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The ectopic F_OF_1 ATP synthase of rat liver is modulated in acute cholestasis by the inhibitor protein IF_1

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Abstract Rat liver plasma membranes contain F_OF_1 complexes (ecto-F_OF_1) displaying a similar molecular weight to the mitochondrial F_OF_1 ATP synthase, as evidenced by Blue Native PAGE. Their ATPase activity was stably reduced in short-term extra-hepatic cholestasis. Immunoblotting and immunoprecipitation analyses demonstrated that the reduction in activity was not due to a decreased expression of ecto-F_OF_1 complexes, but to an increased level of an inhibitory protein, ecto-IF_1, bound to ecto-F_OF_1. Since cholestasis down regulates the hepatic uptake of HDL-cholesterol, and ecto-F_OF_1 has been shown to mediate SR-BI-independent hepatic uptake of HDL-cholesterol, these findings provide support to the hypothesis that ecto-F_OF_1 contributes to the fine control of reverse cholesterol transport, in parallel with SR-BI. No activity change of the mitochondrial F_OF_1 ATP synthase (m-F_OF_1), or any variation of its association with m-IF_1 was observed in cholestasis, indicating that ecto-IF_1 expression level is modulated independently from that of ecto-F_OF_1, m-IF_1 and m-F_OF_1.

Keywords Short-term cholestasis · Rat liver · Ectopic F_OF_1 ATP synthase · Inhibitor protein IF1

Abbreviations

BN-PAGE	Blue Native PAGE
ecto-F_OF_1	ectopic F_OF_1ATP synthase complex
ecto-IF_1	ectopic inhibitor protein
HDL	high-density lipoproteins
HepG2	human hepatocarcinoma cells
HUVEC	human umbilical vein endothelial cells
m-F_OF_1	mitochondrial F_OF_1ATP synthase complex
m-IF_1	mitochondrial inhibitor protein
PM	plasma membranes
SR-BI	scavenger receptor B type 1

Introduction

High-density lipoproteins (HDL) play a major role in cholesterol homeostasis, by transferring cholesterol from peripheral tissues to the liver for elimination into the bile. The hepatic uptake of HDL-bound cholesterol occurs at the level of scavenger receptor-BI (SR-BI), resulting in the release of cholesterol-poor HDL particles into the circulation (Singh et al. 2007). Though SR-BI is essential in reverse cholesterol transport to the liver (Rigotti et al. 2003), evidence suggests that it might not be the only such player. An alternative pathway of HDL-cholesterol uptake into the liver appears to exist, involving HDL binding to a high-affinity receptor and their subsequent internalization into clathrin-coated vesicles (Martinez et al. 2003).

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In a pioneering study using HepG2 cells, a human hepatocarcinoma cell line, the high-affinity HDL receptor was unexpectedly found to be the catalytic β chain of the ectopic ATP synthase complex (ecto-F_OF_1) (Martinez et al. 2003). This complex contains at least some subunits of both F_1 and F_O, the catalytic and membrane sectors, respectively, of mitochondrial ATP synthase (EC 3.6.3.14) (m-F_OF_1) and is localized on cell surface with the F_1 moiety facing outside. Binding of HDL to the β chain of ecto-ATPsynthase (ecto-F_OF_1) occurs via apo-AI (Martinez et al. 2003), the constitutive apoprotein of HDL, and is believed to trigger extracellular ATP hydrolysis and, consequently, ADP-dependent activation of P2Y13, a G protein-coupled purinergic receptor participating in HDL endocytosis (Jacquet et al. 2005). In accordance with this model, more recently it was found that treatment of HepG2 cells with niacin, which is effective in raising plasma HDL level, reduces the surface expression of ecto-F_OF_1 β chain and decreases HDL uptake (Zhang et al. 2008). Other work has also implicated ecto-F_OF_1 in the regulation of lipid metabolism in various cell lines (Arakaki et al. 2007; Lyly et al. 2008; Wang et al. 2006).

Indications are that the ATP handling functions of the ecto-F_OF_1 may parallel those of the mitochondrial enzyme (m-F_OF_1). Ecto-F_OF_1 contains many, if not all, of the multiple subunits of F_1 and F_O, and ecto-F_OF_1 appears to catalyze the synthesis (Burrell et al. 2005; Kim et al. 2004; Mangiullo et al. 2008; Moser et al. 2001) and/or hydrolysis (Martinez et al. 2003) of extracellular ATP depending on cell type. Both these activities are sensitive to inhibitors of m-F_OF_1. Furthermore, IF_1, the endogenous mitochondrial inhibitor protein (Green and Grover 2000), which in mammals plays a prominent role in the regulation of m-F_OF_1 both in vitro and in vivo (Di Pancrazio et al. 2004), has also been detected on the external surface of the plasma membrane in various cell types. Ecto-IF_1 has been identified in human umbilical vein endothelial cells (HUVEC) (Burwick et al. 2005) and human hepatocarcinoma cells (HepG2) (Contessi et al. 2007), where it associates with ecto-F_OF_1. It seems likely that IF_1 might be a regulatory element for the ecto-F_OF_1 as it is for m-F_OF_1.

In the present work, we extended our study of ecto-F_OF_1 and its functional association with ecto-IF_1 into plasma membrane preparations from rat liver rather than in transformed cultured cells. To investigate possible roles in lipoprotein metabolism, data were collected from both sham-operated rats (serving as controls) and acute cholestatic rats (which had undergone ligation of the common bile duct). Obstructive cholestasis is a disease that highlights the role of the liver in HDL-cholesterol homeostasis. It gives rise to a dramatic alteration in plasma lipid profile (Bravo et al. 2007), accompanied by down-regulation of SR-BI receptor (Bravo et al. 2007), of apo-AI (Claudel et al. 2002), and of ABCA1 (Bravo et al. 2007), the ATP-binding cassette transporter promoting active cholesterol efflux at the sinusoidal domain of hepatocyte plasma membrane. Short-term bile duct ligation was, therefore, presumed to serve as a useful model to investigate if ecto-F_OF_1 plays any role in reverse HDL-cholesterol transport.

Our data demonstrate the presence of ecto-F_OF_1 and ecto-IF_1 on the plasma membrane of liver tissue, and show that, while the levels of ecto-F_OF_1 do not change during short term cholestasis, its activity is modulated by variations in the levels of ecto-IF_1.

Materials and methods

Animals, bile duct ligation and isolation of sub-cellular fractions from rat livers

Male Wistar rats (300–350 g) were used for this study. They were fed standard laboratory chow (Harlan Teklad 2018) and tap water ad libitum; they were housed in temperature controlled rooms at 22–24 °C and 50–60% humidity. All animals were maintained and handled at the Animal House of the University of Trieste according to the provisions of the European Community Council Directive (n.86/609/CEE) and of the Italian legislation (D.L.vo. 116/92). The experimental protocol was based on previous established work (Brandoni et al. 2006) and was approved by the University of Trieste Committee for animal studies. Briefly, rats were anesthetized by intra-peritoneal injection of 1 ml/100 g body mass of 2.5% tribromoethanol (mass:vol, dissolved in physiological solution) and of 0.1 ml/100 g body mass of xylazine 2% (mass:vol, dissolved in physiological solution). Then, they underwent laparotomy, resection of the common bile duct between two tight ligatures and suture of the abdominal wall. A group of sham-operated rats, serving as controls, underwent the same treatment, except for ligation and resection of the common bile duct. In the post-operative period both BDL and sham-operated rats received a sub-cutaneous injection of the analgesic and anti-inflammatory drug carprofen (5 mg/kg) and had free access to food and water. Animals were sacrificed 21 h later by decapitation and mitochondria and plasma membrane fractions were obtained by liver sub-cellular fractionation using discontinuous sucrose gradient essentially as described by van Amelsvoort (van Amelsvoort et al. 1978).

IF_1 and F_1 purification

The soluble isolated F_1 domain (F_1) was prepared from beef heart mitochondria according to (Horstman and Racker 1970) and further purified as previously described (Contessi

et al. 2005). The inhibitor protein IF_1 from rat liver was prepared as previously described (Gomez-Fernandez and Harris 1978).

Detergent extraction, immuno-precipitation, gel electrophoresis and immunoblotting

Liver mitochondria (400 µg protein) from cholestatic or non-cholestatic rats were suspended in 40 µl buffer containing 50 mM BisTris, 0.75 M aminocaproic acid, pH7.0 and Triton X-100 added to a final concentration of 2%. The suspension was mixed and incubated for 20 min at 4°C or at 37°C and then centrifuged at 100.000 *g* for 30 min at 4°C, to obtain the supernatant Triton extract. Plasma membranes were treated in the same way, except that Triton incubation was carried out only at 37°C (Kim et al. 2004). Triton extracts were further analysed.

For Blue Native PAGE (BN-PAGE) analyses, 14 µl of the Triton extracts were supplemented with 1 µl Coomassie blue G-250 (Serva) (5% *w*/*v* in 0.75 M aminocaproic acid) and loaded onto a 4–11% polyacrylamide gradient gel (Bisetto et al. 2008). After electrophoresis, gels were either stained for in-gel ATPase activity (Bisetto et al. 2007) or transferred to nitrocellulose membranes for immunoblotting.

For immunoprecipitation, Triton extracts (48 µl, ca. 100 µg protein) were treated with anti-F_OF_1 mAb (MS501 Immunocapture Kit by Mitosciences) in a ratio of 20 µl antibody-conjugated beads per mg of membrane protein, according to the manufacturer's instructions.

For electrophoresis of the dissociated proteins, F_1 and IF1, mitochondria, plasma membranes, and immunoprecipitated fractions were suspended in Laemmli buffer and loaded in 15% SDS-PAGE, followed by Western blotting on nitrocellulose membranes. The membranes were probed with polyclonal anti F_1 α/β subunits antibodies produced in rabbit against the purified bovine heart F_1 (Bisetto et al. 2007; Bisetto et al. 2008) (1:3,000 dilution), monoclonal antibodies against the C-terminal domain of IF1 (since this region is less susceptible to cleavage (Walker et al. 1991)) from Molecular Probes (1:2,000 dilution), monoclonal anti Na^+/K^+ ATPase, monoclonal anti AIF or polyclonal anti SR-BI antibodies from Abcam (1:2,000 dilution) in phosphate-buffered saline (PBS) containing 3% non-fat dry milk. Immunoreactive bands were detected by enhanced chemiluminescence (Pierce) and quantified by densitometry using ImageQuant software, version 2003.03 (Amersham).

ATP hydrolysis assay

Rat liver mitochondria and plasma membranes were treated with 2% (*v*/*v*) Triton X-100 in 50 mM BisTris, 0.75 M aminocaproic acid, and pH7.0 as described above in "Detergent extraction, immuno-precipitation, gel electrophoresis and immunoblotting". Aliquots were withdrawn to determine the maximal ATP hydrolysis rate using an NADH linked ATP-regenerating system (Das et al. 1994) with or without 10 µM aurovertin B, a m-F_OF_1 inhibitor which binds to F_1-β subunits (van Raaij et al. 1996).

Protein quantification

Protein concentration was determined by the method of Lowry (Lowry et al. 1951) for particulate fractions and by method of Bradford for soluble proteins (Bradford 1976).

Results

Ecto-F_OF_1 in rat liver

Although the presence of individual subunits of the mitochondrial ATP synthase at the plasma membrane is well established, their assembly into the whole ecto-F_OF_1 is less certain. In addition, the majority of previous data arises from cultured cells (Bae et al. 2004; Martinez et al. 2003), often transformed cell lines, which may not be representative of normal tissues.

To demonstrate the existence of holo ecto-F_OF_1 in normal tissues, plasma membranes were purified from Wistar rat liver and treated with the mild detergent Triton X-100, which preserves protein associations (Wittig and Schagger 2008). The extraction was performed at 37°C, which favoured the solubilization of the lipid rafts, where ecto-F_OF_1 is thought to be localized (Kim et al. 2004). Mitochondria were similarly extracted with Triton, both at 37°C (as a direct comparison) and at 4°C which is optimal for mitochondrial protein extraction (Bisetto et al. 2007; Bisetto et al. 2008). All extracts were analysed by blue native PAGE (BN-PAGE), which separates the membrane complexes in their native form according to their molecular masses (Wittig and Schagger 2008), and the gel stained for in-gel ATPase activity (Bisetto et al. 2007) to identify the ATP synthase and/or its subcomplexes. Mitochondria extracted at 4°C produced a typical BN-PAGE profile (Bisetto et al. 2008), showing the F_1 complex and the monomeric and dimeric forms of m-F_OF_1 (Fig. 1, lane 2). Figure 1 (lane 3) shows that plasma membrane extract contained two faintly staining bands with ATPase activity, with molecular weights very similar to those of the monomeric m-F_OF_1 and m-F_1. Immunoblotting confirmed that both bands contained F_1 α/β subunits (lane 4). This indicates that the two complexes in plasma membrane extracts can be assigned as ecto-F_OF_1 and ecto-F_1 respectively, and suggests that the plasma membranes of normal liver do contain a complete, functional F_OF_1 ATP synthase complex.

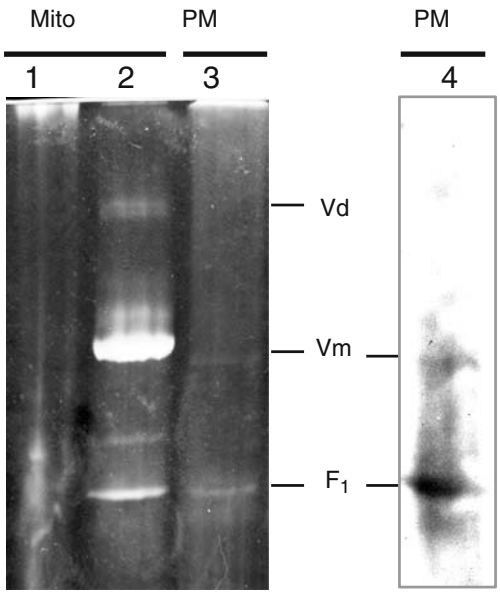

Fig. 1 ecto-F_OF_1 extraction from plasma membranes. Rat liver plasma membrane (PM) and mitochondria (M) (400µg membrane protein) were extracted with Triton X-100 (2%) at 37°C (Lanes 1 & 3) or at 4°C (Lane 2), as described in Methods. All samples were loaded on BN-PAGE, which was stained in-gel for ATPase activity (**a**) or blotted for immunodetection using F_1 α/β antibodies (**b**). Vd: ATP synthase (complex V) dimeric form; Vm: ATP synthase (complex V) monomeric form; F_1: extramembrane region of the ATP synthase. (The panel shows representative results from three independent experiments.)

Figure 1 suggests that the levels of ecto-F_OF_1 were much lower than m-F_OF_1, and the possibility that these observations reflect mitochondrial contamination must be considered. Two lines of evidence suggest that the possibility of such contamination can be discounted. Mitochondrial contamination of plasma membrane preparations was shown to be less than 1% (*w*/*w*) by immunodetection of AIF (Fig. 2). Furthermore, in line with earlier studies, we confirm that mitochondrial m-F_OF_1 and m-F_1 were not stable to Triton extraction at 37°C (Fig. 1, Lane 1), and thus it is unlikely that the two complexes extracted from plasma membranes at 37°C were of mitochondrial origin.

F_1 in mitochondria is associated with a small regulatory protein, IF_1, which inhibits its ATPase activity. The same seems to be true for ecto-F_OF_1 (Burwick et al. 2005; Contessi et al. 2007). The levels of these proteins within plasma membranes were estimated using densitometry on Western blots of denaturing gels. The system was calibrated with purified IF_1 from rat liver mitochondria and purified F_1 from beef heart mitochondria, whose α/β subunits are highly homologous to those of rat liver (Walker et al. 1985). From the calibration at the foot of Fig. 2, F_1 in rat liver mitochondria was estimated at 0.263±0.035 nmol/mg membrane protein, which is a value comparable to that found in beef heart mitochondria (Di Pancrazio et al. 2004) while the total (bound + free) m-IF_1 was 0.305±0.042 nmol/mg protein, comparable to that reported for liver mitochondria (Bravo et al. 2004) (Fig. 2, left hand pane).

By comparing the levels of AIF between the left and right hand panels in Fig. 2, we can deduce the F_1 and IF_1 quantities in plasma membranes which can arise from mitochondrial contamination (and which are less than 20% of the total F_1 and IF_1 found in plasma membranes). Thus, after subtracting these values, we arrive at levels of ecto-F_1 and ecto-IF_1 of 0.016±0.003 and 0.012±0.004 nmol/mg membrane protein, respectively, i.e. about 5% of the m-F_1 and m-IF_1 levels observed in mitochondrial membranes.

Cholestasis is characterized by high levels of cholesterol in the liver, and variations in HDL levels in the plasma (Bravo et al. 2007). If ecto-F_OF_1 were indeed involved in hepatic uptake of HDL, as suggested in (Martinez et al. 2003), cholestasis might be expected to affect its levels in the liver. We therefore investigated the levels of ecto-F_OF_1 and ecto-IF_1 in animals where the bile duct had been ligated, in parallel with measurements of the levels of the scavenger receptor BI (SR-BI).

Plasma membranes were purified from livers of six bile-duct-ligated rats and six sham-operated rats and their content of IF_1, F_1 α/β subunits and OSCP (another F_OF_1 component) measured by immuno-detection. Na^+/K^+ ATPase, a plasma membrane marker, was used as internal standard, since it is known to remain stable in cholestasis (Landmann et al. 1998). Mitochondria contamination was determined by AIF immuno-detection as above. In accordance with

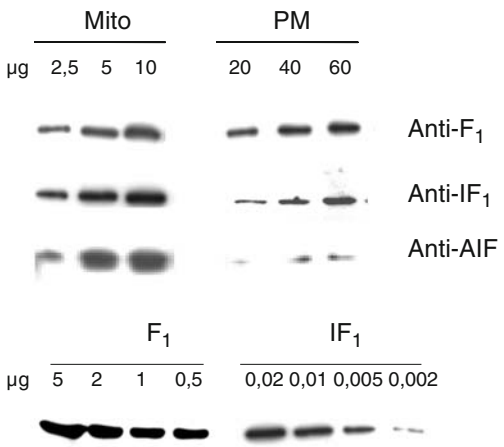

Fig. 2 Immunodetection of F_1, IF_1 and AIF in mitochondria and plasma membranes. Rat liver mitochondria (Mito) and plasma membranes (PM) (quantities as indicated) were subjected to 15% SDS-PAGE followed by immunoblotting using Ab against F_1 α/β subunits, or IF_1 subunits or AIF. The indicated quantities of rat liver purified IF_1 and bovine heart purified F_1 were used for quantitation (foot of figure). Mitochondrial contamination of plasma membranes, evaluated by AIF immuno-detection, was less than 1% (*w*/*w*) in accordance with (Baldini et al. 1986). The panel is representative of five independent experiments

previous work (Bravo et al. 2007), SR-BI decreased significantly relative to the Na^+/K^+ ATPase, falling to 44%±9% of its normal level in the 21 h after bile-duct-ligation. However, the ecto-F_OF_1 level (and the OSCP level as a parallel marker) remained constant. We conclude, therefore, that ecto-F_OF_1 does not change significantly during cholestasis.

However, Fig. 3 shows that, while not affecting ecto-F_OF_1 levels, cholestasis has a clear effect on the ecto-IF_1 level, whose amount associated with the plasma membrane increased greatly. Although some variations through individual samples was evidenced (Fig. 3a, right hand pane), the mean ectopic IF_1/F_1 ratio, determined by densitometry, increased by 90%±26% in acute cholestasis (Fig. 3b, right hand panel), suggesting that the plasma membrane F_OF_1 is associated with a higher level of IF_1 after bile duct ligation. In contrast, the IF_1/F_1 ratio in mitochondria was unchanged between cholestatic and control rats (Fig. 3a and b, left hand panes).

The experiment described in Fig. 3 measures total ecto-IF_1, irrespective of whether or not it is associated with F_1. To establish the state of association between ecto-F_OF_1 and ecto-IF_1, the Triton extract of the plasma membranes was treated with an antibody against F_OF_1 to precipitate the F_OF_1 complex and associated proteins. Figure 4a shows that such a procedure also precipitates ecto-IF1, showing that this protein is indeed associated with F_OF_1 in these membranes. Cholestasis leads to an increase in the ecto-

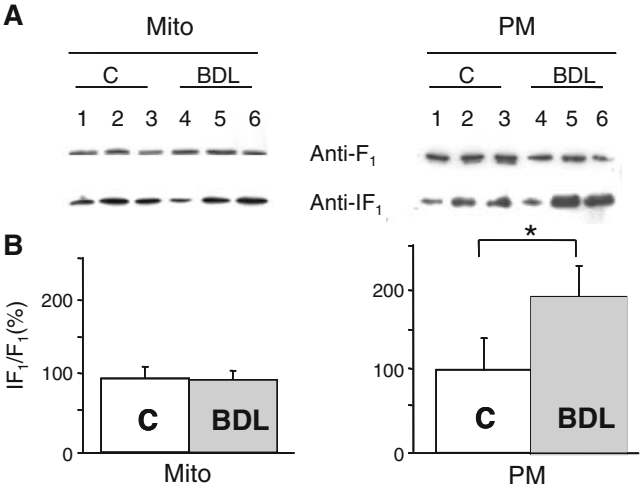

Fig. 3 Effect of cholestasis on mitochondrial and plasma membrane IF_1 content. Mitochondria and plasma membranes (ca 40 µg protein) were subjected to SDS-PAGE and immunoblotting essentially as described in Fig. 2. **a** Representative panel of immunoblotting against α/β and IF_1 subunits of F_OF_1 complex obtained from liver mitochondria (Mito) or plasma membranes (PM) from sham-operated (C) and cholestatic (BDL) rats. **b** The ratio of peak area of IF_1 to that of α/β subunits of Mito or PM from C and BDL rats were measured. The mean value found in C rats in each case was normalized to 100%. Error bars represent SEM (n=6) *P<0.05 vs. control

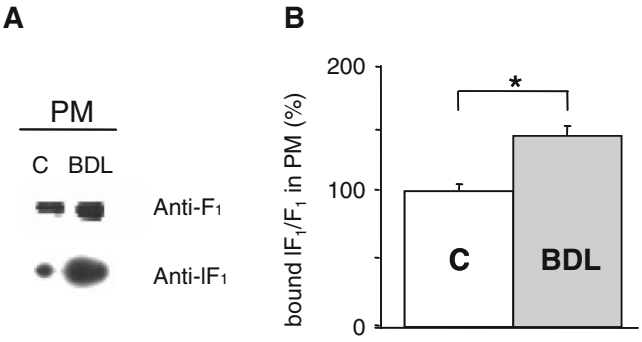

Fig. 4 Immunoprecipitation of the ecto-F_OF_1/IF_1 complex from non-cholestatic and cholestatic rats. **a** Plasma membranes (PM) (400 µg membrane protein) from sham-operated (C) and cholestatic (BDL) rat livers were solubilised using Triton X-100 (2% *v*/*v*) as described in Methods and immunoprecipitated using Immunocapture kit MS501 (MitoScience) as specified in Methods. The pellet was analysed by Western Blotting after SDS-PAGE. No signal was detected when control IgG affinity-purified from pre-immune rabbit or mouse serum was used. **b** Mean ratio between the peak area of IF_1 and that of α/β subunits for the plasma membrane preparations. The ratio found in sham-operated (C) rats was normalized as 100%. Error bars represent SEM (n=3). *P<0.05 vs. control. No difference in the ratio was observed in the mitochondrial samples (data not shown)

IF_1/F_1 ratio of 45%±6% (Fig. 4b). No change in ratio was seen in the immunoprecipitate of m-F_OF_1 (data not shown). We conclude that at least half of the additional ecto-IF_1 associated with plasma membranes in cholestasis is complexed with the ecto-F_OF_1 complex, although part of this additional ecto-IF_1 remains uncomplexed.

IF_1 is a naturally occurring inhibitory protein of the F_OF_1 ATP synthase. It would therefore be expected that higher levels of IF_1 would be associated with lower ATPase activity of ecto-F_OF_1. Consistent with the results of Fig. 4, Table 1 shows that the aurovertin B-sensitive ATPase activity of ecto-F_OF_1 was markedly inhibited in cholestatic rats, to about 45% of that from control rats (n=6). Mitochondrial F_OF_1 showed no such inhibition, indicating

Table 1 Cholestasis effect on ATPase activity of mitochondria and plasma membranes

Sample	C (%)	BDL (%)
Mito	100±14	98±10
PM	100±18	43±8**

ATP hydrolysis rate was determined in Triton extracts of liver mitochondria or plasma membranes from six sham-operated (C) and six cholestatic rats (BDL) in the absence and presence of 10 µM Aurovertin B as described in Methods. Mitochondria (Mito) and plasma membrane (PM) extracts exhibited 85%±5% and 50%±5% aurovertin B sensitivity, respectively. 100% was assigned to the mean value of the Aurovertin B-sensitive ATP hydrolysis rate found in C rats for each membrane preparation. Values given as mean ± SEM (n=6)

**P<0.001vs. control

the existence of a selective modulation of the ectopic complex by ecto-IF_1 upon bile-duct ligation.

Discussion

This study demonstrates for the first time the presence of the whole functionally active F_OF_1 complex in plasma membranes isolated from rat liver, showing that its presence is not restricted to transformed cells (Martinez et al. 2003). In acute cholestasis, ecto-F_OF_1 levels remain unchanged, while ecto-IF_1 is up-regulated with a consequent inhibition of the ATPase activity of ecto-F_OF_1. This increase in the ecto-IF_1 level contrasts with the decreased expression of the SR-BI receptor, although in both cases the net effect would be a decreased activity of the putative HDL receptors in short term cholestasis.

The presence of ecto-F_OF_1 in intact liver was previously indicated by the finding of 9 out of the 16 subunits of the mitochondrial F_OF_1 complex (namely subunits b,d,e,F6 and OSCP of F_O and subunits α,β,γ and δ of F_1) (Bae et al. 2004) in the proteome of lipid rafts from rat liver plasma membranes. However the remaining seven subunits remained undetected, raising the possibility that the ecto-F_OF_1 may not be assembled in a functional complex. Using BN-PAGE and activity staining to reveal functional complexes, the present study demonstrates ectopic and mitochondrial complexes have very similar molecular weights, and aurovertin-sensitive ATPase activities, arguing in favour of a very similar subunit composition and assembly. The mitochondrial complex appears in our experiments to be more sensitive to the extraction temperature than the ecto-F_OF_1, disintegrating at an extraction temperature of 37 °C; this may be related to the peculiar composition of the lipid rafts, which concentrate and incorporate many different proteins (Bae et al. 2004) but it is convenient experimental feature of our system since it makes mitochondrial contamination of ecto-F_OF_1 preparations unlikely. The presence of the holo ecto-F_OF_1 in liver plasma membranes is also supported by recent work on isolated hepatocytes (Mangiullo et al. 2008), although in this case, relative quantities were not measured.

The distribution of both IF_1 and F_OF_1 between mitochondria and plasma membranes measured here is significantly different from that found in the tumour line HepG2. In normal liver, as reported here, the membrane content of ecto-F_OF_1 (0.016±0.003 nmol/mg membrane protein) is only 5% of the level found in mitochondria, much lower than the value of around 20% reported for HepG2 cells (Contessi et al. 2007). Hence the impairment of mitochondrial structure and function which accompanies the neoplastic transformation in liver (Cuezva et al. 2002) appears to be associated with the appearance of increased ecto-F_OF_1 at the plasma membrane, and this might be viewed as a feature of cellular transformation.

The IF_1/F_1 ratio found in liver mitochondria and plasma membranes of control rats was comparable (roughly a 1:1 ratio in untreated animals), so suggesting similar binding properties. However, ecto-F_OF_1 appears not to be saturated with its IF_1 in control conditions, for, as ecto-IF_1 levels rise in cholestasis, the ATPase activity of ecto-F_OF_1 decreases (Table 1). This is consistent with previous observations that perfusion with exogenous IF_1 was able to reduce HDL uptake by rat liver (Martinez et al. 2003).

The data reported here indicate that ecto-IF_1 modulation during cholestasis is independent of that of ecto-F_OF_1. To our knowledge, this is the first demonstration of in vivo modulation of functioning ecto-IF_1. A previous study demonstrated a selective increase in ecto-IF_1 expression in HUVEC upon TNF-α stimulation (Cortes-Hernandez et al. 2005), but no activity measurements were carried out. Conversely, cell surface expression of the F_1 β subunit appears to be regulated by other conditions, such as the deficiency of palmitoyl protein thioesterase in primary mouse neurons (Lyly et al. 2008), the exposure to exogenous cholesterol in HUVEC (Martinez et al. 2003) or to niacin in HepG2 (Zhang et al. 2008). All these studies (Cortes-Hernandez et al. 2005; Lyly et al. 2008; Zhang et al. 2008), including our own, support the existence of specific multi-step pathways for the delivery of mitochondrial proteins to the cell surface. Such export mechanisms had been hypothesized long ago (Soltys and Gupta 1999), but, although extremely interesting, they have not yet been defined (Lebiedzinska et al. 2009).

Other workers have shown that, at the mitochondrial level too, m-IF_1 expression is modulated independently from that of m-F_OF_1. This is indicated by the fact that the ratio between m-IF_1/m-F_OF_1 varies between tissues (Bravo et al. 2004; Campanella et al. 2008; Di Pancrazio et al. 2004). In addition, recent studies show that m-IF_1 is selectively down-regulated in liver of late septic rats (Huang et al. 2007) and may be post-translationally regulated by the Immediate Early response gene X-1 (IEX-1) in different cell lines (Shen et al. 2009). The mechanism for independently controlling these two entities is unknown, but it presumably reflects some role of IF_1 in regulating the energy metabolism of cells under different conditions (Campanella et al. 2008).

In conclusion, this study demonstrates the importance of ecto-F_OF_1 (and its inhibitory protein) in normal tissue, and shows a parallel down-modulation of SR-BI and ecto-F_OF_1 activities in intact rat liver during cholestasis, albeit by very different mechanisms. One crucial question concerns the exact localisation of ecto-F_OF_1 in the polarized hepatocytes, where the ATPase would have to be on the basolateral surface to be directly involved in HDL uptake (Martinez et

al. 2003). This has not yet been defined by electron microscopy, as it has proved difficult to immunostain F_OF_1 in fixed tissues, (probably due to the lability of F_OF_1 epitopes during fixation) (Champagne et al. 2006). Nevertheless, a previous study has identified the $F_1\beta$ subunit selectively on the basolateral domain of liver tissue (Goeser et al. 1990), in accordance with this model. Assuming this to be the case, it may well be possible to reduce liver HDL uptake (and hence increase plasma HDL levels), by manipulating ecto-IF1 function. Considering that HDL raising is an important therapeutic target in atherosclerosis treatment (Castelli et al. 1992; Kapur et al. 2008), the data presented in this study may be significant in future development of HDL-based therapies.

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