motor phenotypes of HSPs. It also remains unclear whether pure HSPs can be linked to cell-autonomous processes or are non-cell autonomous, and indeed whether there are "protective" features in nonspinal targets of upper motoneurons that result in some sparing of neuronal function.

In summary, Beetz and colleagues have generated what promises to be a very useful mouse model of HSP. Future studies of these mice should uncover important features of the pathogenic mechanisms underlying this disease. These mice will also be useful in developing a deeper understanding of ER membrane biology. It is likely that we will "REEP" the benefit of this work for some years to come.

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Zinc, insulin, and the liver: a ménage à trois

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Insulin and Zn^{2+} enjoy a multivalent relationship. Zn^{2+} binds insulin in pancreatic β cells to form crystalline aggregates in dense core vesicles (DCVs), which are released in response to physiological signals such as increased blood glucose. This transition metal is an essential cofactor in insulin-degrading enzyme and several key Zn^{2+} finger transcription factors that are required for β cell development and insulin gene expression. Studies are increasingly revealing that fluctuations in Zn^{2+} concentration can mediate signaling events, including dynamic roles that extend beyond that of a static structural or catalytic cofactor. In this issue of the *JCI*, Tamaki et al. propose an additional function for Zn^{2+} in relation to insulin: regulation of insulin clearance from the bloodstream.

Zinc is an abundant and essential element that plays a number of regulatory roles in biology. Studies in model organisms indicate that zinc receptor proteins control complex networks of genes in Zn^{2+}-responsive manners. Moreover, complex developmental events are controlled by dynamic fluctuations of billions of zinc ions between intracellular compartments and extracellular sites (1, 2). Secretory compartments enriched in Zn^{2+} are found in a number of cell types, including hippocampal and olfactory neurons, oocytes, and pancreatic β cells. Specific stimulation of these cells leads to Zn^{2+} exocytosis; however, neither the physiological nor the biochemical roles of the released Zn^{2+} are yet clear in these systems. In this issue of the *JCI*, Tamaki et al. describe striking connections among zinc compartmentalization, exocytosis, and insulin uptake by the liver (3).

A link between zinc transport and diabetes

The ZnT8 transporter (encoded by solute carrier family 30 member 8 gene; *SLC30A8*) is located on dense core vesicles (DCVs) in β cells and loads Zn^{2+} into these secretory compartments, where it binds with and stabilizes a hexameric form of insulin (4). ZnT8 is an autoantigen in type 1 diabetes

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Table 1
Phenotypes observed in previous *Slc30a8*-KO studies

	Nicolson et al. 2009 (9)[A]	Pound et al. 2009 (11)[A]	Lemaire et al. 2009 (12)[A]	Wijesekara et al. 2010 (13)[B]	Pound et al. 2012 (10)
β cell mass	Normal	Normal			
Granules	Empty/atypical/ rod-shaped		Complete loss of DCVs	Empty/atypical/ rod-shaped	
Proinsulin processing	Normal		Normal	Decreased	
Glucose during GTT	Glucose intolerant in male, normal in female	No change	No change	Glucose intolerant	No change
Plasma insulin	Decreased in male, normal in female	Decreased fasting insulin	No change	No change	50% decrease in male mixed-background KO, 20% decrease in female C57BL/6-background KO
GSIS	Enhanced	33% decrease	No change	Decreased first phase, normal second phase	35% in male mixed-background KO only
Insulin sensitivity after HFD	Decreased	Normal	Normal		

[A]Whole-body KO. [B]β cell–specific KO.

(5), and a W325R *SLC30A8* polymorphism is associated with T2D (6). *Slc30a8* deletion in mice decreases DCV Zn^{2+} content (4, 7–9). A role for decreased Zn^{2+} in DCV functional defects is not obvious, because some species (e.g., guinea pig) produce insulin molecules that do not bind to Zn^{2+} but still maintain normal insulin secretion.

Tamaki et al. address contradictory findings concerning the effect of *Slc30a8* deletion on insulin secretion in mice (3). Using β cell–specific deletion of *Slc30a8*, they report an insulin hypersecretion phenotype and suggest that functional ZnT8 facilitates autocrine and paracrine roles for the Zn^{2+} burst produced by β cells upon glucose stimulation. In the WT animals, the released Zn^{2+} bolus inhibited further insulin release, while loss of *Slc30a8* resulted in sustained insulin secretion. Intriguingly, despite increased insulin secretion, peripheral insulin levels were lower and the C-peptide/insulin ratio was increased in KO mice. These data indicate that lower insulin levels might be due to increased insulin clearance from the bloodstream. Studies of proinsulin/insulin ratio and rates of insulin clearance in humans with the *SLC30A8* W325R polymorphism were also consistent with this idea.

Zinc inhibition of hepatic insulin uptake

Quantitatively, the liver and the kidneys are the most important sites of insulin uptake and degradation. The liver is the first organ exposed to newly secreted insulin and can clear much of this insulin in a single pass. The authors investigated hepatic insulin clearance in mice with pancreas perfusions and pancreas-liver perfusions while measuring insulin levels in the portal vein and the inferior vena cava. The difference between the two perfusions provided a measure of hepatic extraction. Their findings indicated that *Slc30a8*-KO mouse livers had enhanced single-pass insulin clearance (3). A puff of Zn^{2+} accompanies every burst of insulin secretion (7). The authors hypothesized that Zn^{2+} inhibits insulin secretion and hepatic insulin uptake, and, therefore, loss of the ZnT8 transporter should relieve the inhibition of insulin secretion while simultaneously increasing hepatic insulin uptake. In support of this hypothesis, direct Zn^{2+} injection into the portal vein of mice or incubation with a hepatocyte cell line directly inhibited insulin uptake. In agreement with previous studies, deletion of *Slc30a8* resulted in dramatic reduction of insulin crystals. Despite abnormal DCVs, more insulin was secreted, but was balanced by increased hepatic clearance. Conversely, studies in isolated islets and perfused pancreas showed that Zn^{2+} inhibits insulin secretion (3).

It has been proposed that β cell–derived Zn^{2+} acts on α cells to suppress the glucagon secretion that accompanies insulin secretion. However, Tamaki et al. did not observe any difference in glucagon secretion in the *Slc30a8*-KO mice. Together with prior studies (8), these data suggest that the Zn^{2+} cosecreted with insulin is not responsible for the suppressive effect of insulin secretion on glucagon secretion.

Differing phenotypes

There is a lack of consensus on the effect of *Slc30a8* deletion on insulin secretion and circulation in mice (Table 1). In all reports, the consequence of *Slc30a8* deletion appears to be relatively small. Studies using different mouse colonies with whole-body *Slc30a8* deletion all agreed that *Slc30a8* deletion does not affect glucose homeostasis in mice fed a normal chow diet. The studies disagreed on the role of *Slc30a8* on proinsulin processing, glucose-stimulated insulin secretion (GSIS), and glucose tolerance. Nicolson et al. showed that while their male KO mice process proinsulin normally, they are glucose intolerant, secrete significantly less insulin during an intraperitoneal glucose tolerance test, and have increased fasting glucose levels (9). Interestingly, GSIS was significantly elevated in isolated islets. Conversely, Pound et al. found that their *Slc30a8*-KO mice have normal glucose tolerance, but significantly reduced fasting plasma insulin levels, along with a 33% reduction in GSIS (10, 11). The *Slc30a8*-KO mice studied by Lemaire et al. lacked any significant phenotype when on normal chow (12). Another study reported that β cell–specific deletion of *Slc30a8* leads to decreased proinsulin processing, significantly reduced first-phase insulin secretion, and glucose intolerance during an oral glucose tolerance test (13).

Interestingly, there are fewer phenotypic differences between colonies of mice subjected to prolonged high-fat diet (HFD) feeding. The studies agreed that *Slc30a8*-KO mice fed HFD display greater weight gain, fasting hyperglycemia, fasting hyperinsu-

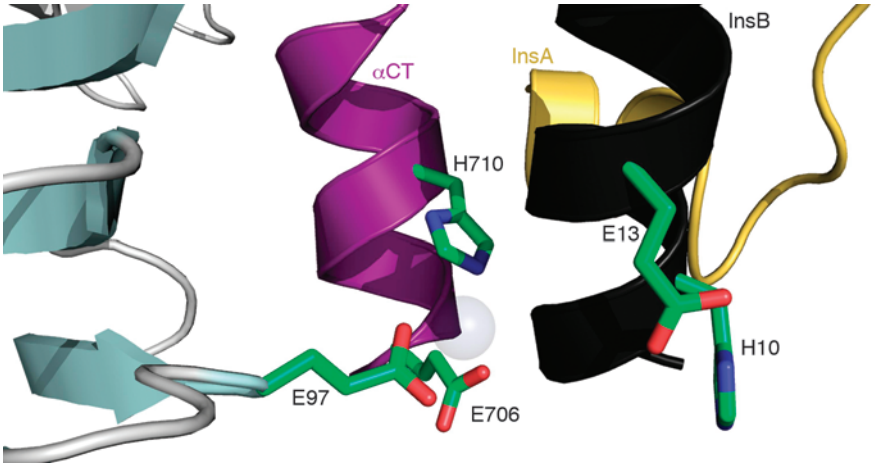

Figure 1
Putative Zn^{2+}-binding residues at the IR-insulin interface. Model of a putative Zn^{2+}-binding site in the insulin-binding site of the IR. The protein coordinates are from the IR-insulin crystal structure (PDB-ID 3W11). Insulin — chains InsA (gold) and InsB (black) are shown — engages with the IR at the αCT (purple). The IR core particle β strands are also shown (light cyan). A Zn^{2+} ion (transparent sphere) has been modeled in the structure based on the environment and known Zn^{2+}-coordinating abilities of the highlighted residues. IR residues (green sticks) H710 and E706 (from the αCT) and E97 form a putative Zn^{2+}-binding site. A water molecule *or* InsB side chains can complete the tetracoordinated Zn^{2+} site after helix rotation/displacement (not shown). Intriguingly, E706 forms a H-bond to a backbone amide on InsB: this docking of the hormone with its receptor could be disrupted by binding of the zinc ion.

linemia, and glucose intolerance (9, 12, 14). The role of *Slc30a8* deletion on insulin sensitivity is still debated. Nicolson et al. (9) and Hardy et al. (14) reported decreased insulin sensitivity in their *Slc30a8*-KO colonies after prolonged HFD feeding; however, Lemaire et al. found insulin sensitivity to be unchanged (12).

Differences in genetic background may explain phenotypic differences. Pound et al. found that *Slc30a8* deletion in two genetic backgrounds renders different phenotypes. They also reported sex-specific effects on resulting *Slc30a8* deletion phenotypes (10). Finally, *Slc30a8* deletion in mouse α cells did not produce a visible phenotype (13).

Linking insulin, zinc, and the liver

Deletion of PCSK1, a proprotein convertase that processes proinsulin, also blocks mature insulin formation and produces β cell DCVs that are essentially devoid of aggregated insulin. The mice have hyperproinsulinemia, but apparently normal glucose tolerance (15). Thus, disruption of DCV maturation does not always lead to impaired regulated secretion. Like *SLC30A8*, *PCSK1* has emerged as a genetic factor of T2D. Deficiency of these genes appears tolerable in animals with a normal demand for insulin secretion, but might produce a bottleneck when challenged by the increased demand posed by insulin resistance.

How might Zn^{2+} influence hepatic extraction of insulin? Tamaki et al. showed that Zn^{2+} levels did not affect the activity of the insulin-degrading enzyme. This observation is consistent with the effect of Zn^{2+} being restricted to insulin internalization. Blockade of insulin internalization was enhanced by pyrithione, a Zn^{2+} ionophore. This suggests that internalized Zn^{2+} is responsible for the inhibitory effect; however, the effect was independent of the Zn^{2+} transporter ZIP-14.

Tamaki et al. make the case that pulsatile release of Zn^{2+} inhibits clathrin-dependent insulin endocytosis via a complex with the insulin receptor (IR). Two physiochemical features of Zn^{2+} binding to proteins may provide an additional and direct antagonistic effect on IR-insulin interaction. First, Zn^{2+} is known to stabilize the hexameric form of insulin in plasma, which may not bind as tightly to the IR. Second, Zn^{2+} may be a competitive inhibitor at the insulin-binding site of IR, acting as an antagonist of insulin binding and/or internalization. In many Zn^{2+}-dependent proteins, Zn^{2+} forms between three and five tight coordinate covalent bonds to side chains such as His, Glu, Asp, and Cys. Inspection of a recent IR structure (16) revealed a cluster of Zn^{2+}-binding histidine and glutamate residues directly adjacent to the insulin-docking site (Figure 1). One of these, H710, was recently shown to be critical in the interaction with insulin (16). We note that this histidine residue is within hydrogen bonding distance to E706, both of which are in the carboxyterminal α chain (αCT) helix of the insulin-binding pocket. With minimal conformational changes, these residues, along with E97 and/or D707 or a water molecule, could form a tetrahedral coordination environment favored for Zn^{2+} binding (Figure 1). This raises the possibility that Zn^{2+} influences insulin clearance in part through direct competition with insulin for IR binding. Intriguingly, insulin monomers contain a potential Zn^{2+} binding site. H10 of the insulin molecule is critical for Zn^{2+} binding the hexameric form, but is absent in insulin variants that do not bind to Zn^{2+} (17). The adjacent E13 is in proximity to coordinate to the same Zn^{2+} ion. It is unknown whether Zn^{2+} occupancy at these putative sites inhibits or perhaps stabilizes the IR-insulin interaction, as is the case for other hormone-receptor interactions (1).

Additional features of Zn^{2+}-histidine coordination chemistry are relevant to insulin biology. The histidine-rich domain of ZnT8 is essential for Zn^{2+} binding. Through binding to two critical histidine residues, Zn^{2+} activates the K_{ATP} channel and hyperpolarizes β cells, which leads to inhibition of insulin secretion (18). This potentially explains how reduced Zn^{2+} uptake by β cells could promote increased insulin secretion.

Future directions

Answering the questions evoked by these findings will require interdisciplinary teams that can pair physical and imaging methods with receptor physiology in model systems. Can Zn^{2+} simultaneously bind histidine and glutamate side chains in the IR and insulin to stabilize the complex and/or to prevent internalization? Are the relative Zn^{2+} affinities of the receptor, hormone, and heterodimeric complex compatible with the transient concentration gradient produced by pulsed Zn^{2+} release into the portal vein? Does Zn^{2+} only block insulin internalization, or does it also block insulin signaling? Answers to this question might help distinguish binding and signaling events. Are there other receptors that bind Zn^{2+} and are affected by the insulin secretion–dependent Zn^{2+} burst? How much Zn^{2+} escapes hepatic clearance and albumin binding to exert actions on extrahepatic tissues?

Although fasting insulin is used as a surrogate measure of insulin resistance, it has a stronger correlation with insulin clearance, which is highly heritable (19, 20). Future human genetic studies will be important for determining whether genetic variation in *SLC30A8* contributes to T2D entirely through its effect on insulin clearance and how much of the heritability of insulin clearance is due to *SLC30A8*.

These studies illustrate how in-depth phenotyping, which requires model organisms, can take clues from human genetics and provide mechanistic explanations for relationships between genetic variation and human disease. Results from these studies can now be used to study subphenotypes associated with diabetes susceptibility. In this case, it may motivate study of the relationship among inorganic physiology (such as the Zn^{2+} fluxes described here), genetic variation at the *SLC30A8* locus, and insulin clearance. Most importantly, these deeper phenotypes should be present in nondiabetics, and thus can be studied independently of the disease.

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Opening lines of communication in the distal nephron

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The distal nephron is composed of two main cell types: principal cells and intercalated cells. These cells have distinct morphologic features that allow them to be readily distinguished by light microscopy, as well as distinct suites of proteins that facilitate cell-specific transport properties. In this issue of the *JCI*, Gueutin and colleagues describe a new mechanism by which β-intercalated cells, via release of ATP and prostaglandin E_2 (PGE_2), influence the activity of transporters in principal cells.

Challenging tradition

The traditional view of the distal nephron considers principal cells to be primarily responsible for reabsorption of filtered Na^+ and for K^+ secretion into the ultrafiltrate, whereas intercalated cells account for urinary acidification. Sodium reabsorption by principal cells occurs via the Na^+/Cl^- cotransporter (NCC) in the early distal nephron and the epithelial Na^+ channel (ENaC) in later nephron segments. Potassium is secreted via the renal outer medullary K^+ channel (ROMK; Figure 1A). Intercalated cells are primarily responsible for urinary acidification, through H^+ secretion by the vacuolar H^+-ATPase or the H^+/K^+-ATPase found in α-intercalated cells. When required, HCO_3^- secretion into the ultrafiltrate occurs via the Cl^-/HCO_3^- exchanger pendrin (also known as SLC4A4) in β-intercalated cells (1, 2). This view presumes limited crosstalk between principal and intercalated cells, as a lack of gap junctions between these cell types limits their communication (3, 4).

As we learn more about properties of the distal nephron, distinctions between principal and intercalated cells are beginning to fade. Recent studies have shown

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