

DRAFT

Working Group 1a. Cell & Molecular Biology of the Liver

Introduction & Background

The liver is the largest organ in the body and the most metabolically active. This complex organ performs multiple interrelated functions that are essential for life, including: 1) uptake, storage, metabolism, and release of nutrients (e.g., amino acids, carbohydrates, lipids, vitamins, and minerals); 2) synthesis of bile salts from cholesterol and their secretion to assist with fat absorption and digestion; 3) synthesis and secretion of plasma proteins necessary for blood clotting and transport of molecules through the circulation; 4) detoxification of drugs, hormones, and the end-products of metabolism and distribution to the bile or urine for excretion; and 5) removal of bacteria and dying red blood cells from the circulation. This functional complexity is attested by the fact that reconstitution of the organ in the form of an artificial or bioartificial liver capable of supporting patients with liver failure has yet to be achieved.

A pivotal organ in metabolism, the liver has a unique structure and position in the body that reflect this important function. The liver receives nutrient-rich blood directly from the small intestine via the portal vein, allowing the liver to control the supply of nutrients to the rest of the body while removing and neutralizing unnecessary and harmful substances. For example, the liver contributes to the maintenance of a steady level of blood glucose by taking up excess glucose after a meal and storing it as glycogen. Furthermore, in the fasting state, the liver releases glucose obtained from breakdown of glycogen or through *de novo* synthesis by gluconeogenesis. The rapid exchange of nutrients and proteins between the blood and liver is accomplished through the unique microarchitecture of the liver, wherein blood flows through sinusoids lined with endothelial cells featuring fenestrae that allow direct contact of hepatocytes with the blood supply.

The functional unit of the liver is the acinus. Blood enters the liver at the base of the acinus from veins and arterioles in the portal area and flows through plates of hepatocytes to the terminal vein. The immediate area around the portal areas is considered zone 1, the intermediate area zone 2, and the area around the terminal vein zone 3. As blood flows through the acinus, substances are removed or added, so that the three zones are exposed to different concentrations of nutrients and oxygen and, therefore, have different metabolic activities. In contrast, bile is formed and flows in the opposite direction, away from the terminal vein and towards the portal areas where it flows into bile ducts. These structural features of the liver are important in understanding local cellular functions and disease processes.

The liver is comprised of a number of cell types that function independently and in concert to perform the manifold tasks that permit survival of the individual. The most abundant cell is the hepatocyte, comprising ~70% of the liver volume and performing the bulk of liver functions. Each hepatocyte is supplied with nutrient-rich portal blood and

oxygen-rich aortic blood, supporting its high metabolic and secretory activity. Like other epithelial cells located at an interface between physiological media and tissue layers, the hepatocyte is polarized to perform unique functions on its cell surfaces that contact blood, bile, or neighboring hepatocytes. This polarization results in a unique distribution of molecules, such as membrane-bound transporters, and subcellular components to support processes occurring at the interface, which requires that the cell's structural elements be arranged so that molecules can be routed across the cell to certain locations through a vesicle trafficking system. Another unique feature of the hepatocyte is its unusually high capacity for proliferation when a portion of the liver is removed or damaged, which underlies the liver's regenerative properties.

Other major liver cell types include sinusoidal endothelial cells, which regulate portal blood flow to hepatocytes and are thought to play a major role in injury associated with storage of donor livers prior to transplantation. Their fenestrations regulate the access of macromolecules in the circulation to the hepatocyte surface. Within the sinusoid are macrophage-derived Kupffer cells, which play important roles in phagocytosis. Another liver cell type, the stellate cell stores fat and vitamin A and, when activated, loses these stores and undergoes morphological transformation into a myofibroblast-like cell that produces collagen, an extracellular matrix protein that has important regulatory consequences for maintenance of liver cell differentiation and function. Stellate cell activation, thus, may play a major role in fibrogenesis and development of cirrhosis. Lastly, cholangiocytes, epithelial cells of the biliary ducts, are essential for bile flow, and their regulation of water permeability by insertion of aquaporins into their surface membrane plays an important role in bile flow.

Liver cells contain abundant, highly developed organelles, which are necessary to perform various cellular processes. For example, mitochondria generate most of the cellular energy. The endoplasmic reticulum (ER), Golgi apparatus, peroxisomes, proteasomes, lysosomes and nucleus also comprise a significant portion of the liver cell and make similarly important contributions to cell function. Loss or disruption of these essential organelles is incompatible with life, and mutation of a single gene, the product of which is involved in a single functional pathway, can often result in a devastating genetic disease. Clearly, it is important to more fully understand the complexity of the basic biological processes of the liver in order to appreciate pathologically important alterations that contribute to the entire range of liver diseases.

Recent Research Advances

For many years, the hepatocyte has served as a classical model for studies of the biology of cell function. Although many cell biologists continue to utilize liver cells as models, their research is not directed primarily towards understanding hepatic physiology or pathobiology. Because relatively few investigators are trained to establish bridges between disciplines such as general cell biology and hepatology, opportunities to advance understanding of liver function and disease have undoubtedly been missed. The future of

hepatology largely rests on better understanding of the cell biology of the liver and how it is integrated into organ physiology and affected by disease.

The past decade has witnessed many advances in understanding the structure, function, and interrelationships of the cells of the liver, and insights into how perturbations may result in disease. For example, cholangiocytes have been shown recently to have primary cilia, structures that can serve as regulatory flow sensors. The basolateral plasma membrane facilitative transporters of foreign compounds (xenobiotics) and selective sterol and bile acids transporters have been identified and characterized functionally. Similarly, many of the key transporters of phospholipids, bile salts and cholesterol on the apical side of hepatocytes have been identified and their function defined in health and disease.

The polarity established in hepatocytes has aspects that distinguish it from other epithelial cells, and the proteins and mechanisms that are required for this process are now being elucidated. Several intracellular processes related to the hepatocyte's actin- and tubulin-based cytoskeletons, including establishment of cell polarity, vesicular transport, and cell division, differentiation, and death, have also been subjects of intense study. A number of motor molecules and regulatory proteins have been identified, providing the first insights into the nanomachinery of the hepatocyte. The function of ATP-dependent pump proteins of the bile canalicular (apical) plasma membrane has also been defined recently. Regulation of function and cellular trafficking of these proteins are areas of intense ongoing study. Other areas in which important studies are ongoing include cell cycle regulation, telomerase activity, energy metabolism, and cell signaling mechanisms (e.g., Ca^{+2} , cAMP, PIP's, DAG, NO, and cytokines). The relationship of these pathways to transcriptional and posttranscriptional regulation of cellular processes is also an important area for current and future investigation. Study of intracellular organellar structure and function, including the ER, Golgi, peroxisomes, and mitochondria, has provided new insights into regulation of important cellular processes in the healthy and diseased liver. It has also been recognized that the cells of the liver do not function in isolation, but communicate with each other via gap junctions as well as autocrine and paracrine mechanisms.

Identification and analysis of genes whose products play important roles in cellular functions of the liver have been facilitated by completion of the Human Genome Project. Genomic approaches to studying gene expression by human liver cells continue to play a central role in accelerating understanding of liver cell biology, including the use of transgenic animal and cell models and DNA microarray technology to permit expression profiling of different cell types and during various physiologic states. Additionally, an international initiative in liver proteomics was launched by the Human Proteome Organization (HUPO) last year. Currently in the pilot phase, the Human Liver Proteome Project aims to catalog all of the proteins expressed by human liver cells, as well as their post-translational modifications and interactions. Another effort by HUPO called the Human Plasma Proteome Project has been established to develop a proteome of all plasma proteins, many of which are secreted by the liver. Additional efforts to develop protein profiles for important subcellular components in the liver cell, such as

mitochondria and peroxisomes, are also underway. Future research in liver cell biology will benefit in important ways from these genomic and proteomic efforts.

Research Goals

Hepatic Lipid Metabolism and Transport: As the metabolic center of the body, the liver actively synthesizes lipids such as phospholipids, triglycerides and cholesterol and directs their transport either for canalicular secretion or for export throughout the body within lipoprotein particles, some of which return to the liver in a modified form for recycling. While much is known about how lipids are transported through the blood by the various types of lipoproteins, efforts to understand the molecular basis for the binding of lipoproteins to liver cells and other cell types for the exchange and metabolism of these lipids are ongoing. An important research goal is to better understand the molecular mechanisms that underlie lipid metabolism and transport in the liver, as it relates to lipid homeostasis throughout the body (Matrix Cell A2). This goal might include studies to elucidate the role of lipoprotein receptors or binding proteins such as SR-B1 in such processes as selective cholesterol ester transfer to and from the hepatocyte, as well as studies of sphingolipid dynamics.

Vesicle Trafficking Across Liver Cells: Transport of molecules across the liver cell membrane and the intracellular space to their appropriate location requires an array of transport proteins within the plasma membrane and an organized network of vesicles and motors within the cell. A goal for furthering research in this area is to analyze the mechanisms of action and physiological importance of specific plasma membrane transporters (Matrix Cell B1), as well as the proteins and structural elements in the cytoskeleton involved in the process of transcellular vesicle trafficking (Matrix Cell C1). In the case of vesicle trafficking, this would require research to isolate and characterize intermediate compartments; identify and characterize molecular machinery (e.g., microtubules, motors, fission/fusion machinery, rab proteins, tethering proteins); and reconstitute the trafficking system *in vitro*. Other research supportive of this goal might involve the development of assays for protein oligomerization and its effect on transport function; protein scaffolds that may regulate transport function; and knock-out animal models that might be used to search for genetic polymorphisms and diseases related to molecular transport in the liver cell.

Liver Cell Signal Transduction: In several other fields, an improved understanding of the signal transduction pathways activated in response to a particular substance, such as a cytokine, has ultimately translated into a therapeutic advance. An important goal in understanding basic liver cell biology and identifying therapeutic targets is to elucidate the molecular participants in signal transduction pathways activated in hepatocytes, Kupffer cells, stellate cells, and cholangiocytes in response to endocrine, paracrine, or autocrine factors (Matrix Cell A1).

Hepatocyte Polarity and Cell Junction Functions: The asymmetrical plan of the hepatocyte and the polarized nature of its intracellular components are necessary to

coordinate the constituents necessary to perform its seemingly disparate functions of nutrient absorption, bile acid secretion, and toxin/waste product elimination. However, much remains to be learned about the processes that govern the establishment and maintenance of this cellular polarity in the hepatocyte. Future research should be directed towards elucidating the intracellular and extracellular events that determine cell polarity and asymmetry (Matrix Cell B3). Research in this area might include a mutagenesis approach to determine the function of specific proteins (e.g., cytoskeletal or transport proteins) in maintaining cell polarity, and efforts to identify and characterize polarity homologues identified in model genetic systems. Additionally, as the junctions between hepatocytes serve important roles in establishing cell polarity by maintaining the integrity of the interface between physiological media and facilitating communications between cells, another important line of research in this area is the characterization of the barrier and signaling functions of cell junctions in hepatocytes (Matrix Cell C2). As a way to apply knowledge gleaned from the Human Liver Proteome Project, a long-term research goal might be to identify all proteins involved in establishing and maintaining polarity in liver cells, including the proteome of cell junctions (Matrix Cell C3).

Liver Cell Interactions: Communication between the liver's multiple cell types is necessary to support coordinated functioning and also underlies some disease-related processes, such as chronic inflammation. However, studies of interacting cells require co-culture methods that provide culture conditions supportive of both cell types while closely mimicking their situation *in vivo*. A worthwhile goal is the development of physiologically based tissue culture models of hepatocytes with another liver cell type (e.g., Kupffer cell, cholangiocyte, stellate cell or endothelial cell) and/or extracellular matrix components to elucidate cellular interactions (Matrix Cell B2). These systems can be used to assess the role of cellular interactions in the development of hepatocyte and cholangiocyte polarity, zonation of gene and protein expression, and changes in signal transduction mechanisms and responses. Extension of this research to animal models could address how the multiple signals received by liver cells through cellular interactions and intracellular events are integrated to result in discrete liver functions (Matrix Cell A1).

Liver Cell and Organelle Proteomics: Studies of the subcellular components or organelles of liver cells and the range of proteins expressed by the liver cell types and their components have the potential to improve understanding of multiple hepatocellular functions. Important goals for future research are to characterize organelle structure, function, and interrelationships as regards specific hepatocellular functions, and to develop a knowledge base of the normal liver proteome, including comprehensive analysis of proteins in the range of cell types, individual subcellular compartments and specific cells along the hepatic acinus (Matrix Cells C3). To achieve these goals, collaborations could be developed with the Human Liver Proteome Project, which seeks to define the array of proteins expressed by the liver cell, including proteins affected by cellular position within the acinus, and its organelles, such as mitochondria and proteasomes (Matrix Cell C3). Cataloguing the proteins produced by liver cells may represent an important first step towards ultimately determining how these proteins interact and function in the healthy and diseased liver.

Steps to Achieve Research Goals

Studies relevant to the cell and molecular biology of the liver require modern tools of science and investigators trained in their use. These tools include mass spectroscopy and proteomic analysis, imaging and microscopy technologies, appropriate molecular probes and inhibitors, and modern bioinformatics technology combined with standardized information processing. To encourage proteomic research and its potential to complement genomic studies in illuminating important aspects of liver cell and molecular physiology, collaborative research relationships could be established and maintained with the Human Liver Proteome Project and Human Plasma Proteome Project, and Special Emphasis Program Announcements could be released in proteomics. The development of resources including appropriate cell culture models, cDNA clones, and recognition molecules, such as antibodies at the necessary scale for proteome-wide investigations, are also important components. Special funding initiatives are appropriate to expand cell biological research into altered mechanisms of signal transduction, cell function, transporters and intracellular vesicular transport in different genetic and induced models of liver disease. Findings in cell and molecular research have the potential to uncover aspects of normal liver cell biology that are altered during disease and may be corrected by pharmacological, cell- or molecular-based therapy. Therefore, a natural extension of the research goals outlined here would be the translation of knowledge gained in basic liver biology into clinically relevant advances in management of patients with liver disease. This translation effort could be aided by enhanced collaboration between basic and clinical liver researchers. To foster these studies and collaborations, support for recruitment and training of students, fellows, and clinical and basic scientists in cell biology, as related to the liver, could be made available. Enhanced recruitment and training of this spectrum of individuals in liver cell biology would facilitate the bridge-building that is required to optimally study liver disease at the cellular and molecular levels and ultimately to apply this knowledge to patient care. Interdisciplinary workshops, courses and collaborative grants could also have a positive impact on this bridge-building effort.

Matrix of Research Goals in Cell & Molecular Biology of the Liver

	Short-term Goals (0-3 years)	Intermediate-term Goals (4-6 years)	Long-term Goals (7-10 years)
High Risk	A3. Determine how intra- and inter-cellular signals are integrated <i>in vivo</i> to regulate liver function.	B3. Elucidate intra- & extra-cellular events that determine hepatocyte polarity.	C3. Develop knowledge base of normal liver proteome including analysis of individual cell types, subcellular compartments and changes along hepatic acinus.
Intermediate Risk	A2. Elucidate the mechanisms of lipid metabolism & transport in liver as it relates to whole body lipid homeostasis.	B2. Develop cell culture model that reflects different liver cell interactions (e.g., hepatocyte with Kupffer cell, cholangiocyte, stellate cell or endothelial cell).	C2. Elucidate how cells interact with each other (e.g., via gap junctions, ECM, paracrine & endocrine signaling).
Low Risk	A1. Define major pathways and molecular participants in signal transduction in liver cells.	B1. Elucidate physiological importance of liver plasma membrane transporters & mechanisms of action.	C1. Elucidate major elements in process of transcellular vesicle trafficking in the hepatocyte.