Review

Michele Bonus, Dieter Häussinger* and Holger Gohlke*

Liver cell hydration and integrin signaling

https://doi.org/10.1515/hsz-2021-0193
Received March 12, 2021; accepted April 12, 2021;
published online April 29, 2021

Abstract: Liver cell hydration (cell volume) is dynamic and can change within minutes under the influence of hormones, nutrients, and oxidative stress. Such volume changes were identified as a novel and important modulator of cell function. It provides an early example for the interaction between a physical parameter (cell volume) on the one hand and metabolism, transport, and gene expression on the other. Such events involve mechanotransduction (osmosensing) which triggers signaling cascades towards liver function (osmosignaling). This article reviews our own work on this topic with emphasis on the role of β_1 integrins as (osmo-)mechanosensors in the liver, but also on their role in bile acid signaling.

Keywords: bile acids; cell swelling; functional selectivity; mechanotransduction; molecular dynamics simulations; tauroursodeoxycholate.

Introduction and general considerations

Liver cell volume can change within minutes under the influence of nutrients, hormones, and toxins due to the creation or dissipation of osmotic gradients and corresponding water fluxes across the plasma membrane (for reviews, see Graf and Häussinger 1996; Häussinger 1996a, b). Although hepatocytes, like virtually every cell type, possess powerful volume-regulatory mechanisms, these mechanisms only prevent excessive volume changes but allow variations of cell volume within narrow limits. Most importantly, such small changes in liver cell volume have been identified as an important and until then not recognized regulator of diverse hepatocyte functions, including metabolism, membrane transport, cell fate, and gene expression (Figure 1; for reviews, see Häussinger and Lang 1991; Häussinger 1996a, b). Many effects of amino acids and hormones can, at least in part, be attributed to cell hydration changes, which correspond on a short-term time scale to cell volume changes.

Indeed, cell volume changes were identified as important mediators of proteolysis control by amino acids and hormones in the liver (Hallbrucker et al. 1991; Häussinger et al. 1991; vom Dahl et al. 1991), and the cellular hydration state, in general, was suggested to be an important determinant of protein catabolism in health and disease (Häussinger et al. 1993).

The effectors depicted in Figure 1 can change within minutes the hepatocyte water content by up to ± 15%. Such volume changes can also be experimentally induced by changing the extracellular osmolarity by ± 80 mosmol/L. Thus, hyper- or hypoosmotic exposure of isolated hepatocytes or perfused rat liver was frequently used to mimic hepatocyte hydration changes and investigate the functional consequences of hepatocyte swelling and shrinkage. Several long-known, but mechanistically unclear effects of amino acids, which could not be related to their metabolism, include the stimulation of glycogen synthesis (Katz et al. 1976; Lavoinne et al. 1987) or inhibition of proteolysis (Mortimore and Pösö 1987). Such effects can quantitatively be mimicked by hypoosmotic hepatocyte swelling to an extent as these amino acids do (Baquet et al. 1990; Hallbrucker et al. 1991; Häussinger et al. 1991). Against this background, it has been suggested that Na^+-dependent amino acid transport systems in the plasma membrane should not merely be viewed as simple amino acid translocators but also as transmembrane signaling systems, which alter cell hydration and, accordingly, cell function in response to amino acid supply (Häussinger 1996b). A cell type- and development-specific expression of concentrative

***Corresponding authors: Dieter Häussinger**, Clinic for Gastroenterology, Hepatology, and Infectious Diseases, Heinrich Heine University Düsseldorf, Moorenstr. 5, D-40225 Düsseldorf, Germany, E-mail: haeussin@uni-duesseldorf.de; and **Holger Gohlke**, Institute for Pharmaceutical and Medicinal Chemistry, Heinrich Heine University Düsseldorf, Universitätsstr. 1, D-40225 Düsseldorf, Germany; and John von Neumann Institute for Computing (NIC), Jülich Supercomputing Centre (JSC), Institute of Biological Information Processing (IBI-7: Structural Biochemistry), and Institute of Bio- and Geosciences (IBG-4: Bioinformatics) Forschungszentrum Jülich GmbH, Wilhelm-Johnen-Str., D-52428 Jülich, Germany, E-mail: gohlke@uni-duesseldorf.de. https://orcid.org/0000-0001-8613-1447
Michele Bonus, Institute for Pharmaceutical and Medicinal Chemistry, Heinrich Heine University Düsseldorf, Universitätsstr. 1, D-40225 Düsseldorf, Germany. https://orcid.org/0000-0003-4411-7342

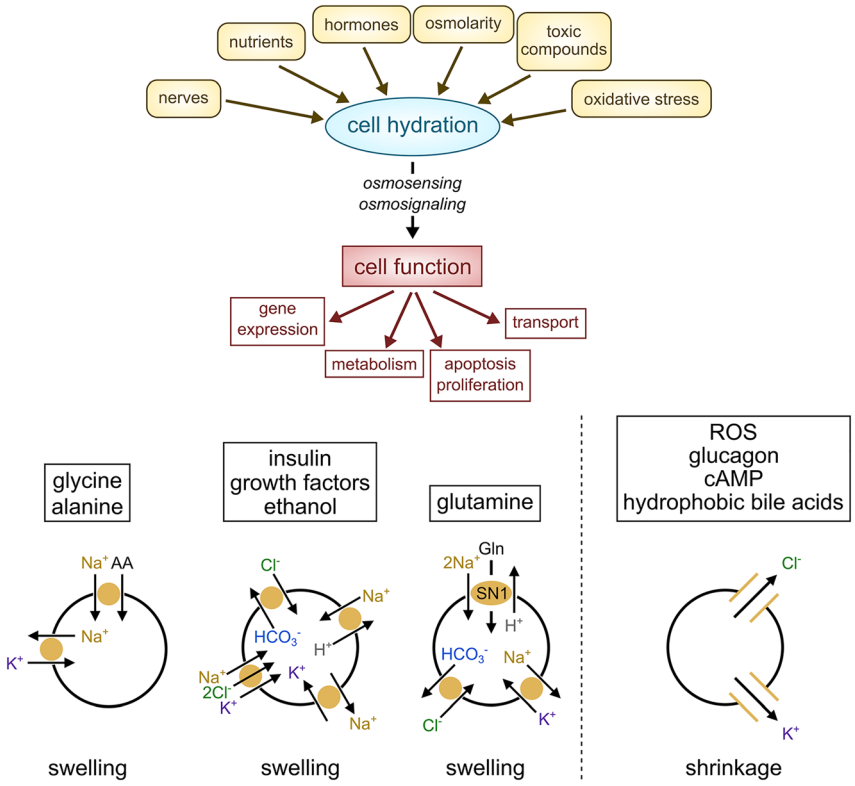

Figure 1: Liver cell volume and hepatocyte function.
Liver cell volume, i.e., hepatocyte hydration, is dynamic and under the influence of hormones, nutrients, nerves, ambient osmolarity either due to cumulative substrate transport or generation or dissipation of ion gradients across the plasma membrane. Osmosensing and osmosignaling triggered by such cell volume changes are potent regulators of hepatocyte function. SN1: system N isoform 1 glutamine transporter (Na^+- and H^+-dependent).

versus simply equilibrating amino acid transporters may add to the complexity of such signaling processes. Roughly spoken, hepatocyte swelling provides a protein anabolic, choleretic, proliferative, and anti-apoptotic signal, whereas hepatocyte shrinkage is catabolic, cholestatic, and proapoptotic.

Much effort has been devoted to the question of how hepatocyte volume changes are sensed („osmosensing“) and how this information signals towards hepatocyte function („osmosignaling“). Several candidates for osmosensing have been discussed in the past, including macromolecular crowding (Minton et al. 1992; Parker and Colclasure 1992), stretch-activated ion channels (for review see Naruse 2018), and in yeast, histidine kinases were identified that might act as putative osmosensors (Maeda et al. 1994). In the liver, however, we identified $\alpha_5\beta_1$-integrins as volume(osmo)sensors in response to hepatocyte swelling (Häussinger et al. 2003; Schliess et al. 2004; vom Dahl et al. 2003), whereas early endosomes were identified as chloride-governed osmosensors that are activated in response to hyperosmotic hepatocyte shrinkage (Reinehr et al. 2006) (for review see Reinehr et al. 2013).

This review summarizes some aspects of our work on osmosensing and osmosignaling in the liver. For further aspects and topics on other mammalian cell types, yeast, bacteria, and plants, the reader is referred to (Häussinger and Sies 2007).

In addition to a volume-regulatory uptake and synthesis of organic osmolytes in response to cell shrinkage and their release in response to cell swelling, an early evolutionary mechanism for the maintenance of cell volume homeostasis is the polymerization of small osmotically active molecules. This polymerization occurs in response to cell swelling in hypoosmotic environments and, conversely, the depolymerization of such macromolecules in response to hyperosmotic cell shrinkage. Such a polymerization/depolymerization strategy is also found in some algae and primitive organisms (Chamberlin and Strange 1989): here, the cellular metabolism is at the service of cell volume maintenance in order to compensate for osmolarity changes in the surrounding watery environment. It seems that this archaic mechanism is still present in higher organisms, such as mammals, whose cells, however, are mostly in an osmotically stable environment. In line with this, experimental hepatocyte swelling favors the “polymerization” of amino acids and glucose to proteins and glycogen, respectively, whereas “depolymerization” (increased proteolysis and glycogenolysis

and inhibition of glycogen and protein synthesis) occurs in response to hyperosmotic hepatocyte shrinkage. A cell volume-stabilizing effect of such maneuvres, however, is doubtful in view of the osmotically stable environment in higher organisms. Instead, it seems that these organisms use the archaic polymerization/depolymerization strategy in an opposite way: changes in cell volume are artificially created by hormones or cumulative substrate uptake in order to regulate metabolism (Häussinger 1996a).

Osmosensing and osmosignaling in response to hyperosmotic hepatocyte shrinkage

Hyperosmotic exposure of hepatocytes leads to hepatocyte shrinkage and an accompanying increase of the intracellular chloride concentration due to water efflux and a volume-regulatory uptake of K^+, Na^+, and Cl^- into the hepatocyte (Graf and Häussinger 1996; Häussinger et al. 1990). Early endosomes were identified as chloride-governed osmosensors, which are activated in response to hyperosmotic hepatocyte shrinkage (Reinehr et al. 2006; for review, see Reinehr et al. 2013). The increase of the intracellular chloride concentration directly activates the vacuolar H^+-ATPase (for review, see Faundez and Hartzell 2004) and triggers a DIDS- and bafilomycin-sensitive endosomal acidification following hyperosmotic exposure, as shown in studies using endocytosed FITC-dextran (Schreiber et al. 1994). Endosomal acidification activates acidic sphingomyelinase, and the accompanying ceramide formation leads to an activation of NADPH oxidase and the formation of reactive oxygen species (ROS) (Reinehr et al. 2006; for review, see Reinehr et al. 2013). The hyperosmotic ROS response was shown to trigger the activation not only of c-Jun N-terminal kinase (JNK) but also of the Src family kinase members Fyn and Yes (Cantore et al. 2011; Reinehr et al. 2004a; Sommerfeld et al. 2015a) and to induce pro-apoptotic miRNA 15/107 (Santosa et al. 2015). Hyperosmotic Fyn activation triggers cholestasis due to retrieval of Bsep and Mrp2 from the canalicular membrane (Cantore et al. 2011) and Ntcp from the sinusoidal membrane (Sommerfeld et al. 2015a; Sommerfeld et al. 2015a) of the hepatocyte. The activation of Yes and JNK triggers a pro-apoptotic state of the hepatocyte (Reinehr et al. 2002, 2003, 2004a; for review, see Reinehr et al. 2013). It should be noted that the hyperosmolarity-induced signaling pathway is also activated by the hydrophobic bile acid glycochenodeoxycholic acid (GCDC) (Becker et al. 2007a, b; Mayer et al. 2019; Reinehr et al. 2004b).

Osmosensing and osmosignaling in response to hypoosmotic hepatocyte swelling

Hypoosmotic hepatocyte swelling inhibits autophagic proteolysis and glycogenolysis but stimulates protein and glycogen synthesis and bile acid excretion and triggers a volume-regulatory decrease (RVD) (for review see Häussinger 1996b). Hepatocyte swelling may also inhibit viral replication, as shown for the duck hepatitis B virus replication (Offensperger et al. 1994), although the underlying mechanisms remained unclear. Concerning the stimulation of glycogen synthesis and acetyl-CoA carboxylase by hepatocyte swelling, a decrease of the intracellular chloride concentration was suggested to be an important trigger (Baquet et al. 1993; Meijer et al. 1992; for review, see Hue 1995). However, concerning hypoosmotic proteolysis inhibition, RVD, and stimulation of bile acid excretion, $\alpha_5\beta_1$-integrins were identified as osmosensors (Häussinger et al. 2003; Schliess et al. 2004; vom Dahl et al. 2003), and the downstream osmosignaling events involve activation of focal adhesion kinase, c-Src, the epidermal growth factor receptor (EGFR), and the mitogen-activated protein kinases Erks and p38 (Figure 2). The latter mediates RVD and proteolysis inhibition, whereas dual MAP kinase activation is required for the insertion of Bsep and Mrp2 into the canalicular membrane and choleresis (Häussinger et al. 2003; Kubitz et al. 1997; Noé et al. 1996; Schmitt et al. 2001).

Swelling-induced activation of $\alpha_5\beta_1$-integrins is also an integral part of insulin action in the liver (Reinehr et al. 2010b; Schliess et al. 2004; for review see Schliess and Häussinger 2003). Activation of the insulin receptor triggers, among many other events, also the activation of PI3 kinase, which activates the $Na^+/K^+/2Cl^-$ cotransporter (NKCC1) leading to hepatocyte swelling (Häussinger and Lang 1992), subsequent integrin-dependent osmosensing, and signaling towards proteolysis and EGFR activation (Reinehr et al. 2010b; Schliess et al. 2004), as schematically depicted in Figure 3. The involvement of osmosensing and osmosignaling events can well explain the prodiabetic action of loop diuretics, hypernatremia, oxidative stress,

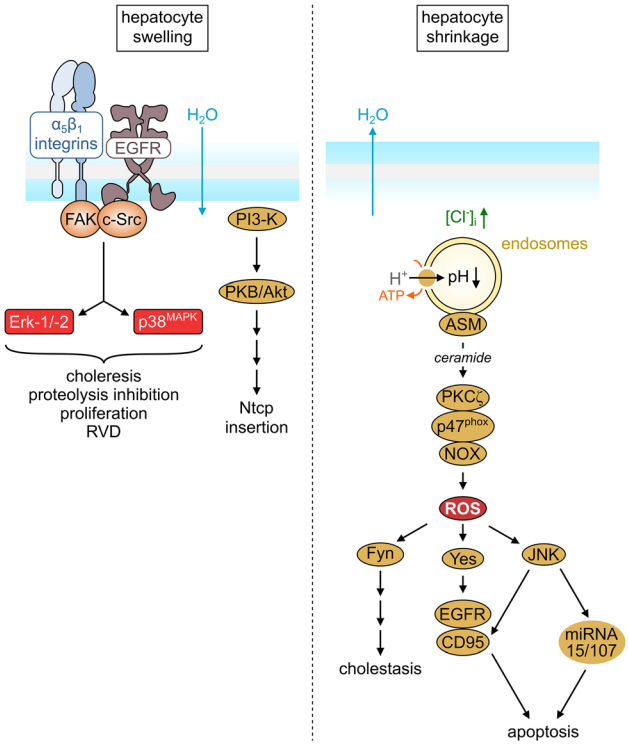

Figure 2: Osmosensing and osmosignaling in response to hypoosmotic hepatocyte swelling and hyperosmotic hepatocyte shrinkage. For details, see text.

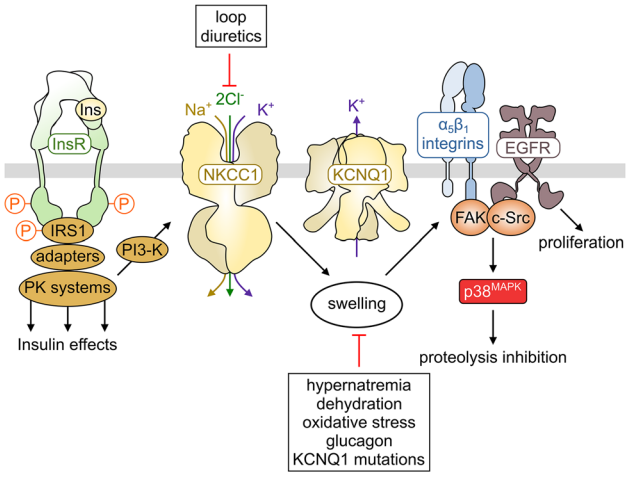

Figure 3: Hepatocyte hydration, insulin action, and resistance. Insulin triggers a PI3 kinase-driven phosphorylation and activation of NKCC1, which triggers cell swelling and activates integrin-dependent osmosensing and -signaling. This results in proteolysis inhibition and EGFR activation. Factors preventing or counteracting insulin-induced hepatocyte swelling include furosemide, oxidative stress, hypernatremia, or KCNQ1 gain of function mutations and are known to be prodiabetic (Boini et al. 2009; Schliess et al. 2001; Unoki et al. 2008; Yasuda et al. 2008).

dehydration, glucagon, and gain of function mutations of KCNQ1 (Boini et al. 2009; Schliess and Häussinger 2003; Schliess et al. 2001; Unoki et al. 2008; Yasuda et al. 2008).

The $\alpha_5\beta_1$-integrins can also be activated non-osmotically/non-mechanically by urea in high concentrations and tauroursodeoxycholate (TUDC), and signaling events similar to those found after hypoosmotic exposure are initiated (Bonus et al. 2020; Gohlke et al. 2013; Reinehr et al. 2010a), which are mechanistically discussed below. The signaling events triggered by TUDC can explain the choleretic and hepatoprotective action of this bile acid (Häussinger et al. 2003; Kurz et al. 2001), which is also a mainstay in the treatment of cholestatic diseases. Interestingly, TUDC also triggers the formation of cyclic AMP (cAMP) in an integrin-dependent manner (Sommerfeld et al. 2015b). Whether cAMP formation also occurs in response to hypoosmotic hepatocyte swelling remains to be investigated.

The TUDC-triggered cAMP formation strongly counteracts the proapoptotic action of the hydrophobic bile acid GCDC by preventing Yes, Fyn, and JNK activation (Sommerfeld et al. 2015b) and triggering an inactivating Ser/Thr-phosphorylation of CD95 (Reinehr and Häussinger 2004). In addition, TUDC prevented GCDC-induced changes in gene transcription (Paluschinski et al. 2019).

Early indications of TUDC-mediated integrin activation

Early studies demonstrated that TUDC activates the mitogen-activated protein kinases (MAPK) extracellular signal-regulated kinase (Erk)-1 and Erk-2 in isolated, cultured hepatocytes and perfused rat liver (Schliess et al. 1997). These TUDC-mediated signal transduction events were similar to those triggered by cell swelling (Noé et al. 1996; Schliess et al. 1995) but, conversely, independent from G protein-mediated pathways and amenable to inhibition by high concentrations of glucagon and CPT-cAMP, i.e., conditions that allow for a rapid induction of MAP kinase phosphatase 1 (MKP 1). For reasons not yet fully understood, this is in contrast to the low levels of TUDC-induced cAMP formation that mediate the hepatoprotective effects of TUDC (Sommerfeld et al. 2015a; see Figure 4). Since Erks can phosphorylate proteins that control microtubule dynamics and in this way regulate vesicular trafficking of canalicular transporters, they were considered early on as a central node for TUDC- and

hypoosmolarity-mediated choleresis. Soon after PI3-K and Ras activation were confirmed as upstream events of the TUDC-mediated, choleretic Erk signal (Kurz et al. 2000), $p38^{MAPK}$ was identified as the second major downstream component to convert a hypoosmolarity- (Häussinger et al. 1999) or TUDC-induced (Kurz et al. 2001) signal into a cellular response. While hypoosmotic cell swelling induced inhibition of autophagic proteolysis via $p38^{MAPK}$ without any involvement of Erks (Häussinger et al. 1999), swelling and liver perfusion with TUDC triggered choleresis only when Erk-1/2 and $p38^{MAPK}$ were both activated. Such dual activation was found essential for vesicular trafficking of the bile salt export pump (BSEP) and multidrug resistance-associated protein 2 (MRP2) (Kubitz et al. 1997; Kurz et al. 2001) to the canalicular membrane.

Considering that the described TUDC-induced signal transduction pathways occurred without any involvement of G proteins (Schliess et al. 1997) and the potency of TUDC toward the farnesoid X receptor (FXR) is only modest (Liu et al. 2003), GPCR and nuclear receptors were ruled out as the primary TUDC sensors (Häussinger and Kordes 2017). A subsequent study (Häussinger et al. 2003) revealed an altered phosphorylation pattern of other kinases associated with the Erk pathway (c-Src and FAK), which turned the focus to integrin $\alpha_5\beta_1$, the most abundant integrin in hepatocytes (Volpes et al. 1993). The same study (Häussinger et al. 2003) demonstrated abrogation of the complete TUDC-induced signal transduction in the presence of the hexapeptide G*RGD*SP, which inhibits RGD-integrins such as $\alpha_5\beta_1$ (Figure 5A). Swelling-induced signal transduction was likewise abolished through inhibition of integrin activation (vom Dahl et al. 2003),

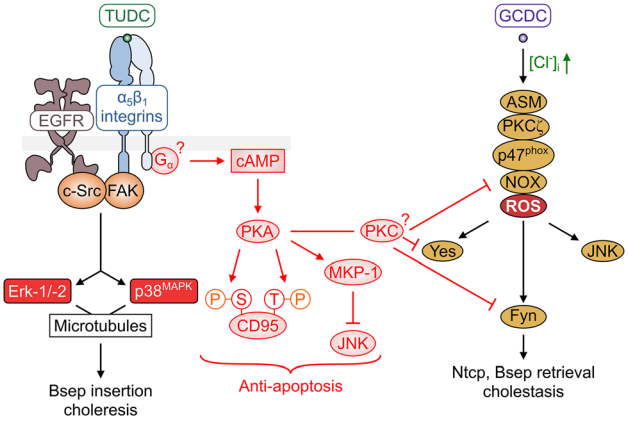

Figure 4: TUDC and GCDC signaling.
GCDC triggers signaling events such as hyperosmolarity, which results in cholestasis and a proapoptotic state. This is counteracted by TUDC, which triggers via cAMP an inhibition of GCDC signaling towards cholestasis and apoptosis.

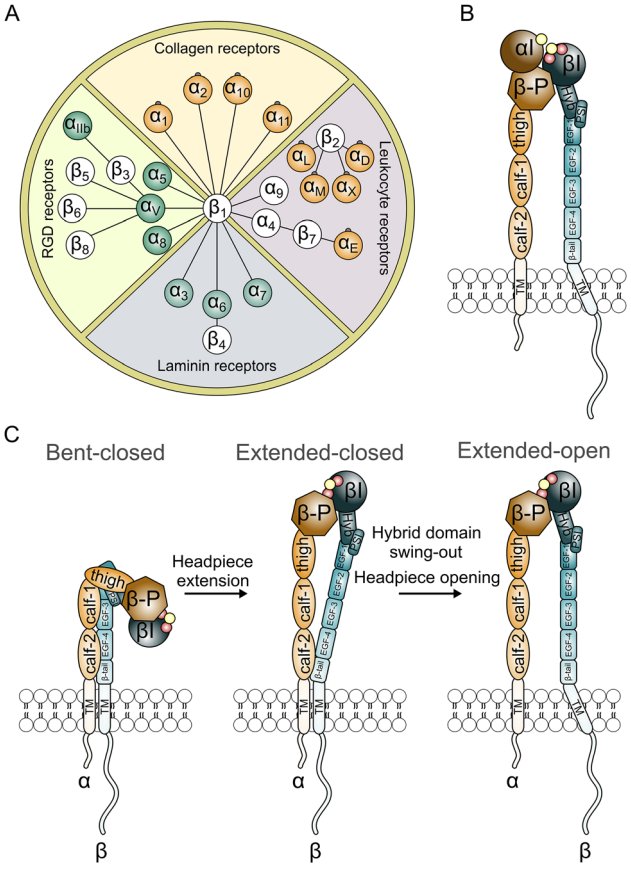

Figure 5: Integrin subunit assemblies and structural aspects of integrin activation.
(A) Representation of known integrin heterodimer pairs in vertebrates and their grouping by ECM ligand. Integrin α subunits are colored according to their evolutionary relationship; α subunits with an αI domain carry a gray attachment (figure modified from Hynes 2002, and Barczyk et al. 2010). (B) Domain architecture of an integrin with an αI domain. The MIDAS is depicted as a yellow sphere, the ADMIDAS (distal) and SyMBS (proximal) are depicted as red spheres. Membrane lipids indicate the membrane region in the background of the transmembrane regions. β-P: β-propeller domain; Hyb: hybrid domain; TM: transmembrane domain. (C) Integrins undergo a large conformational transition from a "bent-closed" (left) to an "extended-closed" conformation (center) by extending their ectodomain headpiece via the "joints" between thigh and calf-1 domains and EGF-1 and EGF-2 domains. The transition between "extended-closed" and "extended-open" conformation (right) is achieved by a swing-out of the hybrid domain and dissociation of the transmembrane helices. The latter has a contributing role in the subunit-specific sensitivity of integrin activation (Pagani and Gohlke 2018). The conformational equilibrium is depicted for an integrin lacking an αI domain (figure modified from Kadry and Calderwood 2020).

leading to the hypothesis that $\alpha_5\beta_1$ is a sensor for TUDC, besides it mediating changes in the hepatocellular hydration state. Likewise, TUDC-induced cAMP formation was inhibited by the integrin inhibitory hexapeptide G*RGD*SP (Sommerfeld et al. 2015b), and β_1-integrin-dependent cAMP

formation has also been described in tumor cell lines (O'Connor and Mercurio 2001; Whittard and Akiyama 2001).

Structural aspects of integrin activation

Structures of integrin ectodomains (Xiong et al. 2001, 2002) and an understanding of how conformational changes are associated with integrin activation opened up the opportunity to complement the *in vitro* and *in vivo* findings with a mechanistic understanding on the structural level. Humans express 18 α and 8 β subunits, which can arrange into 24 distinct integrin heterodimers (Figure 5A). The global architecture of these heterodimers is conserved across all multicellular organisms (Kadry and Calderwood 2020). A large extracellular region (ectodomain) that consists of several domains is connected to two generally short cytoplasmic tails via two single-span transmembrane regions (Figure 5B). α and β subunits are unrelated with respect to their sequence, but the subunits themselves are globally conserved across almost all species. An exception to this is a group of vertebrate α subunits with an additional inserted domain. This insertion, the αI or αA domain, forms the binding site in collagen-binding integrins and some laminin-binding integrins. A divalent cation (usually Mg^{2+}) within the "metal ion-dependent adhesion site" (MIDAS) of this binding site mediates binding of extracellular ligands either via an aspartate or glutamate residue. Regardless of whether an αI domain is present or absent, the ectodomains of all integrin α subunits carry a β-propeller domain, two calf domains, and a thigh domain with an IgG-like structure. Ectodomains of β subunits all bear a βI (or βA) domain homologous to the αI domain, a hybrid domain, a plexin–semaphorin–integrin (PSI) domain, four epidermal growth factor (EGF)-like domains, and a β-tail domain (Xiong et al. 2001). In integrins without an αI domain, the βI domain takes over ligand engagement; however, the βI domain carries three divalent cations instead of one. While the βI MIDAS carries a Mg^{2+} ion, as it does in αI domains, the structural information available to this date suggests that the site "adjacent to MIDAS" (ADMIDAS) and the synergistic metal ion-binding site (SyMBS, formerly referred to as LIMBS for "ligand-associated metal-binding site") both carry a Ca^{2+} ion.

According to the currently accepted model, derived from the first crystal structures of a complete integrin ectodomain (Xiong et al. 2001, 2002) and electron microscopy studies (Nishida et al. 2006; Takagi et al. 2002), integrins adopt at least three stable conformational states during their activation cycle (Figure 5C). The "bent-closed" (Figure 5C, left) conformation with low affinity for extracellular matrix (ECM) ligands represents a resting state of integrins. In contrast, the "extended-closed" conformation (Figure 5C, center) with medium affinity and the "extended-open" conformation (Figure 5C, right) with high affinity are binding-competent (Arnaout et al. 2005; Kadry and Calderwood 2020). Integrin-dependent ligand binding was thought to always follow the same basic multi-step procedure (Bachmann et al. 2019; Kadry and Calderwood 2020; Kechagia et al. 2019; Sun et al. 2019). First, intracellular adaptor proteins such as talin and kindlin are recruited. The binding of these adaptor proteins to the integrin cytoplasmic tails induces the long-range conformational changes described in Figure 5C, which increase the affinity for ECM ligands. Now the integrins engage their respective ECM components with high affinity and simultaneously bind to the actin cytoskeleton. This bilateral connection results in a clustering of integrins and the formation of anchoring cell-matrix connections known as focal adhesions. Numerous intracellular binding partners can subsequently be recruited, which may initiate various downstream signaling pathways (Kim et al. 2011; Morse et al. 2014).

Note that this model neither includes nor excludes the possibility of ligand binding to the low-affinity "bent-closed" conformation (Figure 5C, left). Omitting this scenario is reasonable in the context of binding to voluminous ECM ligands, which are unlikely to reach the binding pocket in the "bent-closed" state for steric reasons. However, the model may imply that the modulation of integrin activity and signaling by extracellular binding partners of lower molecular weight is not possible either. Moreover, the model limits the concept of bidirectionality and reciprocity (Hynes 2002) of integrin signaling to the focal adhesion phase. By contrast, a large body of experimental evidence suggests that the "bent-closed" conformation may very well bind small- and macromolecular ligands (Adair et al. 2005; Zhu et al. 2013). Furthermore, ligand binding to an isolated headpiece induces a conformational change that exactly matches the conformational change following adaptor protein-induced integrin activation (Springer et al. 2008; Zhu et al. 2013). Therefore, ligand binding to the "bent-closed" ectodomain likely influences the integrin conformational equilibrium similar to the binding of intracellular adaptor proteins to the cytoplasmic tail.

Activation of integrin $\alpha_5\beta_1$ by urea

Early studies revealed that high urea concentrations induce a paradoxical regulatory volume decrease with an opening of K^+ channels followed by hepatocyte shrinkage, although the hepatocyte membrane is expected to be freely permeable to urea (Hallbrucker et al. 1994). Cryosections of urea-perfused rat liver immunostained for the active β_1 integrin subunit showed that high urea concentrations (100 μM) cause activation of the hepatocyte β_1 integrin system and trigger the same signal transduction mechanisms as hypoosmolarity and TUDC (Reinehr et al. 2010a). Molecular dynamics (MD) simulations of a model of $\alpha_5\beta_1$ integrin in aqueous urea solution revealed that urea disrupts stabilizing hydrogen bonds between the leg regions of both integrin subunits and between the hybrid domain and the leg region of the α-subunit. Breaking these interactions led to a pronounced integrin unbending, which is part of the conformational transition towards the "extended-closed" state, corresponding to the first step in the activation cycle of integrins (Figure 5C). Hence, urea-mediated $\alpha_5\beta_1$ integrin activation in hepatocytes is suggested to occur through direct urea-integrin interactions, although not at the extracellular ligand binding site of $\alpha_5\beta_1$.

Integrin $\alpha_5\beta_1$ acts as a sensor for TUDC in hepatocytes

Abrogation of the complete TUDC-induced signal transduction in the presence of the hexapeptide G*RGD*SP (Häussinger et al. 2003), the lack of TUDC-induced kinase activation after β_1 integrin knockdown in isolated rat hepatocytes (Gohlke et al. 2013), and the pharmacophoric similarity of TUDC with tirofiban, a small molecule inhibitor binding to the extracellular binding site of integrin $\alpha_{IIb}\beta_3$, led to the suggestion that TUDC can activate integrin $\alpha_5\beta_1$ by specifically binding to its extracellular site. Immunofluorescence staining experiments revealed that only TUDC, but neither taurocholic acid (TC), glycochenodeoxycholic acid (GCDC), taurochenodeoxycholic acid (TCDC), nor taurolithocholic acid 3-sulfate (TLCS), activated β_1 integrins (Gohlke et al. 2013). These bile acids primarily differ from TUDC with respect to substitutions at or configurations of substituents of the cholane scaffold. Furthermore, integrin β_1 was activated predominantly inside hepatocytes and required TUDC uptake through the Na^+/taurocholate cotransporting polypeptide (NTCP) (Gohlke et al. 2013). This mode of activation was in stark contrast to integrin activation by cell swelling, predominantly involving membrane-bound integrins (Gohlke et al. 2013; Reinehr et al. 2010a). Intriguingly, TUDC-mediated integrin activation was also suppressed in the presence of higher TC concentrations, suggesting that both bile acids bind to the RGD binding site but with opposite effects (Gohlke et al. 2013). In line with this data, MD simulations of $\alpha_5\beta_1$ integrin in complex with TUDC, TC, or G*RGD*SP, docked to the RGD binding site, respectively, demonstrated that only TUDC induces conformational changes between the propeller and the βA domains (Figure 6A) and in helices α1 and α7 (Figure 6B) that are consistent with integrin activation (Bonus et al. 2020; Gohlke et al. 2013). Hence, we concluded that TUDC can directly activate intrahepatocytic β_1 integrins, which trigger signal transduction pathways toward choleresis.

Functional selectivity of TUDC and *nor*UDCA for integrin-mediated signaling pathways

Chemical modifications to the ursodeoxycholane scaffold of TUDC led either to a complete loss of activity or, in the case of taurocholic acid (TC), to an antagonist of TUDC-induced $\alpha_5\beta_1$ integrin activation (Gohlke et al. 2013). Whether chemical modifications of the bile acid's side chain – preserving the ursodeoxycholane scaffold – retain or even increase activity towards $\alpha_5\beta_1$ integrin was investigated in a subsequent study (Bonus et al. 2020). Here, MD simulations were conducted first to compare the ability of *nor*UDCA, T*nor*UDCA, GUDC, and UDCA to evoke activation-related conformational changes in $\alpha_5\beta_1$ integrin (Figure 6A, B) with that of TUDC and TC. The extent of these conformational changes, described using three geometric descriptors, was subsequently correlated with an activity ranking of these bile acids (Figure 6C) derived from the amount of immunostained, active β_1 integrin determined at fixed time points (Figure 6D). Based on this data, TUDC and *nor*UDCA were classified as highly active, T*nor*UDCA and GUDC as weakly active, and UDCA and TC as inactive or inhibitory ligands of $\alpha_5\beta_1$ integrin, respectively. A competitive ELISA-based solid-phase assay (Bochen et al. 2013) confirmed direct binding of TUDC and *nor*UDCA to the MIDAS in $\alpha_5\beta_1$ integrins and revealed similar binding affinities of both compounds (Bonus et al. 2020). In view of this, the different extent of $\alpha_5\beta_1$ integrin activation by TUDC and *nor*UDCA could not have been a consequence of different binding site occupancy.

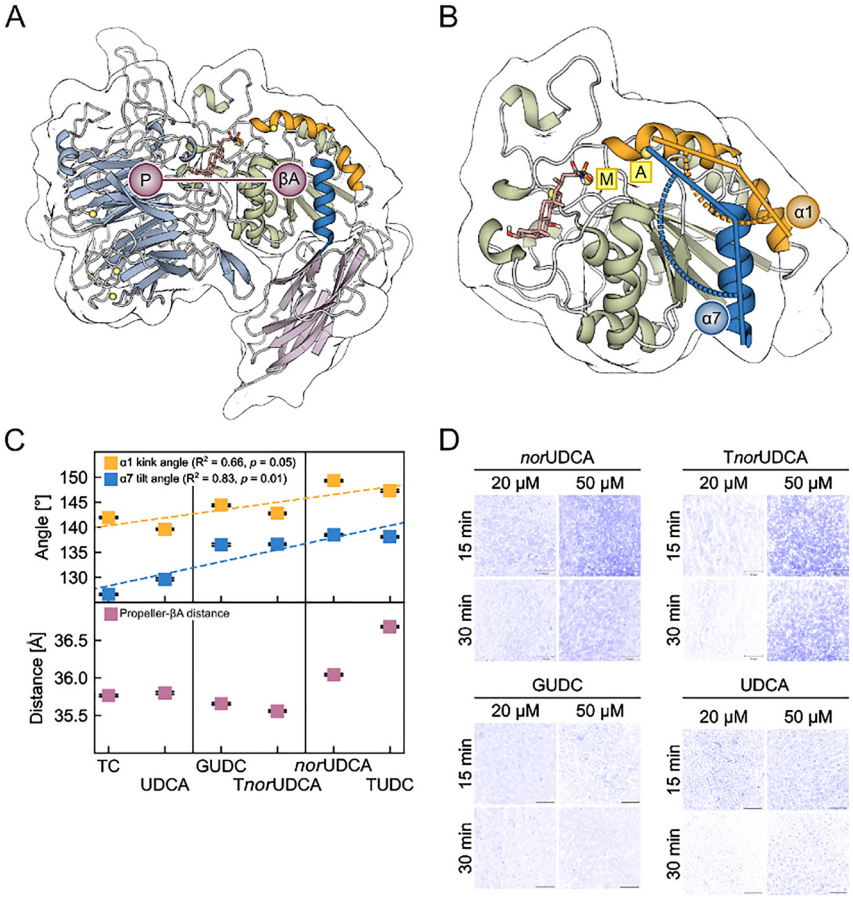

Figure 6: Geometric descriptors and activation of $\alpha_5\beta_1$ integrin in MD simulations and immunofluorescence staining experiments. (A) Part of the $\alpha_5\beta_1$ integrin headpiece in cartoon representation. Helices α1 and α7 are highlighted in orange and blue, respectively. The propeller-βA distance was measured between the respective centers of mass (violet circles). The propeller domain is colored in light blue, the βA domain in pale green, and the hybrid domain in violet. (B) Close-up view of the βA domain with the docked TUDC structure (stick representation) (Gohlke et al. 2013). This complex structure was used to generate the other starting structures by modifying the bile acid side chain. Angles measured during the MD simulations: orange: α1 kink angle; blue: α7 tilt angle. Mg^{2+} ions are depicted as yellow spheres; the metal ion at the MIDAS is labeled *M*, the metal ion at the ADMIDAS is labeled *A*. (C) Average ± SEM values of the α1 kink angle (orange), α7 tilt angle (blue), and propeller-βA distance (violet) over three replicate MD simulations versus the rank of the bile acids according to their agonistic activity towards $\alpha_5\beta_1$ integrin as observed in immunofluorescence staining experiments (panel D). Dashed lines depict linear regressions; fit parameters are given in the figures. Vertical lines separate the dataset into inactive (left), weakly active (middle), and highly active (right) bile acids. (D) Effect of *nor*UDCA, T*nor*UDCA, GUDC, and UDCA on β_1 integrin activation. Rat livers were perfused with the respective bile acids for up to 60 min with the concentrations indicated. Liver samples were immunostained for the active conformation of β_1 integrin (blue, false-color images; for original images cf. Figure 3 in Bonus et al. 2020). Figure and caption were modified from Bonus et al. (2020).

As described before (Kurz et al. 2001; Schliess et al. 1997), TUDC (20 µM)-induced $\alpha_5\beta_1$ integrin activation led to *sustained* activation of Erk-1/-2 and $p38^{MAPK}$ (Figure 7A–C), the critical downstream signal towards choleresis. Perfusion with lower (10 µM) and higher (50 µM) TUDC concentrations did not change this kinetic profile, ruling out a concentration effect (Bonus et al. 2020). In contrast, *nor*UDCA-induced $\alpha_5\beta_1$ integrin activation only led to *transient* activation of these MAPKs (Figure 7C), which may have been a consequence of weaker $\alpha_5\beta_1$ integrin activation. Since TUDC, but not *nor*UDCA, significantly enhanced the EGFR/c-Src association, a c-Src-dependent transactivation of EGFR is likely a determining factor for prolonged MAPK activation (Bonus et al. 2020). Inhibiting PI3-K abolished *nor*UDCA-induced phosphorylation of only Erk-1/-2, but inhibiting c-Src abolished phosphorylation of both Erk-1/-2 and $p38^{MAPK}$, indicating that c-Src activation occurs upstream of PI3-K activation. In an earlier

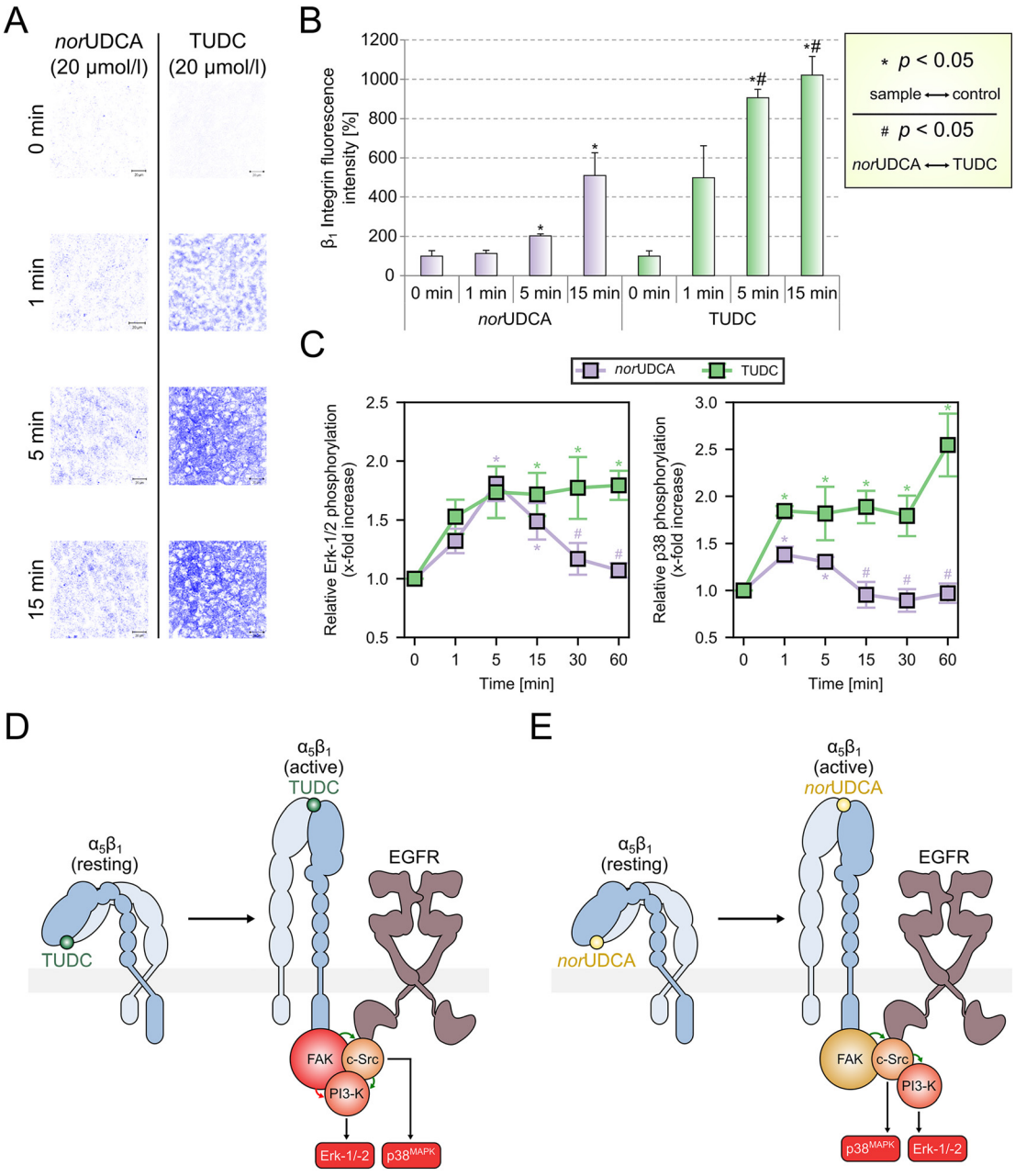

Figure 7: Comparison between TUDC- and *nor*UDCA-induced $\alpha_5\beta_1$ integrin activation and MAPK signaling.
(A) Immunofluorescence staining of active β_1 integrin after liver perfusion with either 20 µM *nor*UDCA (left) or TUDC (right) at timepoints $t = 0, 1, 5,$ and 15 min. The scale bar corresponds to 20 µm. Representative images of three independent experiments are depicted. (B) Quantification of β_1 integrin fluorescence. (C) Densitometric quantification of Erk-1/2 (left) and $p38^{MAPK}$ (right) phosphorylation after liver perfusion with either 20 µM *nor*UDCA (violet) or TUDC (green). Total Erk-1/2 and total $p38^{MAPK}$ served as respective loading controls. Phosphorylation at $t = 0$ min was set to 1. Data represents the mean ± SEM of at least three independent experiments. (D) Activation of $\alpha_5\beta_1$ integrin by TUDC leads to high FAK^{Y397-P} levels, which trigger both a slow, c-Src and EGFR-independent PI3-K activation (red arrow) and a fast, c-Src and EGFR-dependent PI3-K activation (green arrows). The subsequent PI3-K-mediated Erk-1/2 activation is sustained. (E) Activation of $\alpha_5\beta_1$ integrin by *nor*UDCA leads to lower FAK^{Y397-P} levels, which trigger the fast, c-Src and EGFR-dependent pathway of PI3-K activation (green arrows), but abolish the slower FAK-mediated PI3-K activation. The subsequent PI3-K mediated Erk-1/2 activation is only transient. Figure and caption were modified from Bonus et al. (2020).

study (Häussinger et al. 2003), which investigated TUDC-induced signaling towards choleresis, inhibition of c-Src did not prevent Erk-1/-2 phosphorylation but only delayed it by ~8 min. Hence, inhibition of c-Src activity only prevents Erk-1/-2 phosphorylation after *nor*UDCA-induced but not after TUDC-induced $\alpha_5\beta_1$ integrin activation.

Together with further experiments on FAK phosphorylation patterns (Bonus et al. 2020), these observations suggested a ligand-dependent selectivity for signaling pathways induced by $\alpha_5\beta_1$-integrin (Figure 7D and E), a property widely known for G-protein coupled receptors (Violin and Lefkowitz 2007), but that had not yet been described for integrins without an αI domain (Simon 2011). The insights gained from this study also provide a rationale for the different therapeutic applications of UDCA and *nor*UDCA in primary biliary cholangitis and primary sclerosing cholangitis, respectively (Trauner et al. 2015).

Concluding remarks

Hormones, nutrients, and oxidative stress can lead to changes of liver cell hydration (cell volume) within minutes. The changes act as important modulators of cell function and couple the physical parameter cell volume to metabolism, transport, and gene expression. For this to occur, mechanotransduction (osmosensing) is required, which triggers signaling cascades towards liver function (osmosignaling). β_1 integrins have a central role as (osmo-)mechanosensors in the liver, but also are involved in bile acid signaling.

Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

Research funding: The studies reported herein were supported by Deutsche Forschungsgemeinschaft through Sonderforschungsbereiche SFB 575 and SFB 974.

Conflict of interest statement: The authors declare no conflicts of interest regarding this article.

References

Adair, B.D., Xiong, J.-P., Maddock, C., Goodman, S.L., Arnaout, M.A., and Yeager, M. (2005). Three-dimensional EM structure of the ectodomain of integrin $\alpha_V\beta_3$ in a complex with fibronectin. J. Cell Biol. 168: 1109–1118.

Arnaout, M.A., Mahalingam, B., and Xiong, J.-P. (2005). Integrin structure, allostery, and bidirectional signaling. Annu. Rev. Cell Dev. Biol. 21: 381–410.

Bachmann, M., Kukkurainen, S., Hytönen, V.P., and Wehrle-Haller, B. (2019). Cell adhesion by integrins. Physiol. Rev. 99: 1655–1699.

Baquet, A., Gaussin, V., Bollen, M., Stalmans, W., and Hue, L. (1993). Mechanism of activation of liver acetyl-CoA carboxylase by cell swelling. Eur. J. Biochem. 217: 1083–1089.

Baquet, A., Hue, L., Meijer, A.J., van Woerkom, G.M., and Plomp, P.J.A.M. (1990). Swelling of rat hepatocytes stimulates glycogen synthesis. J. Biol. Chem. 265: 955–959.

Barczyk, M., Carracedo, S., and Gullberg, D. (2010). Integrins. Cell Tissue Res. 339: 269–280.

Becker, S., Reinehr, R., Graf, D., vom Dahl, S., and Häussinger, D. (2007a). Hydrophobic bile salts induce hepatocyte shrinkage via NADPH oxidase activation. Cell. Physiol. Biochem. 19: 89–98.

Becker, S., Reinehr, R., Grether-Beck, S., Eberle, A., and Häussinger, D. (2007b). Hydrophobic bile salts trigger ceramide formation through endosomal acidification. Biol. Chem. 388: 185–196.

Bochen, A., Marelli, U.K., Otto, E., Pallarola, D., Mas-Moruno, C., Di Leva, F.S., Boehm, H., Spatz, J.P., Novellino, E., Kessler, H., et al. (2013). Biselectivity of isoDGR peptides for fibronectin binding integrin subtypes alpha5beta1 and alphavbeta6: conformational control through flanking amino acids. J. Med. Chem. 56: 1509–1519.

Boini, K.M., Graf, D., Hennige, A.M., Koka, S., Kempe, D.S., Wang, K., Ackermann, T.F., Föller, M., Vallon, V., Pfeifer, K., et al. (2009). Enhanced insulin sensitivity of gene-targeted mice lacking functional KCNQ1. Am. J. Physiol. 296: R1695–1701.

Bonus, M., Sommerfeld, A., Qvartskhava, N., Görg, B., Ludwig, B.S., Kessler, H., Gohlke, H., and Häussinger, D. (2020). Evidence for functional selectivity in TUDC- and norUDCA-induced signal transduction via alpha5beta1 integrin towards choleresis. Sci. Rep. 10: 5795.

Cantore, M., Reinehr, R., Sommerfeld, A., Becker, M., and Häussinger, D. (2011). The Src family kinase Fyn mediates hyperosmolarity-induced Mrp2 and Bsep retrieval from canalicular membrane. J. Biol. Chem. 286: 45014–45029.

Chamberlin, M.E. and Strange, K. (1989). Anisosmotic cell volume regulation: a comparative view. Am. J. Physiol. 257: C159–173.

Faundez, V. and Hartzell, H.C. (2004). Intracellular chloride channels: determinants of function in the endosomal pathway. Sci. STKE 2004: re8.

Gohlke, H., Schmitz, B., Sommerfeld, A., Reinehr, R., and Häussinger, D. (2013). α5β1-integrins are sensors for tauroursodeoxycholic acid in hepatocytes. Hepatology 57: 1117–1129.

Graf, J. and Häussinger, D. (1996). Ion transport in hepatocytes: mechanisms and correlations to cell volume, hormone actions and metabolism. J. Hepatol. 24: 53–77.

Hallbrucker, C., vom Dahl, S., Lang, F., and Häussinger, D. (1991). Control of hepatic proteolysis by amino acids. The role of cell volume. Eur. J. Biochem. 197: 717–724.

Hallbrucker, C., vom Dahl, S., Ritter, M., Lang, F., and Häussinger, D. (1994). Effects of urea on K+ fluxes and cell volume in perfused rat liver. Pflüger's Arch. 428: 552–560.

Häussinger, D. (1996a). Regulation der Zellfunktion durch den Hydratationszustand. Naturwissenschaften 83: 264–271.

Häussinger, D. (1996b). The role of cellular hydration in the regulation of cell function. Biochem. J. 313: 697–710.

Häussinger, D., Hallbrucker, C., vom Dahl, S., Decker, S., Schweizer, U., Lang, F., and Gerok, W. (1991). Cell volume is a major determinant of proteolysis control in liver. FEBS Lett. 283: 70–72.

Häussinger, D. and Kordes, C. (2017). Mechanisms of tauroursodeoxycholate-mediated hepatoprotection. Dig. Dis. 35: 224–231.

Häussinger, D., Kurz, A.K., Wettstein, M., Graf, D., vom Dahl, S., and Schliess, F. (2003). Involvement of integrins and Src in tauroursodeoxycholate-induced and swelling-induced choleresis. Gastroenterology 124: 1476–1487.

Häussinger, D. and Lang, F. (1991). Cell volume in the regulation of hepatic function: a mechanism for metabolic control. Biochim. Biophys. Acta Rev. Biomembr. 1071: 331–350.

Häussinger, D. and Lang, F. (1992). Cell volume and hormone action. Trends Pharmacol. Sci. 13: 371–373.

Häussinger, D., Roth, E., Lang, F., and Gerok, W. (1993). Cellular hydration state: an important determinant of protein catabolism in health and disease. Lancet 341: 1330–1332.

Häussinger, D., Schliess, F., Dombrowski, F., and Vom Dahl, S. (1999). Involvement of p38MAPK in the regulation of proteolysis by liver cell hydration. Gastroenterology 116: 921–935.

Häussinger, D. and Sies, H. (Eds.) (2007). Osmosensing and osmosignaling. In: *Methods in enzymology*, Vol. 428. Academic Press, San Diego, CA.

Häussinger, D., Stehle, T., and Lang, F. (1990). Volume regulation in liver: further characterization by inhibitors and ionic substitutions. Hepatology 11: 243–254.

Hue, L. (1995). Regulation of liver carbohydrate and lipid metabolism by cell volume. Ann. Endocrinol. (Paris) 56: 599–601.

Hynes, R.O. (2002). Integrins: bidirectional, allosteric signaling machines. Cell 110: 673–687.

Kadry, Y.A. and Calderwood, D.A. (2020). Chapter 22: Structural and signaling functions of integrins. Biochim. Biophys. Acta 1862: 183206.

Katz, J., Golden, S., and Wals, P.A. (1976). Stimulation of hepatic glycogen synthesis by amino acids. Proc. Natl. Acad. Sci. USA 73: 3433–3437.

Kechagia, J.Z., Ivaska, J., and Roca-Cusachs, P. (2019). Integrins as biomechanical sensors of the microenvironment. Nat. Rev. Mol. Cell Biol. 20: 457–473.

Kim, C., Ye, F., and Ginsberg, M.H. (2011). Regulation of integrin activation. Annu. Rev. Cell Dev. Biol. 27: 321–345.

Kubitz, R., D'Urso, D., Keppler, D., and Häussinger, D. (1997). Osmodependent dynamic localization of the multidrug resistance protein 2 in the rat hepatocyte canalicular membrane. Gastroenterology 113: 1438–1442.

Kurz, A.K., Block, C., Graf, D., vom Dahl, S., Schliess, F., and Häussinger, D. (2000). Phosphoinositide 3-kinase-dependent Ras activation by tauroursodesoxycholate in rat liver. Biochem. J. 350 Pt 1: 207–213.

Kurz, A.K., Graf, D., Schmitt, M., vom Dahl, S., and Häussinger, D. (2001). Tauroursodesoxycholate-induced choleresis involves p38(MAPK) activation and translocation of the bile salt export pump in rats. Gastroenterology 121: 407–419.

Lavoinne, A., Baquet, A., and Hue, L. (1987). Stimulation of glycogen synthesis and lipogenesis by glutamine in isolated rat hepatocytes. Biochem. J. 248: 429–437.

Liu, Y., Binz, J., Numerick, M.J., Dennis, S., Luo, G., Desai, B., MacKenzie, K.I., Mansfield, T.A., Kliewer, S.A., Goodwin, B., et al. (2003). Hepatoprotection by the farnesoid X receptor agonist GW4064 in rat models of intra- and extrahepatic cholestasis. J. Clin. Invest. 112: 1678–1687.

Maeda, T., Wurgler-Murphy, S.M., and Saito, H. (1994). A two-component system that regulates an osmosensing MAP kinase cascade in yeast. Nature 369: 242–245.

Mayer, P.G.K., Qvartskhava, N., Sommerfeld, A., Görg, B., and Häussinger, D. (2019). Regulation of plasma membrane localization of the Na^+-taurocholate co-transporting polypeptide by glycochenodeoxycholate and tauroursodeoxycholate. Cell. Physiol. Biochem. 52: 1427–1445.

Meijer, A.J., Baquet, A., Gustafson, L., van Woerkom, G.M., and Hue, L. (1992). Mechanism of activation of liver glycogen synthase by swelling. J. Biol. Chem. 267: 5823–5828.

Minton, A.P., Colclasure, G.C., and Parker, J.C. (1992). Model for the role of macromolecular crowding in regulation of cellular volume. Proc. Natl. Acad. Sci. USA 89: 10504–10506.

Morse, E.M., Brahme, N.N., and Calderwood, D.A. (2014). Integrin cytoplasmic tail interactions. Biochem 53: 810–820.

Mortimore, G.E. and Pösö, A.R. (1987). Intracellular protein catabolism and its control during nutrient deprivation and supply. Annu. Rev. Nutr. 7: 539–564.

Naruse, K. (2018). Mechanomedicine. Biophys. Rev. 10: 1257–1262.

Nishida, N., Xie, C., Shimaoka, M., Cheng, Y., Walz, T., and Springer, T.A. (2006). Activation of leukocyte beta2 integrins by conversion from bent to extended conformations. Immunity 25: 583–594.

Noé, B., Schliess, F., Wettstein, M., Heinrich, S., and Häussinger, D. (1996). Regulation of taurocholate excretion by a hypo-osmolarity-activated signal transduction pathway in rat liver. Gastroenterology 110: 858–865.

O'Connor, K.L. and Mercurio, A.M. (2001). Protein kinase A regulates Rac and is required for the growth factor-stimulated migration of carcinoma cells. J. Biol. Chem. 276: 47895–47900.

Offensperger, W.-B., Offensperger, S., Stoll, B., Gerok, W., and Häussinger, D. (1994). Effects of anisotonic exposure on duck hepatitis B virus replication. Hepatology 20: 1–7.

Pagani, G. and Gohlke, H. (2018). On the contributing role of the transmembrane domain for subunit-specific sensitivity of integrin activation. Sci. Rep. 8: 5733.

Paluschinski, M., Castoldi, M., Schöler, D., Bardeck, N., Oenarto, J., Görg, B., and Häussinger, D. (2019). Tauroursodeoxycholate protects from glycochenodeoxycholate-induced gene expression changes in perfused rat liver. Biol. Chem. 400: 1551–1565.

Parker, J.C. and Colclasure, G.C. (1992). Macromolecular crowding and volume perception in dog red cells. Mol. Cell. Biochem. 114: 9–11.

Reinehr, R., Becker, S., Braun, J., Eberle, A., Grether-Beck, S., and Häussinger, D. (2006). Endosomal acidification and activation of NADPH oxidase isoforms are upstream events in hyperosmolarity-induced hepatocyte apoptosis. J. Biol. Chem. 281: 23150–23166.

Reinehr, R., Becker, S., Höngen, A., and Häussinger, D. (2004a). The Src family kinase yes triggers hyperosmotic activation of the epidermal growth factor receptor and CD95. J. Biol. Chem. 279: 23977–23987.

Reinehr, R., Becker, S., Wettstein, M., and Häussinger, D. (2004b). Involvement of the Src family kinase yes in bile salt-induced apoptosis. Gastroenterology 127: 1540–1557.

Reinehr, R., Gohlke, H., Sommerfeld, A., vom Dahl, S., and Häussinger, D. (2010a). Activation of integrins by urea in perfused rat liver. J. Biol. Chem. 285: 29348–29356.

Reinehr, R., Graf, D., Fischer, R., Schliess, F., and Häussinger, D. (2002). Hyperosmolarity triggers CD95 membrane trafficking and sensitizes rat hepatocytes toward CD95L-induced apoptosis. Hepatology 36: 602–614.

Reinehr, R. and Häussinger, D. (2004). Inhibition of bile salt-induced apoptosis by cyclic AMP involves serine/threonine phosphorylation of CD95. Gastroenterology 126: 249–262.
Reinehr, R., Schliess, F., and Häussinger, D. (2003). Hyperosmolarity and CD95L trigger CD95/EGF receptor association and tyrosine phosphorylation of CD95 as prerequisites for CD95 membrane trafficking and DISC formation. FASEB J 17: 731–733.
Reinehr, R., Sommerfeld, A., and Häussinger, D. (2010b). Insulin induces swelling-dependent activation of the epidermal growth factor receptor in rat liver. J. Biol. Chem. 285: 25904–25912.
Reinehr, R., Sommerfeld, A., and Häussinger, D. (2013). The Src family kinases: distinct functions of c-Src, Yes, and Fyn in the liver. Biomol. Concepts 4: 129–142.
Santosa, D., Castoldi, M., Paluschinski, M., Sommerfeld, A., and Häussinger, D. (2015). Hyperosmotic stress activates the expression of members of the miR-15/107 family and induces downregulation of anti-apoptotic genes in rat liver. Sci. Rep. 5: 12292.
Schliess, F. and Häussinger, D. (2003). Cell volume and insulin signaling. Int. Rev. Cytol. 225: 187–228.
Schliess, F., Kurz, A.K., vom Dahl, S., and Häussinger, D. (1997). Mitogen-activated protein kinases mediate the stimulation of bile acid secretion by tauroursodeoxycholate in rat liver. Gastroenterology 113: 1306–1314.
Schliess, F., Reissmann, R., Reinehr, R., vom Dahl, S., and Häussinger, D. (2004). Involvement of integrins and src in insulin signaling toward autophagic proteolysis in rat liver. J. Biol. Chem. 279: 21294–21301.
Schliess, F., Schreiber, R., and Häussinger, D. (1995). Activation of extracellular signal-regulated kinases Erk-1 and Erk-2 by cell swelling in H4IIE hepatoma cells. Biochem. J. 309: 13–17.
Schliess, F., vom Dahl, S., and Häussinger, D. (2001). Insulin resistance induced by loop diuretics and hyperosmolarity in perfused rat liver. Biol. Chem. 382: 1063–1069.
Schmitt, M., Kubitz, R., Lizun, S., Wettstein, M., and Häussinger, D. (2001). Regulation of the dynamic localization of the rat Bsep gene-encoded bile salt export pump by anisoosmolarity. Hepatology 33: 509–518.
Schreiber, R., Stoll, B., Lang, F., and Häussinger, D. (1994). Effects of aniso-osmolarity and hydroperoxides on intracellular pH in isolated rat hepatocytes as assessed by (2',7')-bis(carboxyethyl)-5(6)-carboxyfluorescein and fluorescein isothiocyanate-dextran fluorescence. Biochem. J. 303: 113–120.
Simon, D.I. (2011). Opening the field of integrin biology to "biased agonism". Circ. Res. 109: 1199–1201.
Sommerfeld, A., Mayer, P.G.K., Cantore, M., and Häussinger, D. (2015a). Regulation of plasma membrane localization of the Na^+-taurocholate cotransporting polypeptide (Ntcp) by hyperosmolarity and tauroursodeoxycholate. J. Biol. Chem. 290: 24237–24254.
Sommerfeld, A., Reinehr, R., and Häussinger, D. (2015b). Tauroursodeoxycholate protects rat hepatocytes from bile acid-induced apoptosis via β1-integrin- and protein kinase A-dependent mechanisms. Cell. Physiol. Biochem. 36: 866–883.
Springer, T.A., Zhu, J., and Xiao, T. (2008). Structural basis for distinctive recognition of fibrinogen gamma C peptide by the platelet integrin alphaIIbbeta3. J. Cell Biol. 182: 791–800.
Sun, Z., Costell, M., and Fässler, R. (2019). Integrin activation by talin, kindlin and mechanical forces. Nat. Cell Biol. 21: 25–31.
Takagi, J., Petre, B.M., Walz, T., and Springer, T.A. (2002). Global conformational rearrangements in integrin extracellular domains in outside-in and inside-out signaling. Cell 110: 599–511.
Trauner, M., Halilbasic, E., Claudel, T., Steinacher, D., Fuchs, C.D., Moustafa, T., Pollheimer, M.J., Krones, E., Kienbacher, C., Traussnigg, S., et al. (2015). Potential of nor-ursodeoxycholic acid in cholestatic and metabolic disorders. Dig. Dis. 33: 433–439.
Unoki, H., Takahashi, A., Kawaguchi, T., Hara, K., Horikoshi, M., Andersen, G., Ng, D.P.K., Holmkvist, J., Borch-Johnsen, K., Jørgensen, T., et al. (2008). SNPs in KCNQ1 are associated with susceptibility to type 2 diabetes in East Asian and European populations. Nat. Genet. 40: 1098–1102.
Violin, J.D. and Lefkowitz, R.J. (2007). Beta-arrestin-biased ligands at seven-transmembrane receptors. Trends Pharmacol. Sci. 28: 416–422.
Volpes, R., van den Oord, J.J., and Desmet, V.J. (1993). Integrins as differential cell lineage markers of primary liver tumors. Am. J. Pathol. 142: 1483–1492.
vom Dahl, S., Hallbrucker, C., Lang, F., Gerok, W., and Häussinger, D. (1991). Regulation of liver cell volume and proteolysis by glucagon and insulin. Biochem. J. 278: 771–777.
vom Dahl, S., Schliess, F., Reissmann, R., Görg, B., Weiergräber, O., Kocalkova, M., Dombrowski, F., and Häussinger, D. (2003). Involvement of integrins in osmosensing and signaling toward autophagic proteolysis in rat liver. J. Biol. Chem. 278: 27088–27095.
Whittard, J.D. and Akiyama, S.K. (2001). Positive regulation of cell-cell and cell-substrate adhesion by protein kinase A. J. Cell Sci. 114: 3265–3272.
Xiong, J.-P., Stehle, T., Diefenbach, B., Zhang, R., Dunker, R., Scott, D.L., Joachimiak, A., Goodman, S.L., and Arnaout, M.A. (2001). Crystal structure of the extracellular segment of integrin αVβ3. Science 294: 339–345.
Xiong, J.-P., Stehle, T., Zhang, R., Joachimiak, A., Frech, M., Goodman, S.L., and Arnaout, M.A. (2002). Crystal structure of the extracellular segment of integrin αVβ3 in complex with an Arg-Gly-Asp ligand. Science 296: 151–155.
Yasuda, K., Miyake, K., Horikawa, Y., Hara, K., Osawa, H., Furuta, H., Hirota, Y., Mori, H., Jonsson, A., Sato, Y., et al. (2008). Variants in KCNQ1 are associated with susceptibility to type 2 diabetes mellitus. Nat. Genet. 40: 1092–1097.
Zhu, J., Zhu, J., and Springer, T.A. (2013). Complete integrin headpiece opening in eight steps. J. Cell Biol. 201: 1053–1068.

Bionotes

Michele Bonus
Institute for Pharmaceutical and Medicinal Chemistry, Heinrich Heine University Düsseldorf, Universitätsstr. 1, D-40225 Düsseldorf, Germany
https://orcid.org/0000-0003-4411-7342

Michele Bonus graduated in pharmacy from Heinrich Heine University Düsseldorf in 2012 and has been working in Holger Gohlke's research

group since 2013. His research focuses on the computational modeling of dynamic protein-ligand interactions in membrane proteins and the computer-aided identification and design of novel small-molecule protein ligands. From 2014 onwards, he studied the activation of $\alpha_5\beta_1$ integrins by bile acids and the translocation mechanism of the lipid floppase ABCB4 as a Research Associate in the Collaborative Research Centre 974 SFB (CRC 974) – "Communication and System Relevance in Liver Injury and Regeneration". During his research work for SFB CRC 974, he was head of the organizing committee of the international PhD symposium "deLIVER – Technology in Hepatology". Since 2017, as a Research Associate in the Research Unit 2518 – "Functional dynamics of ion channels and transporters – DynIon", he has been studying the dynamics of Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels in the presence and absence of cyclic nucleotides. He received several computing time grants from the Jülich Supercomputing Centre (JSC).

Dieter Häussinger
Clinic for Gastroenterology, Hepatology, and Infectious Diseases, Heinrich Heine University Düsseldorf, Moorenstr. 5, D-40225 Düsseldorf, Germany
haeussin@uni-duesseldorf.de

Dieter Häussinger was born on June 22, 1951, in Nördlingen/Bavaria. From 1994 until 2020, he was a full professor in internal medicine at the Heinrich-Heine-University Düsseldorf and the director of the Clinic for Gastroenterology, Hepatology and Infectious Diseases, the Center for Liver and Infectious Disease and of the Hirsch Institute for Tropical Medicine at Arsi University, Ethiopia. He was the coordinator of the Colloborative Research Centers SFB 575 (Experimental Hepatology) and SFB 974 (Liver Injury and Regeneration), and the Clinical Research Group Klinische Forschergruppe 217 (Hepatobiliary Transport). He is also a member of the National Academy of Sciences Leopoldina and the North Rhine-Westphalian Academy for Science and Arts. From 2010 to 2018 he was also a senator of the Scientific Society Leibniz (WGL). He received the Thannhauser-Prize in 1989, the Gottfried Wilhelm-Leibniz-Prize in 1991, the Dr. Robert Pfleger Prize in 2002, the Order of Merit 1st class of the German Federal Republic in 2011, and the Order of Merit of North-Rhine Westfalia (2020).

Holger Gohlke
Institute for Pharmaceutical and Medicinal Chemistry, Heinrich Heine University Düsseldorf, Universitätsstr. 1, D-40225 Düsseldorf, Germany
John von Neumann Institute for Computing (NIC), Jülich Supercomputing Centre (JSC)
Institute of Biological Information Processing (IBI-7: Structural Biochemistry), and Institute of Bio- and Geosciences (IBG-4: Bioinformatics), Forschungszentrum Jülich GmbH, Wilhelm-Johnen-Str., D-52428 Jülich, Germany
gohlke@uni-duesseldorf.de
https://orcid.org/0000-0001-8613-1447

Holger Gohlke is Professor of Pharmaceutical and Medicinal Chemistry at the Heinrich-Heine-University Düsseldorf and head of the NIC Research Group "Computational Biophysical Chemistry" at Forschungszentrum Jülich. He obtained his diploma in chemistry from the Technical University of Darmstadt and his PhD from Philipps-University, Marburg. He then did postdoctoral research at The Scripps Research Institute, La Jolla, USA. After appointments as Assistant Professor in Frankfurt and Professor in Kiel, he moved to Düsseldorf in 2009 and started in Jülich in 2017. He was awarded the "Innovationspreis in Medizinischer und Pharmazeutischer Chemie" from the GDCh and the DPhG, the Hansch Award of the Cheminformatics and QSAR Society, and the Novartis Chemistry Lecturship, and he is the speaker of the DFG-funded Research Training Group GRK 2158 (Natural products and natural product analogs against therapy-resistant tumors and microorganisms). His current research focuses on the understanding, prediction, and modulation of interactions involving biological macromolecules from a computational perspective. His group applies and develops techniques grounded in molecular bioinformatics, computational biology, and computational biophysics.