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Lectins As Effective Tools in the Study of the Biliary Network and the Parenchymal Architecture of the Zebrafish (*Danio rerio*) Liver

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Abstract

Lectins are carbohydrate-binding proteins with specific affinity to glycoconjugates expressed in various tissues. Lectins are of substantial utility as research, histochemical, and diagnostic tools in mammalian systems. Reactivity of 12 commonly used plant-based lectins was studied in zebrafish liver. Four lectins, tomato lectin (TL), wheat germ agglutinin, concanavalin A, and Jacalin showed strong reactivity to hepatic parenchymal structures. Importantly, TL reacted to glycoconjugates within segments of the larval and adult intrahepatic biliary network, from canaliculi to bile ducts. We provide evidence that lectins can serve as important histochemical tools to investigate the structural and functional characteristics of the zebrafish liver.

Keywords: lectin, zebrafish, liver, canaliculi, hepatocytes, bile

The zebrafish is an important model species in the study of liver development and diseases.[1–3] Lectins are carbohydrates or glycan-binding proteins expressed in plants and animals.[4] Plant lectins have historically been used in mammalian model systems as histological reagents for cell and tissue identification, and as diagnostic tools in a variety of cancers.[5–10] Yet, there are few investigations into the usefulness of lectins in the model organism zebrafish.[11,12] Although a number of zebrafish transgenic lines express liver-specific genetically encoded markers, a timely and complete set of these lines is not always readily available. Hence, alternative histological tools can be beneficial.

Previous study from our laboratory has demonstrated that lectins can be used as histochemical tools to identify tissue types in the adult and developing zebrafish heart.[13] We have investigated the utility of lectins as research tools in the zebrafish liver. We used 12 routinely employed plant-based lectins to demonstrate that the zebrafish liver parenchyma displays differential reactivity and staining patterns. Strong levels of reactivity were observed for *Lycopersicon esculentum* (tomato lectin, TL), *Triticum vulgaris* (wheat germ agglutinin, WGA), *Concanavalia ensiformis* agglutinin (Con A), and *Artocarpus heterophyllus* (Jacalin).

Low-to-medium levels of reactivity were noted for *Glycine max* (soybean), *Arachis hypogaea* (peanut), *Vicia villosa* agglutinin, *Solanum tuberosum* (potato), and *Ricinus communis*. No discernable reactivity was detected with *Ulex europaeus*, *Bandeiraea simplicifolia*, and *Dolichos biflorus* agglutinin. The binding specificity and reactivity of these lectins are summarized in Supplementary Table S1. The lectin staining protocol used is described in Supplementary Data S1.

Most notable was the reactivity pattern of TL. In stained wholemounts (Fig. 1A) and 10-μm cryosections (Fig. 1B) of adult liver, a pattern of TL-stained short or long repeating and angled segments could be observed, analogous to previously described biliary network in zebrafish.[14,15] A number of these TL-reactive short segments appear intracellular, spanning the plasma membrane of hepatocytes to perinuclear regions within the cytosol (Fig. 1C). To confirm that the TL-reactive

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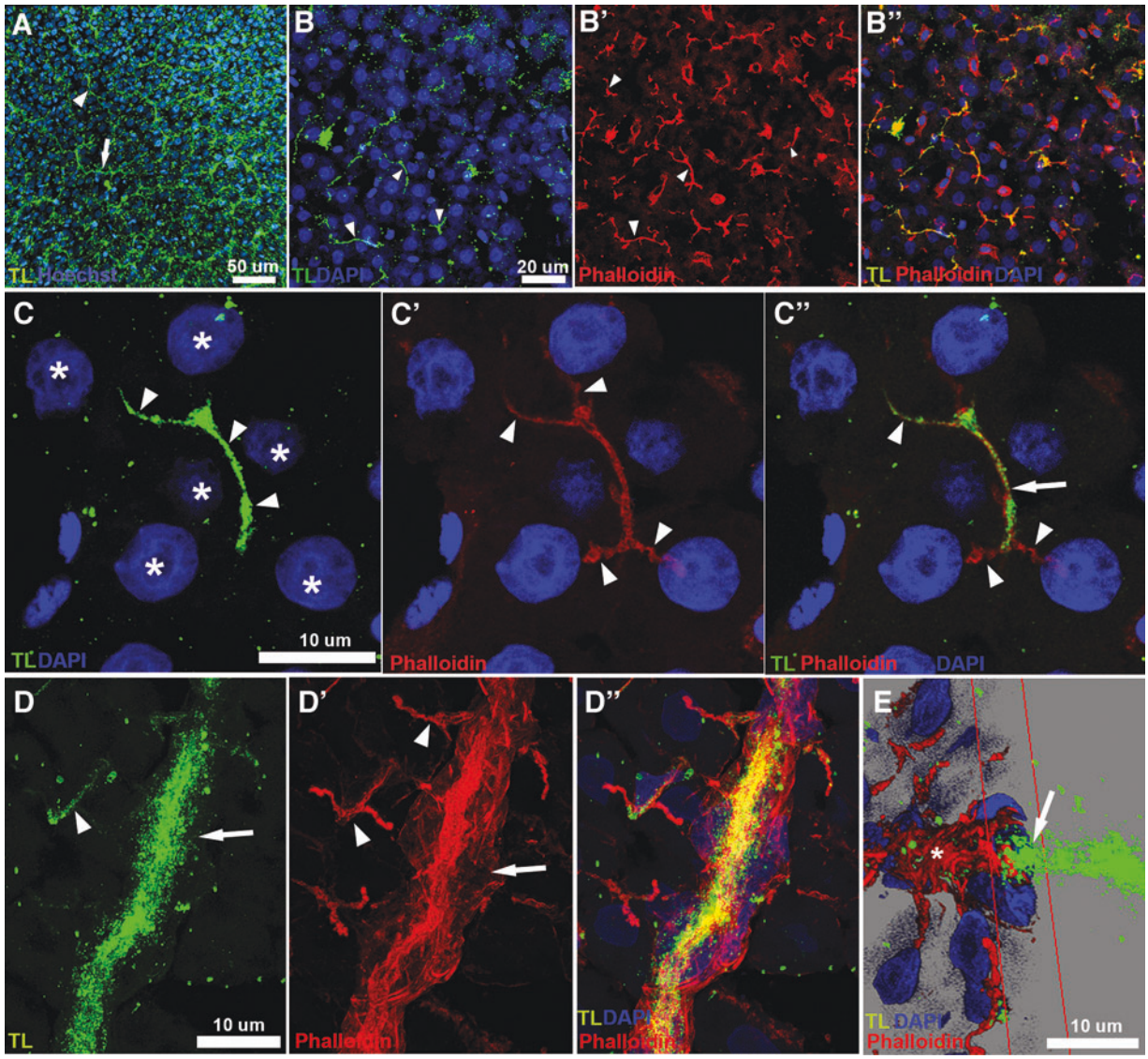

FIG. 1. Binding pattern of TL (*Lycopersicon esculentum*) in the intrahepatic biliary architecture of zebrafish. **(A)** TL reactivity (*green*) in wholemount-stained and imaged adult zebrafish liver displaying interconnected and discontinuous short (*arrowhead*) and long (*arrow*) jagged-line segments analogous to the pattern of biliary structures at low magnification. **(B)** TL reactivity (*green*) in 10-μm cryosections stained and imaged adult zebrafish liver displaying relatively long and short and jagged-line segments (*arrowhead*) at low magnification. **(B')** Actin-enriched short and jagged line segments (*arrowhead*) visualized using rhodamine-labeled phalloidin in the same section in **(B)**. **(B'')** Overlayed images of **(B, B')** showing colocalization (*yellow*) of the TL (*green*) and actin (*red*) line segments. **(C)** TL reactivity (*green*) in 10-μm cryosections stained and imaged adult zebrafish liver displaying strongly reactive short line segments (*arrowhead*) between six hepatocyte nuclei (*) at high magnification. **(C')** Actin-enriched short line segments (*arrowhead*) visualized using rhodamine-labeled phalloidin in the same section in **(C)**. The terminal end of each segment approaches the hepatocyte nuclei and represents hepatic canaliculi (*arrowhead*) that connect to preductules (*arrow*). **(C'')** Overlayed images of **(C,** and **C')** showing colocalization of the TL and actin line segments in a canaliculus (*arrowhead*) and appears contained within the preductule (*arrow*). **(D)** TL staining (*green*) in a bile duct appearing like a partially sinuous, punctate, and dense large diameter *column* (*arrow*) with adjacent ductules (*arrowhead*). **(D')** Phalloidin-stained actin-enriched duct (*arrow*) and ductules (*arrowhead*) are seen. **(D'')** Overlayed images of **(D, D')** showing the smaller diameter TL-stained *column* (*green*) localized along the same longitudinal axis to the center of the larger diameter actin-enriched *column*. **(E)** A 3D reconstruction of TL (*green*) and phalloidin (*red*)-stained section showing a main duct (*) with connecting ductules (*left side* of panel), and a digitally subtracted part of the duct (*right side* of panel) demonstrating that TL-stained glycoconjugates and presumptive bile *column* (*arrow*) located within the bile duct. **(F)** TL staining (*red*) of biliary duct profiles. **(F')** Overlaid image of **(F)** with 2F11 antibody-labeled biliary epithelial cells showing TL-stained glycoconjugates within the biliary duct. **(G)** TL-stained glycoconjugates in larval liver hepatocytes at 7 days postfertilization. **(G')** Overlaid image of **(G)** with actin-enriched canaliculi. TL, tomato lectin.

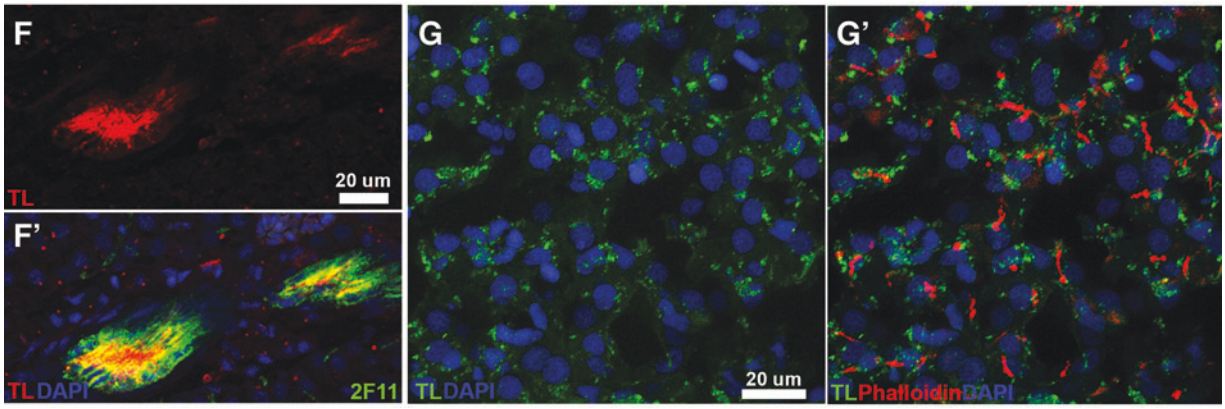

FIG. 1. (Continued).

segments constituted the zebrafish liver biliary tree, we performed lectin staining in combination with phalloidin that identifies the actin-rich cytosolic aspects of canaliculi, biliary preductules, ductules, and ducts (Fig. 1B''').[16]

The close overlapping of lectin-stained segments with phalloidin indicated that TL identified preductules and canaliculi that are deep infoldings of the apical hepatocyte plasma membrane (Fig. 1C''). TL stains biliary glycoconjugates present in the lumen of bile ducts (Fig. 1D, E). In addition, TL reactivity was closely associated with FIS-2F11 antibody immunoreactivity that stains the hepatopancreatic ductal system (Fig. 1F).[17–19] Moreover, TL reactivity could be detected in the hepatocytes of the larval liver at 1-week postfertilization (Fig. 1G), suggesting that it could potentially be used as a functional measure for bile production and flow.

WGA stained glycoconjugates on the surface of hepatocyte membranes and was strong in the endothelium of hepatic sinusoids revealing the tubular nature of the fish's liver (Supplementary Fig. S1A). Con A and WGA had significant overlap in reactivity; however, Con A staining was broader and could be observed on cell membranes other than hepatocytes (Supplementary Fig. S1B, C). Because of their broad reactivity, these two lectins can also be used as counterstains for double staining schemes such as TL with WGA, and TL with Con A (Supplementary Fig. S1D, E). Jacalin displays strong and unique reactivity compared with that of TL, WGA, or Con A.

Although Jacalin appears to stain small cytoplasmic vesicles in hepatocyte (colocalizing with WGA), reactivity was particularly strong in the apical region of biliary epithelial cells facing the lumen of bile ductules and ducts (Supplementary Fig. S1F). Interestingly, clusters of perivascular cells displayed the strongest reactivity to a number of lectins, including TL, WGA, and Con A. Most of these perivascular cells are stained with the pan-leukocyte marker Lcp1 (Supplementary Fig. S2). The specific location of these cell clusters and their morphology are consistent with the phenotype of perivascular macrophages previously described in the zebrafish liver.[20,21]

In conclusion, TL, WGA, Con A, and Jacalin can be used singularly or in combination with other lectins, or in conjunction with immunostaining. They can be utilized as a methodologically simple and highly valuable histological tool for the identification of hepatocytes, hepatic sinusoids, canaliculi, and various segments of the intrahepatic biliary network. The functional correlates of these staining patterns have not yet been elucidated. The utility of lectins in functional studies of the zebrafish liver warrants further investigations.

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Authors' Contributions

H.B., J.M.G.-R., and P.J.L. devised experiments. H.B., Z.R., M.B., F.G., and P.J.L. performed experiments. All authors analyzed data. H.B., F.G., J.M.G.-R., and P.J.L. revised the article. P.J.L. wrote the article.

Authors' Bio

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Disclosure Statement

No competing financial interests exist.

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Supplementary Material

Supplementary Data S1
Supplementary Table S1
Supplementary Figure S1
Supplementary Figure S2

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