internal compartments. Colocalization of HCP1 with endosomal markers such as Rab 5 or Rab 11 or with other markers may help to characterize the internal compartments in which HCP1 resides.

Once the intracellular compartments to which HCP1 relocates in iron-rich animals have been characterized, the larger question will be how is such trafficking regulated? In iron-rich animals, the iron transporter ferroportin in the basolateral membrane of gut epithelial cells moves to an intracellular location (the lysosomes) after binding to hepcidin, a peptide hormone synthesized in the liver (Nemeth et al., 2004). However, when released from the liver into the bloodstream, hepcidin most likely cannot directly access the apical membrane or internal compartments of gut epithelial cells, suggesting a more complicated mechanism for regulation of HCP1 trafficking. Perhaps the time has come when protein trafficking researchers will recognize that identification of targeting motifs, intracellular compartments, and the regulation of iron and heme transporters in polarized epithelial cells represents an important frontier. Similarly, metallobiology researchers should pay attention to the changes in subcellular locations of transporters such as HCP1 that accompany alterations in physiological stimuli and should attempt to understand how this movement of transporters is regulated.

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Osteoblasts Clock in for Their Day Job

The proteins encoded by clock genes regulate circadian variations of various cellular functions. In this issue of *Cell*, Fu, Karsenty, and colleagues (Fu et al., 2005) demonstrate that clock genes control the proliferation of osteoblasts, the cells that promote growth of bone. These findings indicate that the homeostatic regulation of bone mass is subject to circadian control.

Bones are constantly being remodeled by the opposing actions of bone-promoting cells called osteoblasts and bone-degrading cells called osteoclasts (Rodan and Martin, 2000). Osteoclasts degrade the existing bone matrix, whereas osteoblasts synthesize it anew. Through this process, bone mass is kept constant in healthy adult organisms. Bone remodeling is controlled by a complex set of factors including systemic factors (such as steroid hormones, PTH, and leptin) and those secreted by osteoblasts that regulate osteoclast differentiation (such as RANK ligand and Osteoprotegerin, a soluble decoy receptor for the RANK ligand). Synthesis of new bone by osteoblasts is also controlled by several cell-specific transcription factors. The work of Fu, Karsenty, and colleagues (Fu et al., 2005) described in this issue of *Cell* indicates that an important, new regulator of bone remodeling is the circadian cycle.

Many physiological functions in metazoan organisms are regulated by circadian clocks (Schibler and Sassone-Corsi, 2002; Fu and Lee, 2003). In mammals, circadian variations in peripheral tissues are largely synchronized by the suprachiasmatic nucleus of the hypothalamus. Genes that control these diurnal variations are generically called *clock* genes. Fu et al. (2005) hypothesize that *clock* genes might control bone remodeling. Previous work has in fact shown that expression of two major extracellular matrix proteins in bone, type I collagen and osteocalcin, display circadian variation (Simmons and Nichols, 1966; Gundberg et al., 1985).

In the new work, Fu and coworkers (2005) demonstrate that several *clock* genes are indeed expressed in osteoblasts and, not surprisingly, display a circadian pattern of expression. Their work focuses principally on two of these genes, *Per1* and *Per2*. To begin testing their hypothesis, they asked whether the homeostasis of bone remodeling was altered in *Per*-deficient mice. They found that mice deficient in both *Per1* and *Per2* have markedly increased bone mass, which they demonstrated was due to enhanced osteoblast proliferation and not inhibition of bone degradation. The authors noted that this high bone mass phenotype is similar to

that seen in two other strains of mutant mice—animals deficient in leptin and those deficient in the β2-adrenergic receptor—previously characterized by the Karsenty group (Ducy et al., 2000; Takeda et al., 2002). In these studies, Karsenty and colleagues made the important observation that the neuropeptide leptin controls bone mass by binding to leptin receptors expressed by hypothalamic neurons leading to inhibition of osteoblast proliferation. They speculated that *clock* genes might either control leptin expression or mediate the effects of leptin in osteoblasts. Leptin signaling in the hypothalamus is relayed by the sympathetic nervous system through β2-adrenergic receptors expressed by osteoblasts. Importantly, the mediators of leptin's regulation of bone mass do not affect leptin's other activities such as the control of appetite and gonadal functions.

Evidence from their new work argues against a major role of the *Per clock* genes in the control of *leptin* expression because levels of free leptin are similar in wild-type and *Per*-deficient mice. Instead, the results are consistent with the hypothesis that leptin signaling might control *Per* expression in osteoblasts. In agreement with this notion, the high bone mass phenotype persists when the *Per2* gene is specifically inactivated in osteoblasts of a *Per1*-deficient mouse. Two additional sets of experiments also support this view. First, treatment of osteoblasts with the sympathomimetic substance isoproterenol increased expression of *Per1*, *Per2*, and another *clock* gene. Second, intracerebroventricular infusion of leptin, which decreases bone mass in wild-type mice, did not produce this effect in *Per*-deficient mice, but instead had the paradoxical effect of increasing bone mass.

The latter observation prompted Fu et al. (2005) to examine the mechanisms for the increased proliferation of osteoblasts seen in *Per*-deficient mice. They found that expression of the cell cycle regulatory genes *cyclin D1* and *cyclin E* is upregulated in osteoblasts from *Per*-deficient mice. Similarly, the expression of *c-Myc* and of several *AP1* genes encoding the transcription factors c-*fos*, JunB, and c-Jun is also upregulated in bone. These results provide a plausible molecular mechanism for the increase in osteoblast proliferation in *Per*–deficient animals and suggests that the products of the *Per* genes inhibit osteoblast proliferation by blocking expression of genes that promote proliferation. Moreover, Fu et al. (2005) demonstrate from transfection experiments that clock proteins block the *c-Myc* promoter. When they treated osteoblasts from *Per*-deficient mutant mice with isoproterenol, to mimic the action of leptin in vivo, there was no block in the expression of *cyclins D1* and *E* as was the case with wild-type animals. Isoproterenol increased the expression of *c-fos* and other *AP1* genes in wild-type osteoblasts, and this increase was even greater in osteoblasts from *Per* mutant animals. These findings suggest that although activation of β2-adrenergic receptors boosts the expression of the *AP1* gene, Per proteins inhibit this effect. Previous genetic experiments by others strongly suggest that AP1 increases osteoblast proliferation (Kenner et al., 2004). Thus, leptin signaling through β2-adrenergic receptors, which mediates the effects of leptin in osteoblasts, appears to have two antagonistic effects. The first is the inhibition of osteoblast proliferation and *c-Myc* expression through the action of clock proteins, and the second is the stimulation of osteoblast proliferation through the action of AP1 proteins. It is the stimulation of *AP1* expression by leptin signaling via β2-adrenergic receptors that is likely to account for the further increase in bone mass after intracerebroventricular infusion of leptin in *Per*-deficient mice.

The model that emerges from the results of Fu et al. (2005) suggests that signaling by β2-adrenergic receptors first activates the transcription factor CREB. CREB in turn stimulates expression of *clock* genes, which inhibit proliferation of osteoblasts, and *AP1* genes, which stimulate proliferation of osteoblasts. The Fu et al. (2005) work provides evidence that the inhibition of osteoblast proliferation by clock proteins is the dominant effect.

The Karsenty group has also recently reported that leptin signaling increases expression of RANK ligand in osteoblasts resulting in a boost in the differentiation of osteoclasts leading to enhanced degradation of bone. The increased expression of RANK ligand by osteoblasts is mediated by signaling through the β2-adrenergic receptor, but its effect is also counterbalanced by the leptin-induced expression of a hypothalamic neuropeptide called Cart, which inhibits the expression of RANK ligand in osteoblasts (Elefteriou et al., 2005). Perhaps the decrease in bone mass produced by leptin signaling is such a critical function that both the inhibition of osteoblast proliferation and the increased degradation of bone by osteoclasts need to be buffered and counterbalanced by opposing mechanisms.

A number of other questions are raised by the insightful study of Fu et al. (2005). For instance, are the circadian variations in leptin levels and in *Per* expression synchronized? And, if *Per* gene expression in osteoblasts is regulated autonomously, does leptin enhance the physiological oscillations in *Per* gene expression? Also, is *AP1* gene expression in osteoblasts subject to circadian variation that operates independently of circadian variations controlled by clock proteins? Finally, what is the physiological significance for bone tissue of inhibiting osteoblast proliferation during a defined time period each day? One might speculate that such a time period would allow osteoblasts to focus on other functions such as the expression of their genetic program.

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