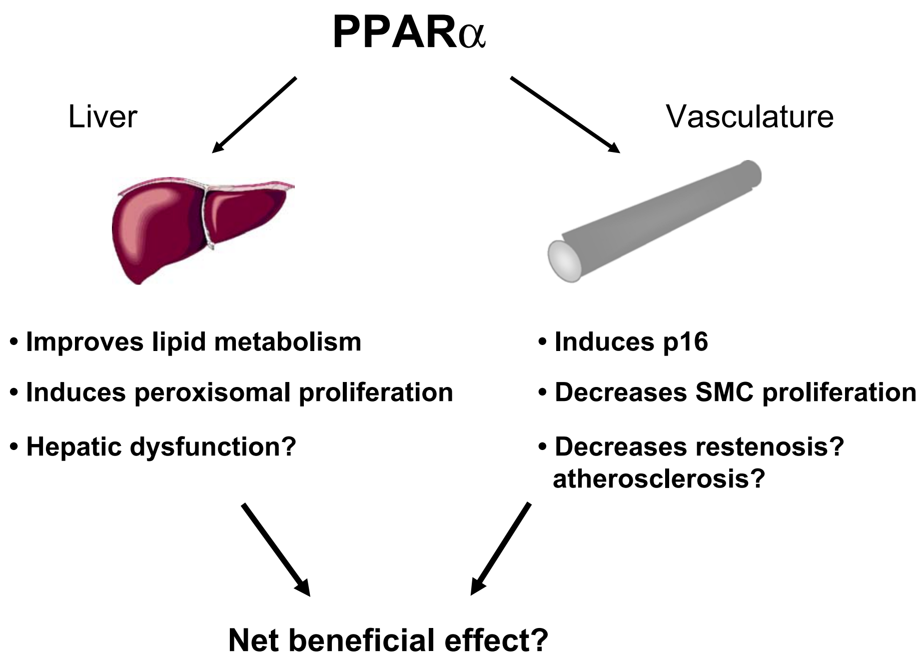

Figure 1. An unexpected process mediated by PPARα

Activation of the nuclear receptor PPARα by fibrate drugs is known to improve systemic lipid metabolism through events occurring predominantly in the liver. Activation of hepatic PPARα can also cause peroxisomal proliferation, liver dysfunction, and tumors (especially in rodents). Recent findings by Gizard and colleagues show that activation of PPARα in vascular smooth muscle cells surprisingly induces the tumor suppressor p16 and decreases smooth muscle proliferation, suggesting a potentially novel strategy for treating restenosis and atherosclerosis.

However, the use of clofibrate (a PPARα agonist no longer available in the United States) was associated with increased total mortality due in part to an increased rate of cancer (Committee of Principal Investigators, 1984).

So is PPARα a savior or a savage? It can probably be both, depending on the degree and site of activation. PPARs are versatile proteins with effects that depend on ligand kinetics, the stoichiometry of available coactivators, and competition with other factors such as repressors. The pleiotropic effects of PPARα could help explain why a recent large fenofibrate clinical trial, the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study, yielded mixed benefits (http://www.americanheart.org/presenter.jhtml?identifier=3035469). By defining a role for PPARα in the control of smooth muscle cell-cycle progression, Gizard and colleagues have extended our understanding of the complex events mediated by this nuclear receptor. Their findings also raise the possibility that local delivery of PPARα activators to sites of vascular disease could be an ideal way to save tissues at risk for vascular disease without savaging other tissues like the liver.

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Selected reading

Chakravarthy, M.V., Pan, Z., Zhu, Y., Tordjman, K., Schneider, J.G., Coleman, T., Turk, J., and Semenkovich, C.F. (2005). Cell Metab. *1*, 309–322.

Committee of Principal Investigators (1984). WHO cooperative trial on primary prevention of ischaemic heart disease with clofibrate to lower serum cholesterol: final mortality follow-up. Lancet *2*, 600–604.

Gizard, F., Amant, C., Barbier, O., Bellosta, S., Robillard, R., Percevault, F., Sevestre, H., Krimpenfort, P., Corsini, A., Rochette, J., et al. (2005). J. Clin. Invest. *115*, 3228–3238.

Issemann, I., and Green, S. (1990). Nature *347*, 645–650.

Mangelsdorf, D.J., Thummel, C., Beato, M., Herrlich, P., Schutz, G., Umesono, K., Blumberg, B., Kastner, P., Mark, M., Chambon, P., and Evans, R.M. (1995). Cell *83*, 835–839.

Peters, J.M., Aoyama, T., Cattley, R.C., Nobumitsu, U., Hashimoto, T., and Gonzalez, F.J. (1998). Carcinogenesis *19*, 1989–1994.

Pietenpol, J.A., and Kastan, M.B. (2004). Control of the cell cycle. In Clinical Oncology, M.D. Abeloff, J.O. Armitage, J.E. Niedehuber, M.B. Kastan, and W.G. McKenna, eds. (New York: Elsevier/Churchill Livingstone), pp. 1–20.

Staels, B., and Fruchart, J.C. (2005). Diabetes *54*, 2460–2470.

Westphal, S., Dierkes, J., and Luley, C. (2001). Lancet *358*, 39–40.

DOI 10.1016/j.cmet.2005.11.006

Collecting new targets in MODY

Transcriptional regulation is crucial in the function of the pancreatic β cell and diabetes, as evidenced by the human MODY families. Work from Fukui and colleagues (Fukui et al., 2005) and Akpinar and colleagues (Akpinar et al., 2005) in this issue of *Cell Metabolism* identifies a target of the MODY3 transcription factor HNF-1α that appears to function both in insulin secretion and β cell proliferation.

Although maturity onset diabetes of the young, or MODY, comprises only a small proportion of all patients with diabetes, it has been extremely useful in elucidating the genetic causes and molecular etiology of diabetes. MODY was originally designated by Tattersall and Fajans (Tattersall and Fajans, 1975), who described the first families with monogenic

diabetes. MODY is characterized by an autosomal dominant mode of inheritance and onset before the age of 25. To date, six MODY genes have been identified (reviewed in Shih and Stoffel, 2002). MODY2 encodes glucokinase, the rate limiting step for glucose metabolism in the pancreatic β cells and liver. Strikingly, the other five MODY genes encode transcription factors: HNF4α [MODY1]; HNF1α [MODY3]; IPF-1 [MODY4]; HNF1β [MODY5]; and *neurogenic differentiation factor 1* (*NeuroD1*) [MODY6], highlighting the importance of transcriptional networks in β cell function. Work from two groups, published in this issue of *Cell Metabolism*, has identified Tmem27 (collectrin) as a HNF-1α target that plays a key role in β cell function.

Although the relative frequency of the MODY mutations in the population varies dramatically, with MODY3 being the most abundant, the penetrance of the phenotype is very high for all known mutations (Froguel and Velho, 1999).Two mouse models have been derived in order to further our understanding of the molecular and physiological analysis of MODY3: mice completely devoid of HNF-1α (also known as Tcf1) function (HNF-1$\alpha^{-/-}$ mice), and transgenic mice expressing a dominant-negative form of HNF-1α in pancreatic β cells (Pontoglio et al., 1996; Yamagata et al., 2002). HNF-1α-deficient islets display a dramatic impairment in stimulus secretion coupling, a phenotype that has been ascribed to defects in glycolysis (Dukes et al., 1998; Pontoglio et al., 1996; Pontoglio et al., 1998; Yamagata et al., 2002). Overexpression of a dominant-negative form of HNF-1α in transgenic mice also causes diabetes, however, this model is characterized by a loss of β cell mass and altered islet structure. Inducible expression of the same dominant-negative HNF-1α mutant in INS-1 insulinoma cells has also been exploited to identify glycolytic and mitochondrial enzymes as HNF-1α targets (Wang et al., 2000). However, it is not clear whether the known HNF-1α targets explain the full spectrum of disease phenotypes found in MODY3.

The two studies published in this issue of Cell Metabolism employ unbiased genome-wide approaches to identify additional targets of HNF-1α that might have an important function in β cell biology (Akpinar et al., 2005; Fukui et al., 2005). While Akpinar and colleagues utilized expression profiling of HNF-1$\alpha^{-/-}$ islets,

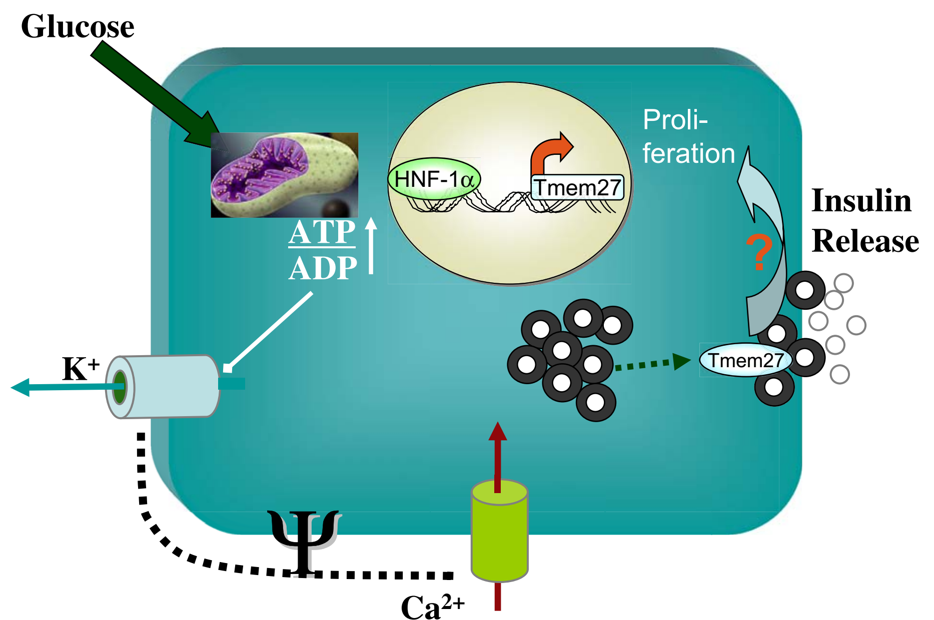

Figure 1. Model for the potential roles of the HNF-1α target Tmem27, also known as "collectrin," in the promotion of insulin exocytosis in pancreatic β cells and β cell proliferation

After glucose enters the β cell through the facilitative glucose transporter GLUT2, it is metabolized by glycolysis and oxidative phosphorylation to raise the cytoplasmic ATP to ADP ratio. This is followed by closure of the ATP-dependent potassium channel, depolarization of the plasma membrane, and Ca^{2+} influx. In the nucleus, HNF-1α is required for the transcriptional activation of Tmem27, which acts in promoting the docking of insulin secretory vesicles through interaction with the SNARE complex, thus facilitating insulin secretion in response to Ca^{2+} influx. The second mechanism of action proposed for Tmem27 is as a promoter of cellular proliferation of β cells through an as yet unknown mechanism.

Fukui and colleagues used suppression subtraction on the INS-1 cell line expressing the dominant-negative mutant of HNF-1α to identify their candidate genes. In a satisfying convergence of functional genomics approaches, both laboratories identified Tmem27, formerly known as Collectrin, as a gene whose expression is critically dependent on HNF-1α in pancreatic β cells. Tmem27 is a glycoprotein that is partly homologous to the C terminus of the angiotensin-converting enzyme-related carboxypeptidase (ACE2). In congruent data from both laboratories, Tmem27 expression was confirmed to be dramatically reduced in HNF-1$\alpha^{-/-}$ islets, and was shown to be a direct HNF-1α target by promoter analysis and co-transfection experiments. Expression of Tmem27 at or near the cell surface was confirmed by both groups by immunostaining. However, beyond this, the results reported by the two groups are strikingly divergent, indicating that Tmem27 might have multiple functions in β cells.

Fukui and colleagues focus on the function of Tmem27 in insulin secretion. Through overexpression of Tmem27 in INS-1 insulinoma cells or in β cells in transgenic mice in vivo, they demonstrate that Tmem27 stimulates insulin secretion by the β cell downstream of Ca^{2+} influx (see Figure 1). Tmem27 was localized to the limiting membrane of secretory vesicles by immuno-electron microscopy. They show further that Tmem27 can bind to the SNARE complex, the mediator of exocytosis, by interacting with snapin, providing a molecular explanation for their discovery. Notably, in their transgenic lines which elevate Tmem27 protein levels about 2-fold, there was no difference in islet morphology or β cell mass.

The findings by Akpinar et al. on Tmem27 function are almost completely opposite. In their transgenic mice with approximately 4-fold overexpression of Tmem27, glucose-stimulated insulin secretion is identical to non-transgenic controls. Instead, they observe a dramatic increase in β cell mass and insulin content in these animals, suggesting a role for this HNF-1α target in β cell proliferation. This notion is supported by their findings that insulinoma cell proliferation is impaired by inhibition of Tmem27 expression via siRNA, and stimulated by Tmem27 overexpression. Akpinar and

colleagues also provide evidence that the N-terminal portion of Tmem27 can be cleaved and shed from β cells. However, this shed fragment showed no biological activity, and the authors conclude that the full-length version of Tmem27 is responsible for cellular proliferation through either a cell-autonomous or cell-cell-signaling effect.

The divergent conclusions drawn on the function of Tmem27 by the two laboratories are difficult to reconcile at present. This is true especially for the observations made on the transgenic mouse models, which in both cases utilize the rat insulin promoter to direct overexpression of Tmem27 to the pancreatic β cell. One possibility for the different phenotypes of the two mouse models is the level of overexpression achieved, which is two- versus 4-fold. Another possibility to consider is that of transgene integration site effects. During the establishment of a transgenic mouse line, the transgene integrates, often as a concatamer of multiple copies, randomly into the genome. On a rare occasion, this integration disrupts the function of another gene, causing a phenotype. Finally, strain differences can also affect the outcome of transgenic mouse experiments. Clearly, a lot more work is required to reconcile these differences and to establish which mechanism of action of Tmem27 is the predominant one.

In summary, it is clear that Tmem27 is a novel and direct target of the MODY3 gene HNF-1α in pancreatic β cells, which has dramatic effects on β cell function. The final evaluation of its molecular mechanism of action will require studies in mice with targeted ablation of this gene. Regardless of the controversy surrounding the function of Tmem27, it is an exciting potential pharmacological target for the treatment of type 2 diabetes.

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Selected reading

Akpinar, P., Kuwajima, S., Krutzfeldt, J., and Stoffel, M. (2005). Cell Metab. *2*, this issue, 385–397.

Dukes, I.D., Sreenan, S., Roe, M.W., Levisetti, M., Zhou, Y.P., Ostrega, D., Bell, G.I., Pontoglio, M., Yaniv, M., Philipson, L., and Polonsky, K.S. (1998). J. Biol. Chem. *273*, 24457–24464.

Froguel, P., and Velho, G. (1999). Trends Endocrinol. Metab. *10*, 142–146.

Fukui, K., Yang, O., Cao, Y., Takahashi, N., Hatakeyama, H., Wang, H., Wada, J., Zhang, Y., Marselli, L., Nammo, T., et al. (2005). Cell Metab. *2*, this issue, 373–384.

Pontoglio, M., Barra, J., Hadchouel, M., Doyen, A., Kress, C., Bach, J.P., Babinet, C., and Yaniv, M. (1996). Cell *84*, 575–585.

Pontoglio, M., Sreenan, S., Roe, M., Pugh, W., Ostrega, D., Doyen, A., Pick, A.J., Baldwin, A., Velho, G., Froguel, P., et al. (1998). J. Clin. Invest. *101*, 2215–2222.

Shih, D.Q., and Stoffel, M. (2002). Curr. Diab. Rep. *2*, 125–134.

Tattersall, R.B., and Fajans, S.S. (1975). Diabetes *24*, 44–53.

Wang, H., Antinozzi, P.A., Hagenfeldt, K.A., Maechler, P., and Wollheim, C.B. (2000). EMBO J. *19*, 4257–4264.

Yamagata, K., Nammo, T., Moriwaki, M., Ihara, A., Iizuka, K., Yang, Q., Satoh, T., Li, M., Uenaka, R., Okita, K., et al. (2002). Diabetes *51*, 114–123.

DOI 10.1016/j.cmet.2005.11.007

Osteoprotegerin, the bone protector, is a surprising target for β-catenin signaling

Osteoblasts influence bone mass by the amount of bone they synthesize and by regulating osteoclasts, the cells that degrade bone. In the December issue of *Developmental Cell*, Kieslinger et al. (2005) show that immature osteoblasts regulate expression of the osteoclast inhibitor, osteoprotegerin, through an early B cell factor and β-catenin signaling.

As baby boomers age, they face many challenges: increasing health insurance costs, diabetes, the metabolic syndrome, Alzheimer's, and the increasing risk of fracture because of thinning bones. Congress, recognizing the need to investigate the molecular mechanisms regulating the function of bone cells, declared a decade of bone and joint research starting in 2002. This has yielded enormous success, with major advances in the understanding of how the skeleton and bone cells are formed and how bone is remodeled (renewed) in adult life. This is exemplified by findings reported earlier this year indicating that Wnt signaling not only determines if mesenchymal progenitors will become chondrocytes or osteoblasts (Hill et al., 2005) but also regulates bone mass through osteoblastic control of bone resorption. Bone is remodeled throughout life. Pockets or trenches of worn out bone are removed during remodeling by osteoclasts, which dig resorption trenches on bone surfaces. Mature osteoblasts fill in the trenches with new stronger bone, in much the same way as workmen repair roadways. Bone mass, strength, and fracture risk are determined by many factors, including the amount of matrix laid down by osteoblasts and removed by osteoclasts.

Somewhat surprisingly, the osteoblast appears to be the foreman controlling bone remodeling. Osteoblasts orchestrate bone destruction by expressing *R*eceptor *A*ctivator of *N*F-κB (RANK) ligand in response to osteoclast-stimulating hormones and cytokines, such as PTH, TNF, and IL-1, but they also protect the skeleton by secreting osteoprotegerin (OPG) (Simonet et al., 1997). RANKL binds to its receptor, RANK, on osteoclast precursors to mediate osteoclast differentiation and subsequent activation; osteoprotegerin, a secreted nonsignaling decoy receptor for RANKL, inhibits both physiological and pathological bone resorption. Surprisingly, *Opg* expression was found to be regulated by β-catenin signaling through TCF1 (Glass et al.,