



Liver function from Y to Z

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In the 1960s, my lab was interested in understanding how bilirubin and other organic anions are transferred from the plasma through the liver cell and into the bile. We performed gel filtration of liver supernatants and identified two protein fractions, designated Y and Z, which bound organic anions including bilirubin, and thus we proposed that they were involved in hepatic uptake of organic anions from plasma. Subsequently, the Y and Z proteins responsible for this binding activity were purified, cloned, and sequenced. Y was identified as a member of the glutathione S-transferase (GST) protein family and Z found to be a member of the fatty acid-binding protein (FABP) family. These proteins have since been shown to have additional surprising roles, but understanding of their full role in physiology and disease has not yet been achieved.

In the 1960s, bilirubin metabolism was a “hot” topic. Along with other groups, my lab was studying various forms of inheritable jaundice in an effort to dissect the mechanism of bilirubin’s transfer from plasma into the hepatocyte and its role in intracellular metabolism and biliary secretion. These processes were eventually identified and found to be related to the basic mechanisms whereby the liver handles many anionic drugs, metabolites, and hormones. Because the mechanism of hepatic uptake of bilirubin was unknown, A.J. Levi, Z. Gatmaitan, and I took advantage of advances in gel permeation chromatography to study this process. In 1969, we described two hepatic cytoplasmic protein fractions, designated Y and Z, that bound bilirubin and various dyes in vivo and in vitro and, based on tissue distribution, abundance, and effects of genetic and pharmacologic models, were proposed to participate in organic anion uptake (1). In the decades since then, the Y and Z proteins have been identified as members of large protein families that were cloned and sequenced. Several surprising functions emerged, whereas others are proposed based on binding properties. Many challenges remain in understanding the full role of these proteins in physiology and disease.

Ligandin

In 1971, our group demonstrated that organic anion binding in the more abundant Y fraction was due to an approxi-

mately 50-kDa protein that has two subunits and is identical to azo dye carcinogen-binding protein (2) and cortisol metabolite-binding protein (3), which had been described by Ketterer and Litwak, respectively. Because each group working on the Y fraction attributed its function to its particular substrate of interest, a generic term, *ligandin*, was agreed upon (4). In 1974, William Jakoby and his colleagues purified a family of approximately 50-kDa liver supernatant glutathione transferases. Ligandin proved identical to glutathione transferase B, the most abundant glutathione S-transferase (GST) in rat liver (5).

Ketterer pursued a role for ligandin in chemical carcinogenesis. Our group went on to study the accumulation of ligandin in newborn liver and its phylogeny. We found that ligandin was induced by phenobarbital and other drugs (6) and that the intracellular concentration of ligandin correlated with the net rate of hepatic organic anion uptake specifically because it reduced hepatic efflux. Using an ingenious method to quantify hepatic influx and efflux rates of organic anions in perfused rat liver, Goresky showed that the concentration of ligandin was inversely related to the efflux rate of organic anions back into plasma after hepatic uptake (7). Circular dichroism revealed that intracellular ligandin could quantitatively transfer bilirubin from albumin. Similar control of organic anion efflux was demonstrated in proximal tubular and small intestinal cells where ligandin is also abundant. Biochemical and structural studies demonstrated that the two subunits of ligandin have differ-

ent functions; one subunit has GST catalytic activity, and the second has specific binding properties (8). Clinically, ligandinuria is a sensitive indicator of proximal tubular injury, and ligandinemia results from hepatocellular damage. No naturally occurring ligandin mutations have been described and neither siRNA nor knockout studies have been performed. Competition between organic anion drugs and bilirubin for binding to ligandin is a cause of unconjugated hyperbilirubinemia.

Discovery of the GST supergene family shifted the focus of ligandin research toward the structural basis of ligand binding and catalytic mechanisms, the ability of GSTs to regulate gene expression, and their role in signaling pathways and carcinogenesis (9). Unfortunately, interest in the transport aspects were sidelined, although recent studies have rediscovered the ligand-binding properties of GSTs and their role in net cellular uptake and, perhaps, downstream events (9).

The Z fraction

In the original 1969 study, we described the distribution of Z fraction in liver, small intestine, kidney, and other tissues and the lack of correlation with organic anion hepatic uptake (1). In 1972, the capacity of Z fraction, and later purified hepatic Z protein, to bind long-chain unsaturated fatty

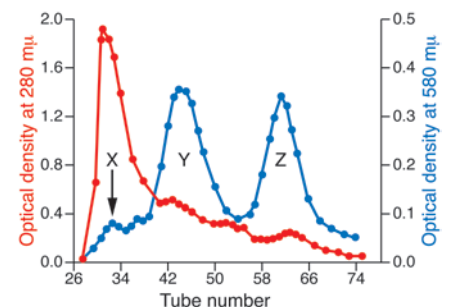


Figure 1

In 1969, my group used gel filtration to fractionate soluble proteins from rat liver. The components of the Y and Z fractions have subsequently been implicated in several hepatic functions, including organic anion uptake, cell signaling, metabolism, and carcinogenesis. Reproduced from ref. 1.

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acids was demonstrated resulting in a name change to *fatty acid-binding protein* (FABP) (10, 11). Because FABP did not participate in fatty acid uptake, a role in intracellular fatty acid utilization was proposed (10, 11).

The FABPs are an evolutionarily conserved family of nine approximately 14-kDa binding proteins for fatty acids and other lipophilic substances, such as eicosanoids and retinoids. The family contains liver, intestinal, heart, adipocyte, epidermal, ileal, brain, myelin, and testes FABPs; however, no FABP is completely tissue specific, and most tissues express several isoforms. In hepatocytes, adipocytes, and cardiac myocytes, where fatty acids are prominent species, FABPs account for 1%–5% of all cytoplasmic proteins, and their expression is further induced after mass influx of lipid (10, 11). Liver-FABP, but not ligandin, is induced by peroxisome proliferators that regulate liver-FABP through PPARs. The crystal structure of synthetic adipose-FABP revealed features of lipid-protein interactions that differ from those present in apolipoproteins and albumin (12). No enzymatic activity has been associated with any FABP.

Despite much research, little is known about the precise biological functions and mechanisms of action of FABPs. Their distribution and regulation suggest possible roles in lipid metabolism. FABPs are proposed to shuttle fatty acids to specific enzymes and cellular compartments, modulate intracellular lipid metabolism, regulate gene expression, and, through their binding of eicosanoids and retinoids, to be linked to metabolic and inflammatory pathways (13). However, most proposals are based on *in vitro* studies. *In vivo* demonstration of the proposed functions is still lacking. Molecular knockout experiments in animals and cells reveal an altered phenotype only when lipid influx is greatly increased (13). A direct link between *in vivo*

data gained from genetic knockout models of FABPs or PPARs remains inconclusive, possibly due to protein redundancy and compensatory mechanisms.

From humble beginnings

Thus, a relatively uncomplicated 1969 gel filtration study of rodent 100,000 g liver supernatant fraction (Figure 1) prompted an unexpectedly wide range of discoveries relating to net hepatic organic anion uptake, glutathione metabolism and detoxification, multifunctional proteins, lipid metabolism, inflammatory pathways, and possibly azo dye carcinogenesis. Current research worldwide focuses on the role of GSTs in protein-protein interaction and signaling, oxidative stress, gene expression and regulation, and renewed interest in net hepatic uptake in physiology and disease. Studies of FABPs continue, particularly regarding their function and transcriptional and epigenetic regulation in metabolic stress, obesity, and other pathologic states. Determining the function of cytoplasmic binding proteins, such as Y and Z, particularly when there are many isoforms and gene products within a single cell, has proven to be a difficult challenge that we hope can be met using high-resolution live-cell imaging of fluorescently labeled ligands and candidate acceptor molecules.

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The studies reported by Jonathan Levi in 1969 were continued by a large number of fellows and colleagues in our laboratory at the Albert Einstein College of Medicine and worldwide. After a distinguished academic career in London, Jonathan Levi died in 1998. This Hindsight article is dedicated to the memory of his scholarship and friendship.

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