among them central regulators of cholesterol metabolism. Accordingly, a more comprehensive appraisal of DHR96 function in lipometabolism has to await continuative, functional studies on its other target genes. As it is the baton which grants the conductors control over the orchestra, it is the ligand which empowers nuclear receptors. As yet DHR96 is an orphan nuclear receptor but belongs to a family in which some members made their career as prominent drug targets. Accordingly, the identification of the endogenous DHR96 ligand(s) is an outstanding future challenge in view of the potential functional conservation among the xenobiotic receptors of flies and man with respect to the presented novel mode of fat storage control.

Showing that Orlistat slims *Drosophila* is not only good news for flies concerned about their "wasp waists." This finding also provides proof of concept for small compound in vivo screens to identify modulators of dietary fat digestion using the fly model. Collectively, this study underscores the value of *Drosophila* as a rising model system for energy metabolism research (Baker and Thummel, 2007; Schlegel and Stainier, 2007) with relevance for the understanding of physiological and pathophysiological processes in fat storage regulation of mammals and man.

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How Iron Controls Iron

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Cells regulate iron homeostasis by posttranscriptional regulation of proteins responsible for iron uptake and storage. This requires RNA-binding activity of iron-regulatory proteins, IRP1 and IRP2. Two studies recently published in *Science* by Vashisht et al. (2009) and Salahudeen et al. (2009) reveal how cells adjust IRP2 activity.

Iron-containing enzymes are essential for the survival of both uni- and multicellular organisms, as they function in energy-producing redox reactions, oxygen transport, DNA synthesis, and cellular detoxification. Iron associates with proteins most commonly by its insertion into a porphyrin ring as heme or its assembly with sulfur in Fe-S clusters. In some proteins, di- or trivalent iron is bound directly to specific pockets in the secondary structure. Prior to its incorporation, iron needs to be bioavailable as "free" iron. This free iron is potentially harmful because of its ability to generate reactive oxygen species through Fenton chemistry. Thus, cells must carefully regulate iron homeostasis to ensure sufficient iron supply while limiting iron toxicity.

In mammals, two distinct regulatory circuits control body and cellular iron homeostasis. Body iron is sensed by the liver, which in response to high iron synthesizes and secretes hepcidin. This peptide hormone negatively regulates iron export from intestinal cells to limit iron absorption from the diet. Cellular iron homeostasis is achieved by the cytoplasmic RNA-binding proteins IRP1 and IRP2, which regulate posttranscriptionally the fate of mRNAs encoding proteins crucial for iron metabolism, such as transferrin receptor 1 (TfR1) and ferritin H and L (Figure 1). At low cellular iron concentrations, IRPs are active and bind to conserved RNA hairpin structures, known as iron-responsive elements (IREs). Binding to five IREs in the 3′ untranslated region of TfR1 mRNA inhibits mRNA degradation, thereby increasing TfR1 expression and iron uptake. Binding to one IRE in the 5′ untranslated region of ferritin mRNA inhibits ferritin translation, thereby reducing cellular iron storage. Increased iron uptake and reduced iron storage cumulatively augment the free iron pool. High iron levels, in turn, inactivate IRP1 and IRP2 RNA-binding activity. IRP1 inserts a 4Fe-4S cluster, which converts it into a cytosolic aconitase, while IRP2 is targeted for proteasomal degradation. Initial studies concluded that a unique 73 amino acid region of IRP2, which is absent in IRP1, was modified by iron-dependent oxidation and then recognized by heme-oxidized IRP2 ubiquitin ligase 1 (HOIL-1) (Yamanaka et al., 2003). These conclusions were, however, contradicted by studies showing that deletion of the 73 amino acid region or RNA interference against HOIL-1 did not abrogate iron-dependent IRP2 degradation (Hanson et al., 2003; Wang et al., 2004; Zumbrennen et al., 2008). In addition, a constitutive apo-IRP1 mutant was sensitive to iron-dependent proteasomal degradation,

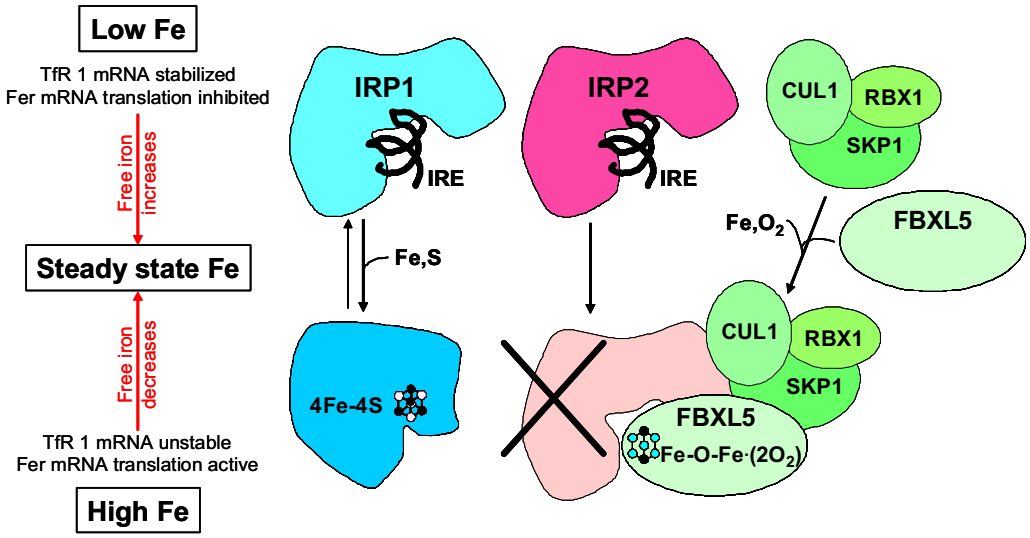

Figure 1. Mechanisms that Control Cellular Iron Homeostasis

The scheme depicts the two iron regulatory proteins, IRP1 and IRP2, which are active RNA-binding proteins at low-free-iron conditions. They control posttranscriptionally the fate of mRNAs encoding the most essential proteins in iron metabolism, TfR1 and ferritin (Fer), which function in cellular iron import and storage, respectively, thus adjusting the free iron pool. Free iron contributes to the assembly of the 4Fe-4S cluster that inactivates IRP1 (light blue), converting it to a cytoplasmic aconitase (dark blue) and to the di-iron-oxygen center in the hemerythrin domain of FBXL5, which then binds IRP2 (fuchsia pink) and induces its degradation by the proteasomal pathway. Thus, free iron acts on its own level through these elaborate feedback loops. Its steady state will equilibrate at the concentration required for the iron center synthesis.

suggesting a conserved mechanism of degradation for IRP2 and apo-IRP1 (Clarke et al., 2006).

Now, two laboratories have independently identified an E3 ubiquitin ligase complex that is required for IRP2 degradation (Vashisht et al., 2009; Salahudeen et al., 2009). Salahudeen et al. used an siRNA screen to identify proteins required for the iron-dependent degradation of IRP2. They identified FBXL5, SKP1, cullin1 (CUL1), and RBX1, which form the newly discovered E3 ligase complex, as critical proteins in IRP2 iron degradation. Vashisht et al. generated an FBXL5 mutant lacking the F-box domain (FBXL5-ΔF-box), which is unable to interact with SKP1 and CUL1 and therefore functions as a substrate-trapping reagent. Mass spectrometry analysis of FBXL5-ΔF-box-interacting proteins showed that FBXL5 interacted with both IRP2 and IRP1. Both laboratories showed that the interaction between FBXL5 and IRP2 or IRP1 is iron dependent and that in vitro or in vivo IRP2 ubiquitination is increased in the presence of FBXL5. Furthermore, RNA interference against FBXL5 eliminated the iron-dependent degradation of both IRP2 and apo-IRP1, indicating that SCF^{FBXL5} functions as the E3 ligase for both proteins.

The mechanism by which iron regulates the interaction between FBXL5 and IRP2 was revealed by studying the stability of FBXL5 under high- and low-iron conditions. Both groups showed that FBXL5 is degraded by the proteasome when cellular free iron was low, which presumably requires yet another uncharacterized ubiquitin ligase. FBXL5 was also destabilized under low oxygen concentration. The iron- and oxygen-dependent stability of FBXL5 was located at its N-terminal 199 amino acids. This region is predicted to fold into a hemerythrin-like domain and was shown to bind iron. Hemerythrin domains utilize histidine and carboxylate-containing residues to coordinate a di-iron core that can reversibly bind oxygen (Stenkamp, 1994). Mutation of predicted iron-binding residues within the hemerythrin domain of FBXL5 showed that the domain folded appropriately only in the presence of iron and oxygen (Salahudeen et al., 2009). Deletion of the hemerythrin-like domain eliminated the iron-dependent regulation of FBXL5. Hemerythrin domains have not previously been observed in mammalian proteins, but are known to play an important role in oxygen sensing in bacteria (Stenkamp, 1994). Future studies will have to characterize this unique mammalian hemerythrin domain. Since IRP1 also interacts with FBXL5 (Vashisht et al., 2009), one wonders why IRP1 escapes protein degradation. Maybe a swift 4Fe-4S cluster insertion alters its structure to the extent that it is no longer recognized by FBXL5.

We know now that two parallel mechanisms control iron homeostasis, both mediated by the insertion of iron into iron centers (Figure 1). Why would cells need two IRPs? IRP1 and IRP2 seem rather redundant and show similar affinity for known target mRNAs. However, they also have unique in vitro binding specificities (Henderson et al., 1996) that might be reflected in unique target mRNAs in vivo. For $IRP2^{-/-}$ mice, anemia and mild locomotor dysfunction were observed, but the phenotype is partially compensated by IRP1, while $IRP1^{-/-}$ mice showed only mild changes that are well compensated by IPR2 (Meyron-Holtz et al., 2004a). Yet both proteins are essential, as double deletions are embryonic-lethal and phenotypic changes are severely increased in $IRP2^{-/-}IRP1^{+/-}$ mice. The new results incite us now to ask whether the IRP1-inactivating 4Fe-4S cluster and the di-iron-oxygen center of FBXL5 serve the same purpose. Since Fe-S clusters are synthesized in mitochondria, it seems

possible that IRP1 inactivation may depend on the mitochondrial iron concentration, whereas IRP2 would rather sense cytoplasmic iron. In addition, the two iron centers show different properties with respect to oxygen. Assembly of 4Fe-4S clusters is favored by low oxygen concentrations, as they occur in tissues (Meyron-Holtz et al., 2004b). In contrast, stability of the hemerythrin domain of FBXL5 and hence IRP2 degradation is enhanced at high oxygen concentrations (Salahudeen et al., 2009). Therefore, having two IRPs with different modes of regulation provides cells with the opportunity to control iron homeostasis over a wide range of oxygen concentrations.

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