DNA or RNA) selected from a pool of random DNA or RNA sequences for their ability to bind to a given molecular target. The in vitro procedure involves several rounds of selection and amplification. We focused on RNA aptamers because RNAs adopt specific, sometimes complex, 3-dimensional structures. Using PrPSc purified from hamster brains, in collaboration with the Gene Center, Munich, Germany, we selected the aptamer Ap9, which binds selectively to PrPSc. We are testing the ability of the aptamer to interfere with prion replication and formation of PrPSc in cells infected with the prion agent that causes scrapie. Moreover, because Ap9 can be used to distinguish PrPSc from PrP without the need for a protease digestion step, we are exploring use of the aptamer as a fluorescent probe for detecting PrPSc on a variety of samples, including tissue sections and living cells. We are also developing lentiviral vectors to optimize in vitro and in vivo delivery of the PrPSc-specific aptamer or other PrP ligands.

A functional histology core facility for standard tissue processing and immunolabeling and classical or confocal microscopy analysis is being set up as a service for the Department of Infectology and Scripps Florida. Another goal of the facility is to develop innovative molecular labeling and imaging techniques.

### Protein-Protein Interactions in Hepatitis C

A.D. Strosberg, C. Coito, S. Kota, N. Ayad,* C. Nahmias**

* Department of Biochemistry, Scripps Research
** CNRS, Paris, France

**INTERACTIONS BETWEEN HEPATITIS C VIRUS PROTEINS**

We are interested in interactions between the proteins of hepatitis C virus (HCV). Our goals are to better understand the respective roles of the proteins and to identify small-molecule inhibitors that might actually have an effect on HCV replication. Previously, we identified 5 pairs of interacting HCV protein domains and tagged the domains with glutathione-S-transferase or the octapeptide FLAG. We have now synthesized and cloned 4 of the 10 cDNA constructs that encode these HCV protein domains. The corresponding proteins were expressed in *Escherichia coli* and were affinity purified by using nickel-containing agarose, which recognizes the 6-histidine–containing epitope attached at the C terminus of the interacting domains.

Interactions within one of these pairs of domains, composed of the first 106 residues of the HCV core labeled with FLAG and with glutathione-S-transferase or the octapeptide FLAG, have been confirmed. Attempts to inhibit this core-core interaction by using 10 core-derived 18-residue peptides or a mixture thereof have been unsuccessful. A more sensitive and high-throughput homogenous time-resolved fluorescence assay will soon be implemented to further extend screening of peptide and small-molecule inhibitors of the protein-protein interactions. Once
this assay is operational for the core protein domains, it will used for studies of the other pairs of domains.

**INTERACTIONS BETWEEN HCV AND HOST PROTEINS IN HUMANS**

To identify the human host proteins that interact with individual HCV proteins, in collaboration with N. Ayad, Department of Biochemistry, we are using S35 translation and a collection of more than 7000 individually sequenced human cDNA clones available at Scripps Florida. Using this method, we searched for human protein partners of the NS5A domain I of HCV labeled with glutathione-S-transferase. The proteins that bound to this HCV polypeptide were recovered and identified (Fig. 1). After several independent experiments, we found that 7 proteins reproducibly bound to the NS5A protein domain I. We are further identifying and characterizing these proteins.

**HEPATOMA CELL CULTURE SYSTEMS FOR HCV SUBTYPES**

To further evaluate potential inhibitors of interactions between HCV proteins or interactions between HCV proteins and human host proteins, we have assembled the components necessary to re-create a culture system in which HCV subtype 1b replicates at low levels in hepatoma cells. Corresponding replicons have been produced, and transfected cells have been subcloned to isolate producers of HCV particles.

**PUTATIVE TUMOR SUPPRESSOR GENE IN HEPATOCELLULAR CARCINOMA**

The end stage of HCV infection is hepatocellular carcinoma. Despite its rare occurrence, HCV infection is nevertheless the leading cause of liver cancer in the United States. Recently, in collaboration with C. Nahmias, CNRS, Paris, France, we found that more than 10% of patients with hepatocellular carcinoma have mutant forms of the gene that encodes ATIP, a protein that specifically interacts with the C-terminal region of the AT2 receptor for angiotensin II. This putative tumor suppressor gene is also underexpressed in other forms of cancer. We are expanding these studies to investigate how the mutations affect the function of ATIP, in particular its capacity to activate the tyrosine phosphatase regulated by binding of angiotensin II to the AT2 receptor. In addition, using the system implemented with Dr. Ayad for the HCV NS5A protein, we are evaluating protein partners of ATIP tagged with glutathione-S-transferase.

**PUBLICATIONS**


**Hepatitis C Virus RNA Replication**

T.L. Tellinghuisen, J.C. Treadaway, K.L. Foss

Hepatitis C virus (HCV) is a human pathogen of global importance; according to some estimates, nearly 3% of the world’s population is chronically infected with HCV. Long-term viral replication in these individuals leads to severe liver disease, including cirrhosis and often hepatocellular carcinoma. The current treatment regimen with agents nonspecific for HCV is poorly tolerated and is ineffective in about half of the patients, emphasizing the need for effective antiviral drugs specific for HCV.

Extensive structural and functional characterization of the HCV proteins protease/helicase (NS3) and RNA polymerase (NS5B) has led to the development of potent small-molecule inhibitors of HCV replication. Despite these advances, the high mutation rate inherent in HCV RNA replication and the subsequent evolution of resistance dictate the need for new antiviral agents. Study of the protein NS5A, another replicate component, has lagged behind research on NS3 and NS5B. NS5A remains enigmatic, with no known function in HCV replication, except its requirement in this process.

We have been characterizing NS5A. Our goal is to understand the role of this protein in replication and, more generally, the replicase itself. Using biochemical
and genetic methods, we have defined NS5A as an absolutely required 3-domain metalloprotein component of the replicase. Our recent crystal structure of the amino-terminal domain of NS5A (domain I) has provided a glimpse of the potential membrane, protein, and RNA interactions of NS5A in the viral replicase and has suggested potential antiviral targets.

In addition, using genetic screens, we recently discovered an interaction between the membrane anchor of NS5A and the HCV protein NS4B, another component of the replicase. This interaction appears to localize NS5A to the replicase and is essential for RNA replication. We are conducting biochemical, genetic, and structural experiments to evaluate the potential interaction surfaces of the NS5A observed in the NS5A domain I structure and our genetic screens. These experiments include using the HCV replicon system, the HCV infectious cell culture system, mass spectrometry, nuclear magnetic resonance, molecular modeling, and crystallography. A parallel focus of our research is the development of small-molecule inhibitors that disrupt these putative interactions.

Our ultimate goal is to understand, at the molecular level, the HCV RNA replication machinery. Greater insight into the poorly understood replicase components, such as the NS5A protein, will provide a more complete view of the replicase complex and will fuel new drug design.

**PUBLICATIONS**

