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Catherine Decaens, Marjorie Durand, Brigitte Grosse, Doris Cassio. Which in vitro models could be best used to study hepatocyte polarity?: Hepatocyte polarity models. *Biology of the Cell*, 2008, 100 (7), pp.387-98. 10.1042/BC20070127 . inserm-00270868

HAL Id: inserm-00270868

<https://inserm.hal.science/inserm-00270868>

Submitted on 19 Jun 2008

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TITLE Which in vitro models could be best used to study hepatocyte polarity?

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Running title Hepatocyte polarity models

Keywords hepatocyte, polarity, cytoskeleton, IP3R, in vitro models

ABSTRACT

The correct functioning of the liver is ensured by the setting and the maintenance of hepatocyte polarity. The complex polarity of hepatocyte is characterized by the existence of several basolateral and apical poles per cell. Many *in vitro* models are available for studying hepatocyte polarity. But which are the more suitable? To answer this question, we wished to identify criteria which determine the typical hepatocyte polarity. Therefore, we compiled a battery of protein markers of membrane domains in rat hepatocytes and looked for their involvement in hepatocytic functions. Then, we focused on the relationship between hepatic functions and the cytoskeleton, Golgi apparatus and endoplasmic reticulum. Subsequently, we compared different cell lines expressing hepatocyte polarity. Finally, to demonstrate the usefulness of some of these lines, we presented new data on reticulum organization in relation to polarity.

INTRODUCTION

Although the Ancient Greeks already knew that the liver is able to regenerate, nowadays many people are still working hard to understand how this main organ functions. The liver is made up of 60% hepatocytes that produce 90% of the whole hepatic proteins and which are responsible for most of the main liver functions such as bile production, detoxification... As all epithelial cells, hepatocytes must be polarized to be functional. This implies the formation of specific membrane domains and special cytoskeletal and endoplasmic reticulum (ER) networks. Hepatocyte polarity is very peculiar and complex (Hubbard et al., 1994) compared to the so-called «simple polarity» of most epithelial cells such as intestinal cells (Figure 1). «Simple» polarized cells have one apical pole located at the cell apex and one basolateral pole corresponding to the remaining domain of the plasma membrane. In contrast, hepatocytes that are organized in plates in the liver have several apical and basolateral poles. In contact with the external environment, the apical poles of front-facing and adjacent hepatocytes form a continuous network of bile canaliculi (BC), into which bile is secreted. In contact with the blood, the basal membrane domain called sinusoidal pole, secretes various components -proteins, drugs- into the circulation and takes up recycled biliary salts. In both types of polarized cells, the tight junctions make the frontier between apical and lateral poles.

The first studies on hepatocytes were performed with isolated perfused organ and subcellular fractionations (Bartles et al., 1987). Later, easier *in vitro* models were obtained by enzymatic digestion of the liver—freshly isolated hepatocytes induced to repolarize (Musat et al., 1993) and hepatocytes isolated as couplets that keep a bile canalicular space between the two cells (Graf et al., 1984). Both models have been widely used to study bile secretion (Graf and Boyer, 1990), protein trafficking (Maurice et al., 1988) and calcium waves (Combettes et al., 1994). However, variations

occur between different isolations and both models have a limited life span allowing only short-term experiments. Primary hepatocyte repolarization is slow and variable.

To circumvent these disadvantages, hepatic cell lines were used. A few human lines exhibit polarity and some rat polarized lines were generated. The aim of this review is to make a survey of these cell lines. First, we will sum up data from literature concerning the relationship between liver functionality and hepatocyte polarity. Then, we will compare the cell lines. To exemplify their usefulness, some new data on inositol 1,4,5-trisphosphate receptors (IP₃Rs) distribution in relation to polarity will be presented.

HEPATIC FUNCTIONALITY AND POLARITY

Which are the contributors in hepatic polarity? We will look at the membrane domain proteins, the cytoskeleton and two cytoplasmic organelles, focusing on their relationship with hepatic functionality.

MEMBRANE PROTEINS

Poles of polarized cells are characterized by a special plasma membrane protein distribution (Simons and Fuller, 1985). A list of membrane protein markers of polarity in rat hepatocyte is given in Supplementary Table 1. Among hepatocyte membrane markers, the bile salt export pump (Bsep/BSEP for rodent/HUMAN forms), the Na⁺/taurocholate cotransporting polypeptide (Ntcp/NTCP) and the asialoglycoprotein receptor are liver-specific. The other markers are also expressed in «simple» polarized cells of other tissues and are similarly localized in both types of polarized cells.

Biliary function is ensured by a set of transporters that need to be properly settled at their specific pole. At the apical pole of hepatocytes, several ATP-binding cassette (gene symbol *Abc/ABC*) transporters are localized, in particular, two multidrug resistance proteins Mdr1/MDR1 and Mdr2/MDR3, one multidrug resistance-associated protein Mrp2/MRP2 and Bsep/BSEP. Bsep/BSEP is the major bile salt transporter that ensures bile secretion at the BC (Trauner and Boyer, 2003). Several clinical forms of cholestasis have been related to mutations in genes encoding for bile salt transporters (Pauli-Magnus et al., 2005). (Definition **cholestasis** = reduction or suppression of bile flow resulting from liver injury and diseases). For example, various BSEP mutations prevent the protein from trafficking to the apical membrane and account for the syndrome of progressive familial intrahepatic cholestasis of type 2 (Strautnieks et al., 1998). Protein sorting is a prerequisite for polarized membrane domains. Most apical proteins of the hepatocyte are «indirectly» sorted, first addressed to the basolateral membrane prior to their subsequent transcytosis to the apical membrane (Bartles et al., 1987). But apical Abc transporters such as Bsep

are directly sorted to the apical pole through an endosomal compartment from which they can be released when increased bile secretion is needed (Kipp and Arias, 2002). Myosin Vb and Rab11 participate in apical targeting of these recycling endosomes, thereby contributing to canalicular biogenesis (Wakabayashi et al., 2006).

At the sinusoidal pole of hepatocyte, Ntcp/NTCP and organic anion transporting polypeptide proteins are in charge of bile salt uptake from portal circulation. These transporters are essential for bile formation as 95% of the biliary secreted bile salts re-enter into portal circulation. Some members of the Mrp/MRP family, also localized at the sinusoidal pole, are responsible for the basolateral efflux of bile salts and organic anions. The Mrp3 up-regulation observed under cholestatic conditions facilitates clearance of anionic conjugates in the blood when canalicular secretion is impaired (Donner and Keppler, 2001).

Among polarity markers, proteins of the apical junctional complex -the adherens and tight junctions- (Nelson, 2003) and of the gap junctions (Kojima, T. et al., 2003) are of special interest. Cell junctions are essential for several hepatocyte-specific functions (Vinken et al., 2006), including bile secretion (Nathanson et al., 1999), cytochrome P-450 isoenzyme expression (Hamilton et al., 2001) and glycogenolysis (Nelles et al., 1996). According to their gate function in paracellular diffusion, tight junctions in hepatocytes can be called the blood-biliary barrier as they serve to keep bile in the BC away from blood circulation. These junctions are composed of transmembrane constitutive proteins and of various cytoplasmic proteins directly bound to the constitutive ones, such as the Zonula Occludens (ZO)-protein family. Recently, two of these proteins have been found to play a direct role in human hereditary liver diseases—claudin 1 in neonatal sclerosing cholangitis associated with ichthyosis syndrome (Hadj-Rabia et al., 2004) and ZO-2 in familial hypercholanemia (Carlton et al., 2003). Tight and gap junctions are closely associated in hepatocytes. Connexin 32, specifically expressed in liver gap junctions, partly colocalizes with occludin and claudin 1 (Kojima, T. et al., 2003). These interactions could indicate some regulatory mechanism of the blood-biliary barrier by the gap junctions. In cholestasis, a loss of gap junctions is always observed in association with leaky tight junctions (Anderson, 1996).

CYTOSKELETON

Polarity also means special cell shape and architecture. The cytoskeleton, widely responsible for this three dimensional organization, has a fundamental role in the generation of cell polarity (Yeaman et al., 1999). The cytoskeletal components, microfilaments (MF), microtubules (MT) and intermediate filaments (IF), provide structural asymmetry in the plasma membrane; the three major cytoskeletal proteins -actin, tubulin and cytokeratin- are connected, directly or via protein scaffolds, to the plasma membrane especially at the intercellular junctions. How is the cytoskeletal network involved in hepatocyte polarity and functionality?

Studies on human isolated BC membrane preparations have provided clues on the organization of the BC cytoskeleton (Tsukada et al., 1995). The BC is surrounded by two circumferential MF belts of actin and actin-binding proteins. One belt containing myosin II and associated with the adherens junctions is responsible for the contractility of BC implicated in the bile flow; the other belt regulates vesicle transport near the plasma membrane (Tsukada and Phillips, 1993). Experimental cholestasis models always show alterations in the pericanalicular MF. When cholestasis was induced in rats by treatment with phalloidin (which prevents F-actin depolymerization), bile flow and bile acid secretion decreased and tight junction permeability was altered. This demonstrates the role of MF on the hepatocyte junctional barrier and on bile production (Elias et al., 1980). Among the proteins associated with MF, the ezrin-radixin-moesin proteins are implicated in apical protein retention. Kikuchi et al. have shown that radixin-null mice have a loss of Mrp2 transporter at the BC membrane and hyperbilirubinemia (Kikuchi et al., 2002). In human primary biliary cirrhosis, a large reduction in radixin expression is associated with a partial loss of canalicular MRP2 (Kojima, H. et al., 2003) .

MT are polarized filaments with the fast-growing minus-ends facing the apical pole of polarized cells (Figure 1). Highly concentrated near the BC (Novikoff et al., 1996), MT are involved in hepatic protein trafficking (Pous et al., 1998). MT are also essential in BC formation : during epithelial polarity establishment, MT are reorganized according to an apico-basal polarity axis; this allows transport of apical proteins to the MT minus-ends which contributes to the apical pole formation (Cohen et al., 2004a). MT also organize signaling as demonstrated by the specific involvement of the dynamic MT sub-population in the growth hormone pathway (Phung-Koskas et al., 2005).

IF are arranged as a pericanalicular sheath, inserted into desmosomes, around the BC that they stabilize. IF network perturbation by nickel leads to abnormal canalicular function (Kawahara et al., 1990). IF are also targets and modulators of toxic stress and apoptosis in hepatocyte (Zatloukal et al., 2004). In hepatocyte, the keratin expression pattern is rather simple, exclusively composed of cytokeratins 8 and 18. In cytokeratin 8-null mice, the apical HA4 protein is not only found in BC as usual but also at the basolateral pole (Ameen et al., 2001). Mutations in cytokeratin 8 and 18 genes were shown to be risk factors in patients for developing liver cirrhosis (Ku et al., 2003).

GOLGI APPARATUS AND ENDOPLASMIC RETICULUM

In addition to the cytoskeleton components, organelles are not randomly distributed in the cytoplasm. The Golgi apparatus, involved in protein selective targeting to the poles, is localized between nucleus and the apical pole in polarized cells (Figure 1), namely between the nucleus and BC in hepatocytes (Musat et al., 1993). During cell polarization, Golgi apparatus undergoes a

complex rearrangement, moving along the new apico-basal polarity axis of MT towards their minus-ends in the apical region (Bacallao et al., 1989).

The ER is a continuous and dynamic membrane system, connected to the cytoskeleton and closely associated with plasma membrane and organelles such as Golgi apparatus (Voeltz et al., 2002). It exhibits structural and functional distinct sub-regions (Baumann and Walz, 2001) which fulfil specialized biological functions, such as calcium (Ca^{2+}) signalling. Stored in the smooth ER, Ca^{2+} is released in response to specific signals through two types of Ca^{2+} channels, the IP_3Rs and the ryanodine receptors, organized in specific domains of the ER (Meldolesi and Pozzan, 1998). The subcellular distribution of the three IP_3R isoforms depends on cell types, physiological status (Vermassen et al., 2004) and even correlates with polarity state in Madin-Darby canine kidney (MDCK) cells (Colosetti et al., 2003).

Hepatocytes are exceptionally active in protein and lipid synthesis. Accordingly, their ultrastructural examination reveals quantities of rough and smooth ER, occupying 15% of the total cell volume. Ca^{2+} signalling regulates diverse hepatocyte functions, in particular bile secretion (Leite and Nathanson, 2001) and a predominant role of IP_3Rs was evidenced. Hepatocytes express only the $\text{IP}_3\text{R1}$ and $\text{IP}_3\text{R2}$ isoforms (Dufour et al., 1999). The preponderant $\text{IP}_3\text{R2}$ isoform is concentrated at the BC level, precisely where calcium signals originate (Nathanson et al., 1994). This enrichment might be essential for the formation of Ca^{2+} waves in hepatocytes *in vivo* and could also play a role in the formation of intercellular Ca^{2+} waves because some gap junctions, necessary for the cell-to-cell wave propagation, are also located in that region (Hernandez et al., 2007). So, although still poorly understood, ER could take part in polarity.

IN VITRO HEPATOCYTE MODELS: MAIN USES, INTERESTS AND LIMITS

A comparison of these various lines is given in Supplementary Table 2.

HUMAN LINES

Huh7 cells are poorly polarized. They make few BC structures according to the localization of apical aminopeptidase N (immunohistochemistry and immunoelectron microscopy) but the protein is also present in numerous cytoplasmic vesicles and on the remainder of the cell membrane (Chiu et al., 1990). ZO-1 protein does not localize at the BC structures but makes belts at the cell apex as in «simple» polarized cells (Harada et al., 2000). Huh7 cells have been used in the study of hepatitis C virus (Lohmann et al., 1999) and of copper excretion in Wilson's disease (Harada et al., 2000).

HepG2 cells have polarity features. BC like-spaces, microvilli-lined and sealed by tight junctions are present. Apical markers, dipeptidyl peptidase IV (DPP IV), B442 (BC antigen) and ABC

transporters, especially BSEP, are properly addressed (Zegers and Hoekstra, 1997; Kubitz et al., 2004). Various cytoskeletal components (actin, villin, myosin II and α -tubulin) localize as in hepatocytes (Sormunen et al., 1993). HepG2 cells express some efflux transporters (Cantz et al., 2000; Le Vee et al., 2006) and accumulate cholyglycylamidofluorescein (a fluorescent bile acid-derivative) in more than 70% of their BC structures (Figure 2). However, only 20-40% of HepG2 cells are polarized (Theard et al., 2007); their BC structures are hard to detect in phase contrast and mislocalization of apical markers and microvilli may occur (Zegers et al., 1998). Recently, HepG2 cell polarity was improved by growth in multilayering clusters on their predeposited extracellular matrix; this led to the formation of elongated canalicular lumens that span several cells and appear as large acinar structures (Herrema et al., 2006). Since HepG2 cells express numerous metabolizing enzymes, they are an excellent tool for genotoxic studies (Knasmuller et al., 2004). They are currently used for polarity development and lipid and protein trafficking studies (Theard et al., 2007).

The **HepaRG** cell line displays a heterogeneous morphology with hepatocyte-like and biliary-like epithelial phenotypes (Gripon et al., 2002). Hepatocyte-like cells have BC-like structures (phase contrast and electron microscopy), that are functional as evidenced by fluorescein excretion (Cerec et al., 2007). They correctly localize DPP IV, E-cadherin, ZO-1 and cytokeratin 18 (Parent et al., 2004). More polarity markers have to be looked for to better define the phenotype. The HepaRG cells express drug transporters and various active P450 cytochromes at levels comparable to those found in cultured human hepatocytes. Thus, the HepaRG cell line is a good tool for xenotoxic and genotoxic studies (Guillouzo et al., 2007). As bipotent progenitors, HepaRG cells are of great interest for differentiation studies. However, no more than 55% of the cells display the hepatocytic phenotype, favoured by growth with 2% DMSO and epidermal growth factor.

CELL LINES DERIVED FROM THE Fao RAT HEPATOMA

Several polarized lines have been obtained by hybridization and various treatments.

WIF-B and WIF-B9 were derived from hybrid cells obtained by fusion of Fao with human fibroblast (Cassio et al., 1991; Ihrke et al., 1993). More than 90% of the cells create BC structures, well visible in phase contrast microscopy (Figure 2), microvilli-lined and sealed by typical tight junction continuous strands (freeze-fracture replica) (Decaens et al., 1996). These BC are surrounded by sheaths of actin, myosin IIA, ezrin, α -tubulin and cytokeratins 8 and 18, as in hepatocytes in situ (Decaens and Cassio, 2001). WIF-B and WIF-B9 cell lines express and correctly settle a large pool of the polarity markers listed in supplementary Table 1 (Decaens et al., 1996; Bender et al., 1998). These cells communicate via connexin 32-constituted gap junctions (Chaumontet et al., 1998). Their Golgi apparatus is located between the BC region and the nucleus

(Pous et al., 1998). They make vectorial transport of bile acids (Figure 2) (Bravo et al., 1998) and express several rat and even human hepatobiliary transporters (Briz et al., 2007). WIF-B and WIF-B9 cell lines are now used as *in vitro* models for many hepatocytic trait studies. The respective role of stable and dynamic MT classes in specialized transport steps was evidenced using WIF-B9 cells (Pous et al., 1998). These cells permit hepatocytic traffic studies because they sort most of their apical proteins just as hepatocytes do (Ihrke et al., 1998; Sai et al., 1999; Nyasae et al., 2003; Wakabayashi et al., 2004; Guo et al., 2005; Ramnarayanan et al., 2007). Overcoming drug resistance (Briz et al., 2003) and the hepatotoxicity profile of chemicals (Biagini et al., 2006) were evaluated using WIF-B9 cells. Bile acid metabolism (Monte et al., 2001) and biliary cholesterol transport (Harder et al., 2007) have been studied, since WIF-B and WIF-B9 cells correctly localize transporters for bile secretion and aquaporin water channels (Nies et al., 1998; Gradilone et al., 2005). Recently, alcohol-induced hepatotoxicity was investigated using WIF-B cells which efficiently metabolize ethanol into acetaldehyde as hepatocytes *in situ* (Kannarkat et al., 2006). WIF-B9 cells can also be used to study both types of polarity (Figure 1) since they express the «simple» polarized phenotype before expressing the typical hepatocytic phenotype (Decaens et al., 1996; Cohen et al., 2004a).

11-3 is a mouse monochromosomal-Fao rat hepatoma hybrid. More than 90% of the cells form functional BC-like structures and have an hepatocyte polarized phenotype according to the localization of many membrane polarity markers. Before expressing the hepatocyte phenotype, 11-3 cells express the «simple» polarized phenotype. Two subclones, 11-3-5 which only displays the simple polarized phenotype, and 11-3-1 which is unpolarized (Peng et al., 2006) might be useful to compare protein expression profile with polarity state. 11-3 cells were used to study the regulation of alcohol dehydrogenase (Majewski and Yang, 1995).

Fao-BA1 and C2rev7-BA1 were generated by Fao or C2rev7 induction with chenodeoxycholic acid (Ng et al., 2000). They form functional BC-like structures easily detected in phase contrast, microvilli-lined, and delimited by tight junctions (electron microscopy). Apical protein markers (γ-glutamyl transferase, aminopeptidase N, Bsep and Mrp2, but not DPP IV), 1-18 basolateral marker and ZO-1 protein are correctly localized. However, some polarity traits are missing: Ntcp and organic anion transporting polypeptide 1 are localized in the cytoplasm rather than at the basolateral membrane—apical markers are not present in all the BC. As hepatocytic phenotype is maintained only in the presence of bile acids, Fao-BA1 and C2rev7-BA1 cells may be suitable for studies on hepatocyte polarization and bile salt trafficking.

Can 3-1 and Can 10 were generated by Fao culture in spheroids, which favours contacts between cells, followed by enrichment in BC-forming zones in monolayers.

More than 95% of Can 3-1 cells form BC structures, easily detectable in phase contrast. Numerous apical, basolateral and tight junction protein markers are correctly localized. Can 3-1 cells make vectorial transport of bile acids but the expression of their hepatobiliary transporters is not bile-acid inducible (Cassio et al., 2007). They polarize only when plated at high density, which makes them particularly suitable for studies on hepatocyte polarization.

Can 10 cells form long and branched BC structures made by union of the apical poles of tens of adjacent cells (Figure 3). These long BC, hardly detectable in phase contrast, are delimited by a complex network of tight junctions (freeze-fracture replica) (unpublished data) and surrounded by actin, radixin, ezrin, α -tubulin and β -tubulin (Figures 3-4). According to the correct localization of many polarity markers, more than 90% of Can 10 cells are polarized and vectorially transport bile acids. Their transporters are correctly localized (as illustrated for multidrug resistance protein in Figure 3) and are inducible by bile-acids (Cassio et al., 2007). Therefore, Can 10 is the first model with functional highly developed BC, reminiscent of those found *in vivo*. According to their protein repertoire close to that of the hepatocyte, especially for claudins (unpublished data), Can 10 cells can be a useful tool in tight junction studies. They may contribute to understanding the way that neighbouring cells are able to form long BC.

CELL LINES DERIVED FROM LIVER OF TRANSGENIC MICE

mhAT3F is derived from the liver of a transgenic mouse expressing SV40. It forms organoids in a Matrigel-based 3D culture with intercellular lumens having polarized traits: they are microvilli-lined with tight junction complexes clearly visible in electron microscopy; ZO-1, α -catenin, actin and radixin are localized at membranes delimiting the lumens. However, these apical lumens are more related to acini than to BC-structures. mhAT3F cells have been used to study apoptotic signalling (Haouzi et al., 2005).

AML12 and AML14 are derived from the livers of transgenic mice overexpressing transforming growth factor β . Adjacent cells form BC-like structures with microvilli and tight junctions (electron microscopy). They express the liver-specific connexins 32 and 26. The stable AML12 line expresses numerous hepatocyte secretory proteins and enzymes. This line is used for studying the role of transforming growth factor β 1 (Yang et al., 2006) and of cytokines (Brooling et al., 2005) on various cellular processes and lipogenesis in steatotic hepatocytes (Schadinger et al., 2005).

EMK1 OVEREXPRESSING MDCK CELLS

Finally, we want to point to MDCK cells, of kidney origin but able to express the typical hepatocyte polarity by overexpression of EMK1. EMK1 is the mammalian homologue of PAR-1, an essential kinase for the establishment of polarity in *Caenorhabditis elegans*, regulating MT organization.

Increased expression of EMK1 promotes hepatocytic-type lumen formation between more than 90% of neighbouring MDCK cells during their polarization (Cohen et al., 2004a). These lumens are microvilli-lined and express the MDCK apical markers gp135 and gp114, ZO-1 and actin. E-cadherin localized on the plasma membrane is excluded from these lumens. MT are oriented with minus-end towards the lumens. According to these criteria, these lumens look like BC structures. The transcytotic trafficking route for apical markers, typical of hepatocyte, is promoted in those cells (Cohen et al., 2004b). So EMK1-overexpressing MDCK cells constitute an excellent model to study the regulation of epithelial lumen morphogenesis and the candidates involved in the branching decision between simple or hepatocytic-like phenotypes.

PERSPECTIVES

All the cell lines described above have interesting features and deserve to be further characterized. Some aspects of cell organization have not been yet approached, for example Ca^{2+} signalling. Very little information is available on their ER organization and IP_3R distribution. We have used two of these cell lines to study the distribution of the three IP_3R isoforms. In WIF-B9 and Can10 cells, the three isoform mRNA were detected (K. Schmidt, J.F.Dufour, personal communication). In WIF-B9 cells, $\text{IP}_3\text{R}2$ and $\text{IP}_3\text{R}3$ have an enriched pericanalicular localization whereas $\text{IP}_3\text{R}1$ is homogeneously distributed in the cytoplasm (Figure 4). The expression of $\text{IP}_3\text{R}3$, an isoform absent from hepatocytes but present in cholangiocytes, was noticed in several cells in culture (Blondel et al., 1993). Sehgal et al. have also found the expression of the three IP_3R s in WIF-B cells but they did not observe a polarized distribution for either $\text{IP}_3\text{R}2$, or for $\text{IP}_3\text{R}3$ (Sehgal et al., 2005). As we both used the same commercial antibodies for $\text{IP}_3\text{R}3$, this discrepancy could be due to a difference in the polarity state of the cells; we have previously shown that several days in culture are needed before WIF-B9 cells express a fully polarized hepatocyte phenotype. Can 10 cells express the three IP_3R s with a pericanalicular enrichment for $\text{IP}_3\text{R}2$ (not shown) and $\text{IP}_3\text{R}3$ (Figure 4). Thus, in WIF-B9 and Can 10 cells, two IP_3R s exhibit a polarized distribution as observed in hepatocytes. These first results indicate that polarized cell models could be very useful to study the relationship between ER organization, Ca^{2+} signalling and polarity.

Numerous studies have been carried out to find a way to keep the hepatocytes isolated from normal liver in well differentiated and polarized status. For example, hepatocyte culture on and in various matrices preserves more or less their polarized functional features (Moghe et al., 1996). However polarity establishment study with normal hepatocytes is limited because these cells do not divide.

An *in vivo* model with chimeric human livers in mice was recently developed (Kneteman and Mercer, 2005) in which the human hepatocytes repopulated the diseased mouse liver and formed

functional BC. These chimeric human-mouse livers were used to study hepatitis B and C virus infection. This model of great interest has also limits as pointed out by users who stated : «*While the scope of utility of the chimeric mouse model is vast, the model is not without its limitations. Investigators should continue to work towards developing stable cell lines that maintain a differentiated hepatocyte phenotype.*» (Kneteman and Mercer, 2005).

The development of new cell lines -a fully polarized human line would be necessary- can lead to useful tools in hepatocyte polarity and functionality studies, as shown with the models described in this review.

Online supplementary material

Table 1: MEMBRANE PROTEIN MARKERS OF POLARITY IN RAT HEPATOCYTE

Table 2: COMPARISON OF HEPATIC CELL LINES

Acknowledgments We greatly thank B.R. Stevenson and D. Haouzi for kindly providing antibodies, J.G. Marin for cholyglycyamidofluorescein and K. Schmidt and J.F. Dufour for unpublished results on IP₃R mRNA levels. We also thank V. Nicolas (IFR141-ITFM, Chatenay Malabry, France) for help with confocal analysis and L. Mery for critical reading of the manuscript. This work has been funded by institutional grants from INSERM and supported by a grant from Egide (PAI Picasso) and the Acción Integrada Hispano-Francesa (HF2003-0089).

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LEGENDS

Figure 1 □ Diagrams of a simple epithelial cell and of an hepatocyte.

The apical pole  is separated from the lateral pole by tight junctions .

The Golgi apparatus  is always facing the apical pole.

Microtubules are oriented with minus-end at the apical pole .

BC □ bile canaliculus.

Figure 2 □ Vectorial transport of cholyglycylamidofluorescein (a fluorescent bile acid analogue) by HepG2 and WIF-B9 cells.

The transport of cholyglycylamidofluorescein was performed according to Bravo et al., 1998. A field of 18 cells is shown for each line. Cells accumulate in their BC the fluorescent bile acid analogue added to the medium (3 out of 4 BC for HepG2 and 7 out of 10 for WIF-B9 cells). Microscopic images in phase contrast (left) and in fluorescence (right). Arrows point at BC. *Scale bar* 10 μ m.

Figure 3 □ Immunolocalization of α -tubulin with radixin, of α -tubulin with radixin and of Mdr with ZO-1 in Can 10 cells.

Radixin (anti-radixin polyclonal antibodies, D. Haouzi) is expressed at the BC level where the cytoplasmic α -tubulin (anti- α -tubulin Mab, Sigma, USA) network is reinforced (a). The punctuated staining of α -tubulin (anti- α -tubulin Mab, Sigma, USA) underlines the BC stained by radixin (b). Mdr (anti-Mdr1/2 Mab C219, Zymed, USA) transporters are present at the BC level encircled by the tight junction protein ZO-1 (anti-ZO-1 Mab, B. Stevenson) (c). Can 10 BC structures are composed of either long branched BC implicating dozen of cells or of simple BC implicating two-three cells. Confocal microscopy □ compilations of xy sections taken at 0.2 □ 0.3 μ m steps. *Scale bar* 10 μ m.

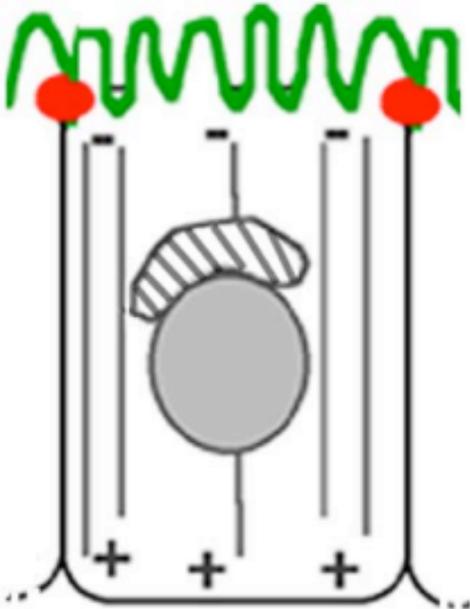
Figure 4 : Immunolocalization of IP3Rs in WIF-B9 and Can 10 cells.

In WIF-B9 cells (a-d''), IP3R1 (b) is present in whole cytoplasm whereas IP3R2 (b') and IP3R3 (b'') are enriched at the BC level (short arrows) marked by the tight junction ZO-1 protein (c, c', c''). In Can 10 cells (e-k), IP3R3 (f, g) is enriched at the BC level where actin (stained by fluorescent phalloidin) (h, i) also localizes. (Anti-IP₃R1 and IP₃R2 polyclonal antibodies, ABR and CovaLab, France, anti-IP₃R3 Mab, BD Biosciences, France). Corresponding images in Nomarski contrast (a, a', a'' and e) and merged images (d, d', d'', j and k). Note the long branched BC formed by union of the apical poles of six Can 10 cells compared to the round BC formed by union of the

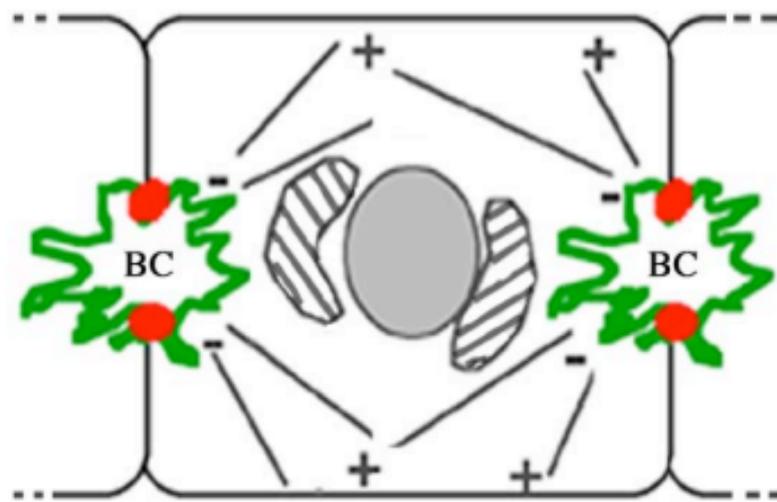
apical poles of two WIF-B9 cells. Confocal microscopy compilations of 3-8 xy sections taken at 0.4 μ m steps and, for Can 10, xz sections (g, i, k) taken as indicated (long arrows) on f, h, j images. *Scale bar* 10 μ m.

Figure 5 possible cover figure

Abbreviations used : ER, endoplasmic reticulum; BC, bile canaliculus; IP₃R, inositol 1,4,5-trisphosphate receptor; Bsep/BSEP, bile salt export pump; Ntcp/NTCP, Na⁺/taurocholate cotransporting polypeptide; *Abc/ABC*, ATP-binding cassette; Mdr/MDR, multidrug resistance protein; Mrp/MRP, multidrug resistance-associated protein; ZO, Zonula Occludens; MF, microfilaments; MT, microtubules; IF, intermediate filaments; Ca²⁺, calcium; MDCK, Madin-Darby canine kidney; DPP IV, dipeptidyl peptidase IV.



simple polarity



hepatocyte polarity

Figure 1

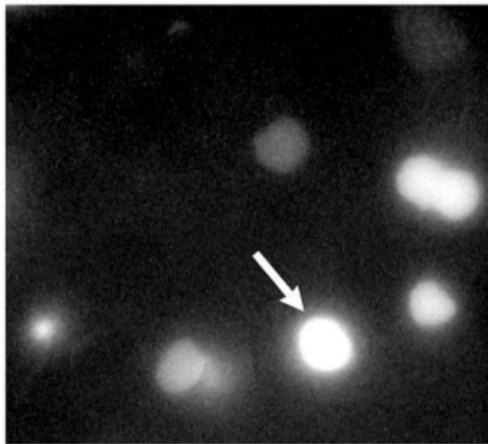
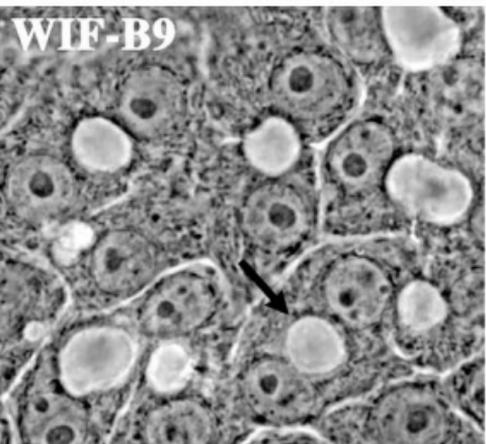
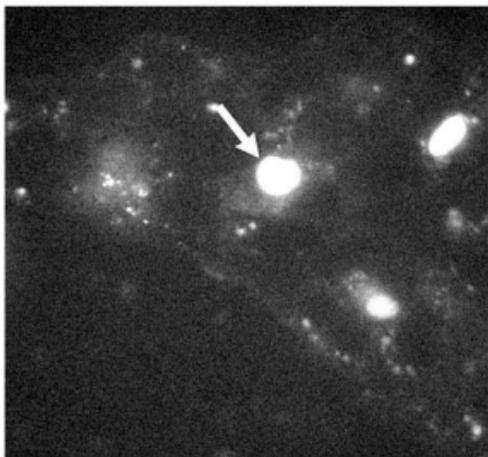
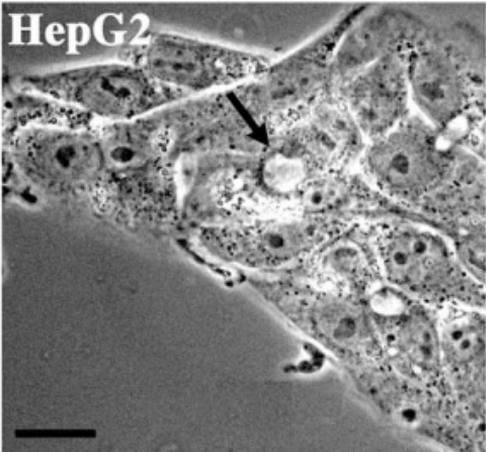
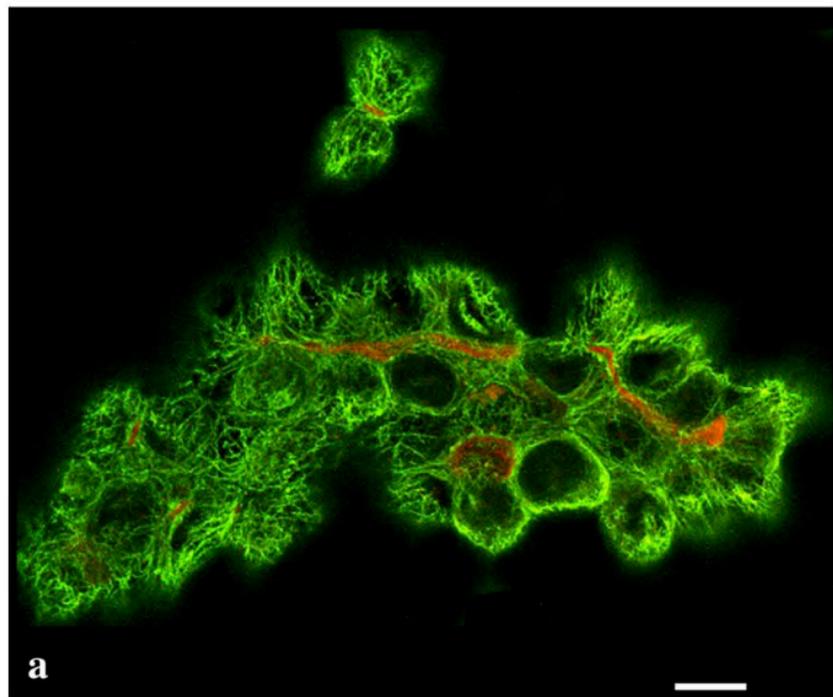
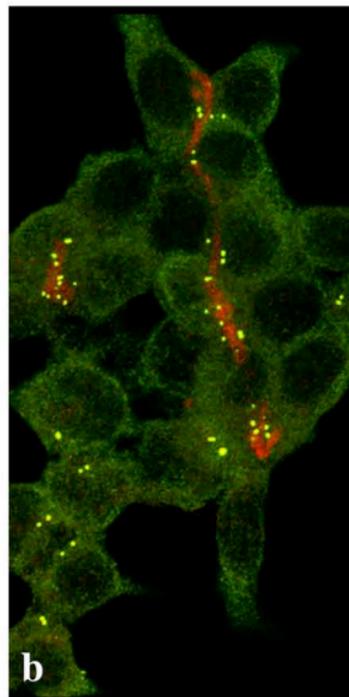


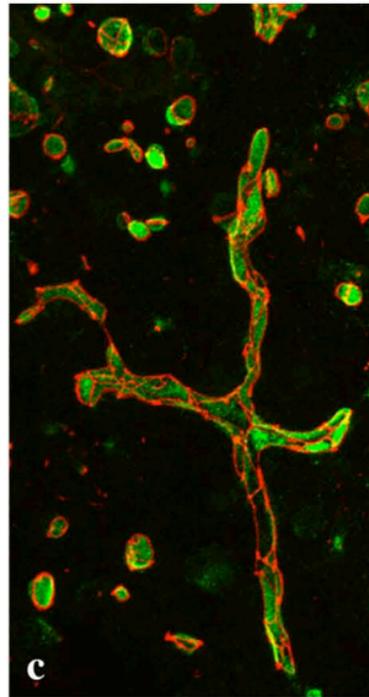
Figure 2



α -tubulin + radixin



γ -tubulin + radixin



Mdr + ZO-1

Figure 3

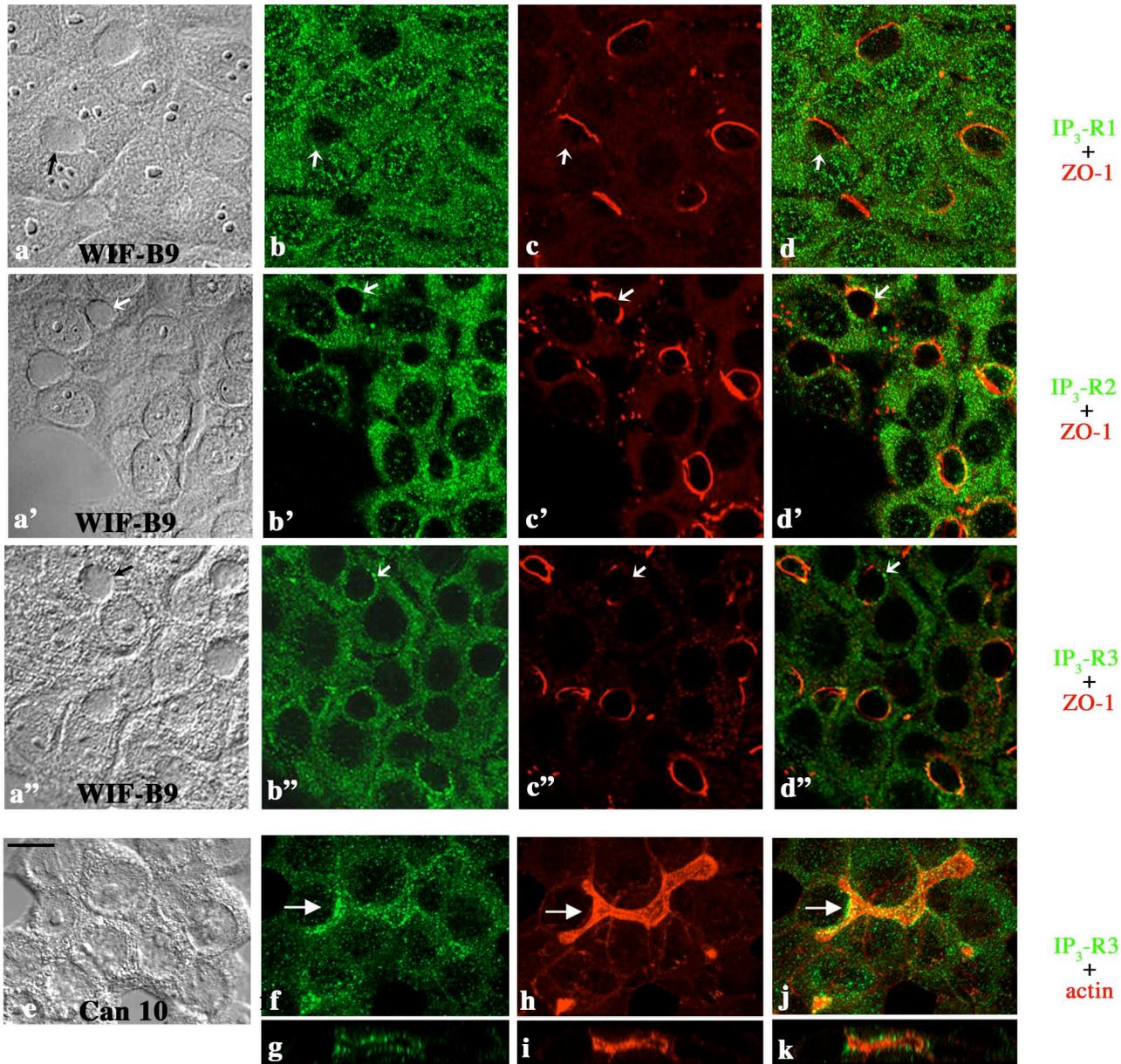


Figure 4

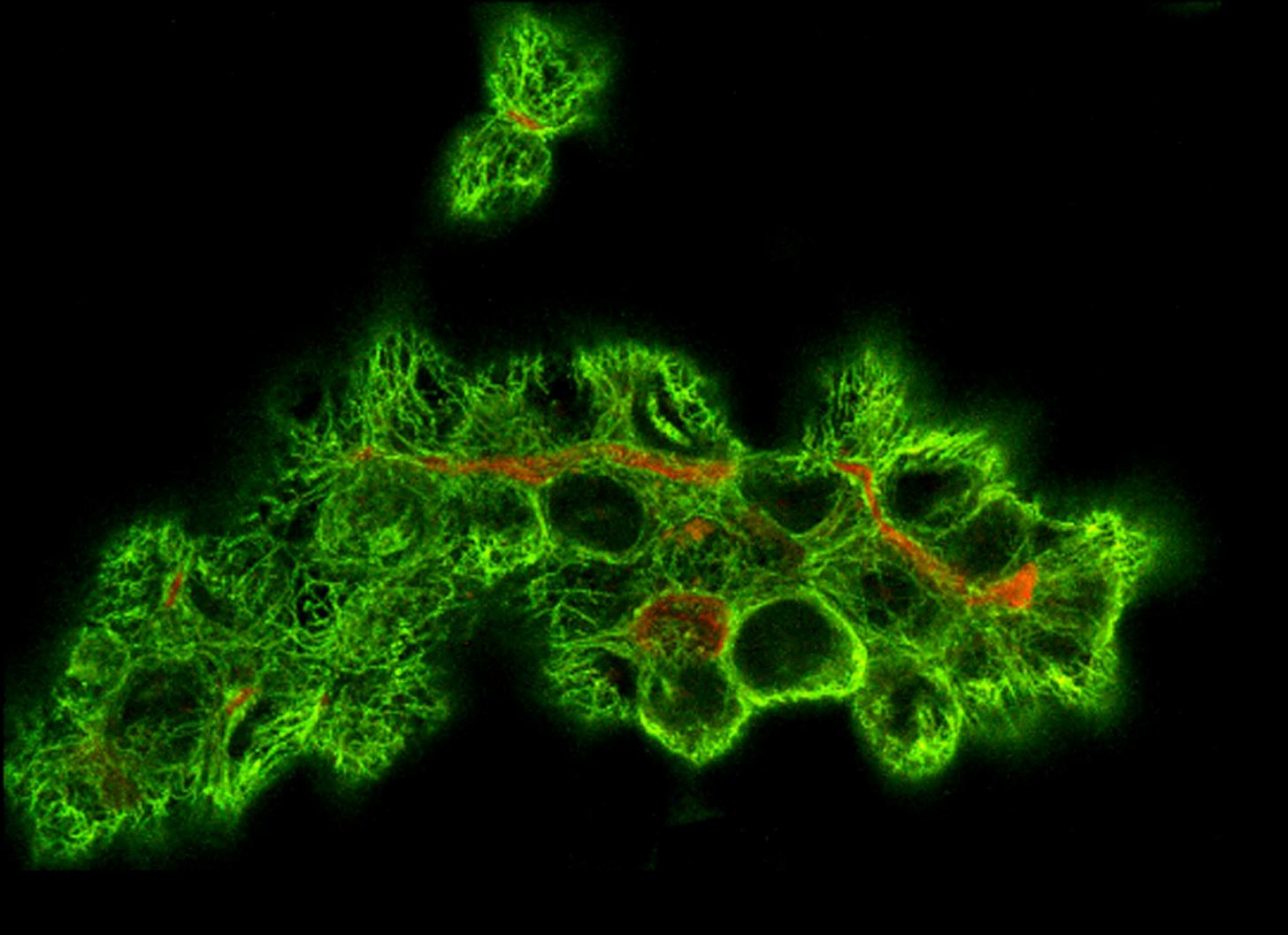


Figure 5
Bile canalicular structures stained by radixin (red) and highlighted by α -tubulin (green)
in Can 10 hepatic cell line.