Review

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# Structural and functional hepatocyte polarity and liver disease

Paul Gissen[1,2,3,*], Irwin M. Arias[4]

[1]MRC Laboratory for Molecular Cell Biology, University College London, London, UK; [2]UCL Institute of Child Health, London, UK; [3]Great Ormond Street Hospital, London, UK; [4]Cell Biology and Metabolism Program, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, United States

## Summary

Hepatocytes form a crucially important cell layer that separates sinusoidal blood from the canalicular bile. They have a uniquely organized polarity with a basal membrane facing liver sinusoidal endothelial cells, while one or more apical poles can contribute to several bile canaliculi jointly with the directly opposing hepatocytes. Establishment and maintenance of hepatocyte polarity is essential for many functions of hepatocytes and requires carefully orchestrated cooperation between cell adhesion molecules, cell junctions, cytoskeleton, extracellular matrix and intracellular trafficking machinery. The process of hepatocyte polarization requires energy and, if abnormal, may result in severe liver disease.

A number of inherited disorders affecting tight junction and intracellular trafficking proteins have been described and demonstrate clinical and pathophysiological features overlapping those of the genetic cholestatic liver diseases caused by defects in canalicular ABC transporters. Thus both structural and functional components contribute to the final hepatocyte polarity phenotype. Many acquired liver diseases target factors that determine hepatocyte polarity, such as junctional proteins. Hepatocyte depolarization frequently occurs but is rarely recognized because hematoxylin-eosin staining does not identify the bile canaliculus. However, the molecular mechanisms underlying these defects are not well understood. Here we aim to provide an update on the key factors determining hepatocyte polarity and how it is affected in inherited and acquired diseases.



Keywords: Hepatocyte polarity; Inherited liver diseases; Hepatocyte biology; Cholestasis; Canalicular diseases.

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* Corresponding author. Address: MRC Laboratory for Molecular Cell Biology, Gower Street, London WC1E 6BT, UK. Tel.: +44 7979547619; fax: +44 020 7679 7806.

*E-mail address:* p.gissen@ucl.ac.uk (P. Gissen).

*Abbreviations:* TJ, Tight junctions; BC, Bile canaliculi; ATP, Adenosine triphosphate; ABC, ATP binding cassette; TGN, Trans Golgi Network; RE, Recycling endosomes; ECM, Extracellular Matrix; cAMP, Cyclic Adenosine Monophosphate; PKA, Protein Kinase A; GPI, Glycophosphatityl Inositol; SNARE, Soluble N-ethylmaleimide-sensitive factor attachment Protein Receptor; ARC, Arthrogryposis Renal dysfunction and cholestasis syndrome; MVID, Microvillus Inclusion Disease; NISCH, Neonatal ichthyosis-sclerosing cholangitis syndrome; FHC, Familial hypercholanemia; HBV, Hepatitis B Virus; HCV, Hepatitis C virus.

## Introduction

A defining feature of metazoans is the existence of polarized layers of epithelium which give rise to the three dimensional shapes of body parts and types. The formation and maintenance of a polarized epithelium is complex and requires specific cell adhesion molecules, cytoskeletal factors and intracellular trafficking components [1]. These give rise to apical and basolateral plasma membrane domains which separate interior from external environments and permit directional absorption and secretion of proteins and other solutes. Most epithelial cells, such as intestinal and renal tubular cells, are polarized in the plane of the tissue [2]. In contrast, hepatocytes have a unique polarization arrangement in which each of two adjacent cells contributes an apical plasma membrane that form one or more capillary-like structures, the bile canaliculus (BC), which is the smallest branch of the bile ductal system (Fig. 1) [3]. The BC is functionally sealed by tight junctions (TJs) and, with its microvilli, constitutes ~13% of total hepatocyte plasma membrane [4]. Defects in hepatocyte polarization leads to major pathophysiological consequences.

### Key points

**Hepatocyte polarity**

Structural polarity

- Each hepatocyte contributes apical membrane domain to one or more canaliculi.
- Bile canaliculi are sealed by tight junctions and form a complex interconnected network.
- Microvillae dramatically increase the surface area of canalicular membrane.

Functional polarity

- Polarized flow of various molecules across canalicular and basolateral (sinusoidal) membranes.
- Intrahepatocyte retention of bile components due to the genetic defects in canalicular ATP binding cassette (ABC) transporters leads to structural depolarization.

Review

**Key points**

**Polarization elements**

- Extracellular matrix provides a scaffold for adhesion of hepatocytes and a signaling platform that determines hepatocyte differentiation.
- Cell junctions including tight and adherens junctions, desmosomes and gap junctions connect hepatocytes. Loss of tight junctions results in hepatocyte depolarization.
- Intracellular protein trafficking: newly synthesized proteins are sorted at Trans Golgi Network into specific trafficking pathways. Rab11a positive recycling endosomes, dynamic microtubules and actin filaments play crucial roles in determining localization of apical transmembrane transporters.
- Mitochondrial energy production is essential for polarization and is regulated by AMPK.

**Key points**

**Protein defects in inherited disorders of polarity**

Tight junction proteins:

- Claudin 1 (NISCH syndrome)
- TJP2 (PFIC-4 and Familial Hypercholanaemia)

Intracellular trafficking proteins:

- VPS33B or VIPAR (ARC syndrome)
- Myosin 5B (MVID)

Canalicular membrane transporters:

- ATP8B1 (PFIC-1)
- ABCB11 (PFIC-2)
- ABCB4 (PFIC-3)
- ABCC2 (Dubin Johnson syndrome)

Basolateral membrane transporters:

- Combined OATP1B1 and OATP1B3 defect (Rotor syndrome)

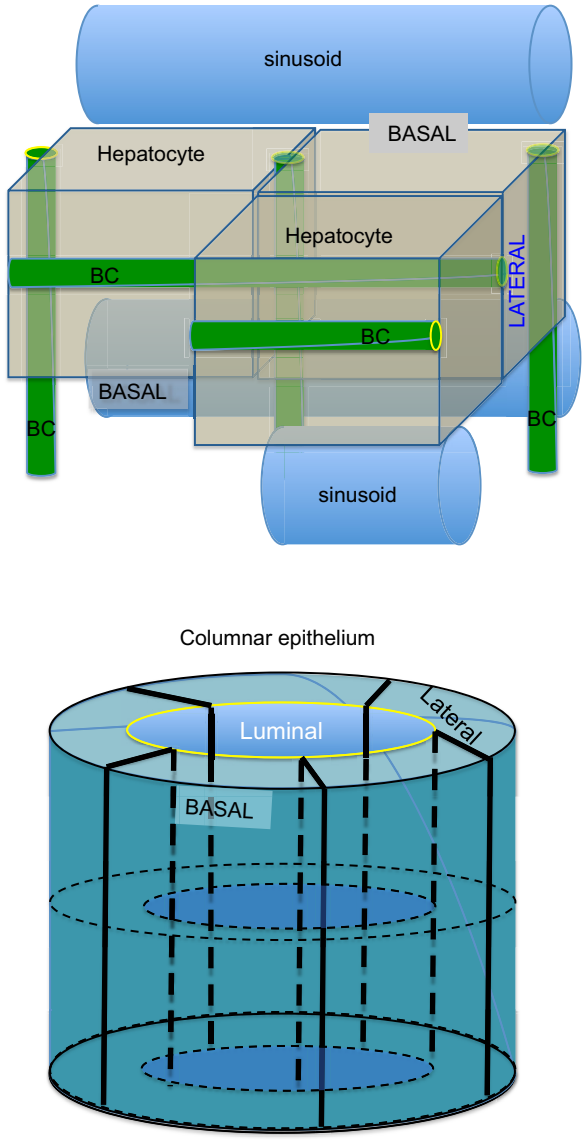


**Fig. 1. Comparison of hepatocyte and columnar epithelial phenotypes.** (A) Adjacent hepatocytes form bile canaliculi (green) at their cell-cell contacting domains (blue) and are strengthened by surrounding tight junction belt (yellow). A single hepatocyte can form bile canalicular lumina with three neighbours (BC). Hepatocytes can also have two basal domains that face the adjacent sinusoids. (B) Columnar epithelia feature a central lumen formed by the apical domains of individual cells, which are perpendicular to their cell-cell contacting domains (black) and separated from the latter by tight junctions (yellow). The basal domains are in contact with a basal lamina (adapted with permission from Müsch A. Exp Cell Res, 2014) [153].

## Basic understanding of hepatocyte polarity

Hepatocyte polarity mechanisms may be divided into structural and functional components. Structural polarity includes morphologic integrity of TJs and apical plasma membranes with their microvilli, and BC network formation. In cultured hepatocytes and cell lines, reversion to planar polarity phenotype and loss of BC has been demonstrated as a result of deletion or inhibition of individual components of the complex polarization machinery [5–7]. In contrast, functional polarity is predominantly defined by the action of canalicular ATP binding cassette (ABC) transporters. The scope is expanding to include acquired diseases although pathologists rarely comment on hepatocyte polarization because BC cannot be visualized by hematoxylin and eosin staining and