Becoming fat

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Most people do not wish to become fat, for cosmetic as well as medical reasons. Despite this, there is an international epidemic of obesity fueled by sedentary lifestyles and high caloric consumption among people living in industrialized societies. Obesity is characterized by excess adipose tissue, and rational intervention requires an understanding of adipocyte genes, development, and function. The peroxisome proliferator activated receptor γ (PPARγ), a member of the large family of nuclear hormone receptors, has received enormous attention as its role has emerged in the formation of adipose tissue, as well in the pathogenesis and treatment of diabetes, cardiovascular disease, and cancer [Kersten et al. 2000]. In this issue of Genes & Development, Rosen et al. [2002] and Ren et al. [2002] provide answers to two fundamental questions regarding the role of PPARγ in the making of a fat cell.

Shortly after its initial description as an orphan receptor related to PPARα [Chen et al. 1993; Klauser et al. 1994], PPARγ was found to be particularly abundant in adipose tissue and induced during adipocyte differentiation [Chawla et al. 1994; Tontonoz et al. 1994a]. PPARγ has since been shown to be sufficient [Tontonoz et al. 1994b] as well as necessary [Barak et al. 1999; Rosen et al. 1999] for adipocyte differentiation. A variety of adipocyte genes are transcriptional targets of PPARγ [Auwerx 1999]. PPARγ ligands, most notably the thiazolidinedione (TZD) class, ameliorate insulin resistance and represent exciting new therapies for type 2 diabetes. This effect of TZDs is likely to be multifactorial, including numerous effects on adipocyte gene expression that lead to altered secretion of adipocyte proteins and fatty acids [Olefsky and Saltiel 2000].

In addition to PPARγ, the bZip-containing C/EBP transcription factors have established roles in adipogenesis [Loftus and Lane 1997]. C/EBPβ and C/EBPδ are transiently increased early in adipogenesis [Cao et al. 1991]. When constitutively expressed, these factors are sufficient to induce adipocyte differentiation [Yeh et al. 1995], in part owing to induction of PPARγ [Wu et al. 1995; Schwarz et al. 1997]. C/EBPs is induced later. Like PPARγ, expression of C/EBPα is sustained in the mature adipocytes [Fig. 1A; Birkenmeier et al. 1989; Christy et al. 1989]. C/EBPα has been shown to be critical for adipogenesis [Freytag et al. 1994; Lin and Lane 1994], and C/EBPα and PPARγ positively regulate each other’s expression [Shao and Lazar 1997; Hamm et al. 1999]. However, previous studies have shown that PPARγ can induce adipogenesis in the absence of C/EBPα [Fig. 1B; Hamm et al. 1999, Wu et al. 1999].

Until now it was unknown whether C/EBPα could induce adipogenesis in the absence of PPARγ. This is the fundamental question addressed by Rosen et al. [2002], who mutated the PPARγ gene in mouse embryonic stem cells, which were used to generate embryonic fibroblasts (MEFs) incapable of expressing PPARγ. Normal MEFs can be induced to differentiate into adipocytes, and the PPARγ null cells allowed Rosen and colleagues to spotlight the role of PPARγ in the hierarchy of adipogenic transcription factors. Their study shows that ectopic expression of C/EBPα could not rescue the failure of PPARγ null MEFs to differentiate into adipocytes (Fig. 1C). Therefore, rather than being an equal codirector of the adipocyte differentiation program, PPARγ has a leading role in the adipogenic hierarchy. C/EBPα undoubtedly plays a supporting role in maintaining specific aspects of the adipocyte phenotype, including insulin sensitivity [Hamm et al. 1999; Wu et al. 1999] and lipid accumulation [Wang et al. 1995]. The dominance of PPARγ also sheds light on the mechanism of lipoatrophy in mice expressing a dominant-negative bZip polypeptide from an adipocyte-specific promoter [Moittra et al. 1998]. In this setting, loss of function of all C/EBP proteins most likely interferes with adipocyte differentiation by blocking the induction of PPARγ.

There are two main isoforms of PPARγ: PPARγ1 and PPARγ2 [Fig. 2A]. These are generated from a single gene by differential promoter usage. PPARγ1 protein is the translation product of PPARγ1 and PPARγ3 mRNAs, which differ only in their 5′ untranslated regions [Fajas et al. 1998]. A distinct mRNA encodes PPARγ2 [Tontonoz et al. 1994a]. PPARγ2 contains an additional 31 amino acids at its amino terminus, after which it is completely identical to PPARγ1. The amino terminus of PPARγ functions as a ligand-independent transcriptional activation domain [Adams et al. 1997]. The activation function of the extended amino terminus of PPARγ2 is somewhat more potent than that of PPARγ1, and its activity is affected by insulin [Wernan et al. 1997]. An amino-terminal serine that is phosphorylated by MAP

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kinase and negatively regulates PPARγ activity (residue 112 in mouse PPARγ2) is present in both PPARγ1 and PPARγ2 (Hu et al. 1996; Adams et al. 1997; Shao et al. 1998). Other properties of PPARγ1 and PPARγ2, including DNA binding, ligand binding, and interaction with coactivators, are mediated by identical domains and are also quite similar (Fig. 2A).

In addition to adipocytes, PPARγ is expressed in other cell types, most abundantly in macrophages and colono-
cytes. PPARγ1 is the isoform expressed in extra-adipose tissues, whereas PPARγ2 expression is adipocyte-specific. In macrophages, PPARγ ligands inhibit cytokine gene expression [Jiang et al. 1998; Ricote et al. 1998] and induce the expression of another nuclear receptor, LXR, that enhances the expression of ABC reverse cholesterol transporters [Repa et al. 2000; Chawla et al. 2001b; Chinetti et al. 2001]. Together these effects are antiatherosclerotic, as empirically shown in rodents [Li et al. 2000] and humans (Minamikawa et al. 1998). In colono-
cytes, activation of PPARγ has been suggested to increase proliferation in the min mouse model of intestinal neoplasia (Lefebvre et al. 1998; Saez et al. 1998) yet to antiprolifera-
tive in other colon cancer models [Brockman et al. 1998; Sarraf et al. 1998]. Ligands for PPARγ also have beneficial effects in inflammatory bowel disease [Su et al. 1999; Desreumaux et al. 2001]. Although some of the anti-inflammatory effects of TZD ligands may be PPARγ-independent [Chawla et al. 2001a], PPARγ-depen-
dent effects on nonadipose tissues are almost cer-
tainly mediated by PPARγ1 because that is the predomi-
nant isoform. In contrast, the relative importance of the two PPARγ isoforms for adipogenesis has remained an open question because PPARγ1 and PPARγ2 are ex-
pressed at comparable levels in adipocytes.

Ren and coworkers (2002) have elegantly addressed this question. First, PPARγ expression was eliminated in

Figure 1. Role of PPARγ and C/EBPα in adipogenesis. (A) PPARγ and C/EBPα are both induced during normal adipogenesis. (B) Expression of PPARγ overcomes the absence of C/EBPα. (C) Expression of C/EBPα fails to overcome the absence of PPARγ. (TG) Fat stored as triglycerides.
mouse 3T3-L1 preadipocytes, a classic model for studying adipogenesis, using a novel strategy. Transcriptional repressors were engineered to bind specifically to the PPARγ gene via novel zinc fingers, and inhibit PPARγ gene expression via a potent KRAB repression domain. Lack of PPARγ was established and found to prevent adipocyte differentiation, confirming previous work in PPARγ null models (Barak et al. 1999; Rosen et al. 1999). Ren and coworkers then used retroviruses to restore expression of either PPARγ1 or PPARγ2. Although both proteins could be expressed at comparable levels, only PPARγ2 was able to rescue the adipogenic phenotype [Fig. 2B]. This experiment conclusively shows that PPARγ2 is uniquely suited for adipogenesis.

This result at first seems inconsistent with previous studies suggesting that the amino terminus of PPARγ is unnecessary for adipogenesis (Tontonoz et al. 1994b; Shao et al. 1998). However, those studies involved ectopic expression of wild-type and mutant PPARγ in cells that, unlike those used by Ren et al., could express PPARγ2 from the endogenous gene. Because PPARγ positively regulates its own expression, it is very possible that induction of endogenous PPARγ2 by ectopic PPARγ1 might reconcile the earlier studies with the present result. It would actually be quite interesting and important to confirm the divergent adipogenic functions of PPARγ1 and PPARγ2 in the PPARγ null MEFs used by Rosen et al. (2002), because endogenous PPARγ2 could...
not be generated after PPARγ1 expression in this model system.

A positive feedback role for PPARγ1 on PPARγ2 might be an important function of endogenous PPARγ1, whose protein levels are actually higher than those of PPARγ2 in adipocytes [Xue et al. 1996]. This would be analogous to the role of C/EBPs in up-regulating PPARγ expression during normal adipogenesis. The model of PPARγ gene expression knockout developed by Rosen et al. (2002) could also be used to confirm the dominance of C/EBPs over PPARγ in adipose conversion of 3T3-L1 cells, the system in which the importance of C/EBPs in adipocyte differentiation has been most rigorously evaluated.

The dramatic difference in adipogenic potential of PPARγ1 and PPARγ2 is surprising, given their highly similar properties when studied in vitro. However, there are other examples of transcription factors whose biologically relevant functional domains were not predictable from molecular studies. For example, the transactivation domain of the GATA-1 transcription is dispensable for erythropoiesis, whereas another domain is critical [Weiss et al. 1997]. C/EBPs itself has cellular functions that are independent of DNA binding [McKnight 2001]. The unique role of PPARγ in adipogenesis is particularly interesting in light of the observation that a common human genetic variation in PPARγ affecting only PPARγ2 (the Pro12Ala mutation) provides protection from insulin resistance [Deeb et al. 1998; Altshuler et al. 2000]. This variation has very modest effects on the measurable in vitro functions of the PPARγ2 protein [Deeb et al. 1998].

Together, these new findings move the field forward by illuminating the role of the γ2 isoform of PPARγ as a critical regulator of adipogenesis. PPARγ1 and C/EBPs proteins are induced during adipogenesis and are highly expressed in mature adipocytes, where C/EBPs and probably PPARγ1 play important functional roles. However, PPARγ2 would seem to be the preferred target for treatments aimed at stopping a cell, and ultimately a person, from becoming fat.

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References

Adams, M., Reginato, M.J., Shao, D., Lazar, M.A., and Chatterjee, V.K. 1997. Transcriptional activation by PPARγ is inhibited by phosphorylation at a conserved mitogen-activated protein kinase site. J. Biol. Chem. 272:5128–5132.

Altshuler, D., Hirschhorn, J.N., Klannemark, M., Lindgren, C.M., Vohl, M.C., Nemesh, J., Lane, C.R., Schaffner, S.F., Bolk, S., Brewer, C., et al. 2000. The common PPARγ Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. Nat. Genet. 26:76–80.

Auwerx, J. 1999. PPARγ, the ultimate thrifty gene. Diabetologia 42:1033–1049.

Barak, Y., Nelson, M.C., Ong, E.S., Jones, Y.Z., Ruiz-Lozano, P., Chien, K.R., Koder, A., and Evans, R.M. 1999. PPARγ is required for placental, cardiac, and adipose tissue development. Mol. Cell 4:585–595.

Birkenmeyer, E.H., Gwynn, B., Howard, S., Jerry, J., Gordon, J.I., Landschulz, W.H., and McKnight, S.L. 1989. Tissue-specific expression, developmental regulation, and genetic mapping of the gene encoding CCAAT/enhancer binding protein. Genes & Dev. 3:1146–1156.

Brockman, J.A., Gupta, R.A., and DuBois, R.N. 1998. Activation of PPARγ leads to inhibition of anchorage independent growth of human colorectal cancer cells. Gastroenterology 115:1049–1055.

Cao, Z., Umem, R.M., and McKnight, S.L. 1991. Regulated expression of three C/EBP isoforms during adipose conversion of 3T3-L1 cells. Genes & Dev. 5:1538–1552.

Chawla, A., Schwarz, E.J., Dimcaulangan, D.D., and Lazar, M.A. 1994. Peroxisome proliferator-activated receptor γ [PPARγ]: Adipose predominant expression and induction early in adipocyte differentiation. Endocrinology 135:798–800.

Chawla, A., Barak, Y., Nagy, L., Liao, D., Tontonoz, P., and Evans, R.M. 2001a. PPARγ dependent and independent effects on macrophage-gene expression in lipid metabolism and inflammation. Nat. Med. 7:48–52.

Chawla, A., Boisvert, W.A., Lee, C.-H., Laffitte, B.A., Barak, Y., Joseph, S.B., Liao, D., Nagy, L., Edwards, P.A., Curtiss, L.K., et al. 2001b. A PPARγ-LXR-ABCA1 pathway in macrophages is involved in cholesterol efflux and atherogenesis. Mol. Cell 7:161–171.

Chen, F., Law, S.W., and O’Malley, B.W. 1993. Identification of two mPPAR related receptors and evidence for the existence of five subfamily members. Biochem. Biophys. Res. Commun. 196:671–677.

Chiaretti, G., LeStavel, S., Bocher, V., Remaley, A.T., Neve, B., Torra, I.P., Teissier, E., Minnich, A., Jaye, M., Duberger, N., et al. 2001. PPARα and PPARγ activators induce cholesterol removal from human macrophage foam cells through stimulation of the ABCA1 pathway. Nat. Med. 7:53–58.

Christy, R.J., Yang, V.W., Ntambi, J.M., Geiman, D.E., Landschulz, W.H., Friedman, A.D., Nakabppu, Y., Kelly, T.J., and Lane, M.D. 1989. Differentiation-induced gene expression in 3T3-L1 preadipocytes: CCAAT/enhancer binding protein interacts with and activates the promoters of two adipocyte-specific genes. Genes & Dev. 3:1323–1335.

Deeb, S.S., Fajas, L., Nemoto, M., Phihlajamzki, J., Myekkanen, L., Kuusisto, J., Laakso, M., Fujimoto, W., and Auwerx, J. 1998. A Pro12Ala substitution in PPARγ2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. Nature Genet. 20:284–287.

Desreumaux, P., Dubuxqouy, L., Nutten, S., Peuchmaur, M., Englaro, W., Schoonjans, K., Desvergne, B., Wahl, W., Champon, P., Leibowitz, M.D., et al. 2001. Attenuation of colon inflammation through activators of the retinoid X receptor [RXR]/peroxisome proliferator-activated receptor γ heterodimer. A basis for new therapeutic strategies. J. Exp. Med. 193:827–838.

Fajas, L., Fruchtart, J.C., and Auwerx, J. 1998. PPARγ3 mRNA: A distinct PPAR mRNA subtype transcribed from an independent promoter. FEMS Lett. 148:55–60.

Freytag, S.O., Pauci, D.L., and Gilbert, J.D. 1994. Ectopic expression of the CCAAT/enhancer binding protein α promotes the adipogenic program in a variety of mouse fibroblastic cells. Genes & Dev. 8:1654–1663.

Hamm, J.R., el Jack, A.K., Pilek, P.F., and Farmer, S.R. 1999. Role of PPARγ in regulating adipocyte differentiation and insulin-responsive glucose uptake. Ann. NY Acad. Sci. 892:134–145.
Hu, E., Kim, J.B., Sarraf, P., and Spiegelman, B.M. 1996. Inhibition of adipogenesis through MAP kinase-mediated phosphorylation of PPARγ. *Science* 274: 2100–2103.

Jiang, C., Ting, A.T., and Seed, B. 1998. PPARγ agonists inhibit production of monocyte inflammatory cytokines. *Nature* 391: 82–86.

Kersten, S., Desvergne, B., and Wahli, W. 2000. Roles of PPARs in health and disease. *Nature* 405: 421–424.

Lefebvre, A.M., Chen, I., Desrumaux, P., Najib, J., Furehurt, J.-C., Geboes, K., Briggs, M., Heyman, R., and Auwerx, J. 1998. Activation of the peroxisome proliferator activated receptor γ promotes the development of colon tumors in mice. *J. Clin. Invest.* 102: 253–531.

Lin, F.-T. and Lane, M.D. 1994. CCAAT/enhancer binding protein α is sufficient to initiate the 3T3-L1 adipocyte differentiation program. *Proc. Natl. Acad. Sci. USA* 91: 8757–8761.

Lodish, T.M. and Lane, M.D. 1997. Modulating the transcriptional control of adipogenesis. *Curr. Opin. Genet. Dev.* 7: 603–608.

McKnight, S.L. 2001. McBindall—A better name for CCAAT/enhancer binding proteins? *Cell* 107: 259–261.

Minamikawa, J., Tanaka, S., Yamauchi, M., Inoue, D., and Olefsky, J.M. and Saltiel, A.R. 2000. PPARγ/H9253—Li, A.C., Brown, K.K., Silvestre, M.J., Willson, T.M., Palinski, W., and Glass, C.K. 2000. Peroxisome proliferator-activated receptor γ ligands inhibit development of atherosclerosis in LDL receptor-deficient mice. *J. Clin. Invest.* 106: 523–531.

Morgan, C.P., et al. 1999. Differential expression and activation of a family of murine peroxisome proliferator-activated receptors. *Proc. Natl. Acad. Sci. USA* 91: 7355–7359.

Marcus-Samuels, B., Feigenbaum, L., Lee, E., Aoyama, T., Endocrinol. Metab. 83: 608.

Marcus-Samuels, B., Feigenbaum, L., Lee, E., Aoyama, T., Endocrinol. Metab. 83: 608.

Olefsky, J.M. and Saltiel, A.R. 2000. PPAR γ and the treatment of insulin resistance. *Trends Endocrinol. Metab.* 11: 362–368.

Ren, D., Collingwood, T.N., Rebar, E.J., Wolfe, A.P., and Camp, H.S. 2002. PPARγ knockdown by engineered transcription factors: Exogenous PPARγ2 but not PPARγ1 reactivates adipogenesis. *Genes & Dev.* 16: 27–32 [this issue].

Repa, J.J., Turley, S.D., Lobaccaro, J.A., Medina, J., Li, L., Lustig, K., Shan, B., Heyman, R.A., Dietschy, J.M., and Mangelsdorff, D.J. 2000. Regulation of absorption and ABC1-mediated efflux of cholesterol by RXR heterodimers. *Science* 289: 1524–1529.

Ricote, M., Li, A.C., Willson, T.M., Kelly, C.J., and Glass, C.K. 1998. The peroxisome proliferator-activated receptor-γ is a negative regulator of macrophage activation. *Nature* 391: 79–82.

Sarraf, P., Mueller, E., Jones, D., King, F.J., DeAngelo, D.J., Partridge, J.B., Holder, S.A., Chen, L.B., Singer, S., Fletcher, C., et al. 1998. Differentiation and reversal of malignant changes in colon cancer through PPARγ. *Nat. Med.* 4: 1046–1052.

Schwarz, E.J., Regnato, M.J., Shao, D., Krakow, S.L., and Lazar, M.A. 1997. Retinoic acid blocks adipogenesis by inhibiting C/EBPβ-mediated transcription. *Mol. Cell. Biol.* 17: 1552–1561.

Shao, D. and Lazar, M.A. 1997. PPARγ, C/EBPa, cell cycle status and the commitment to adipocyte differentiation. *J. Biol. Chem.* 272: 21473–21478.

Shao, D., Rangwala, S.M., Bailey, S.T., Krakow, S.L., Regnato, M.J., and Lazar, M.A. 1998. Interdomain communication regulating PPARγ ligand binding. *Nature* 396: 377–380.

Su, C.G., Wen, X., Jiang, W., Rangwala, S.M., Keilbaugh, S.A., Flanagan, A., Murthy, S., Lazar, M.A., and Wu, G.D. 1999. A novel therapy for colitis utilizing PPARγ ligands to inhibit the epithelial inflammatory response. *J. Clin. Invest.* 104: 383–389.

Tontonoz, P., Hu, E., Graves, R.A., Budavari, A.I., and Spiegelman, B.M. 1994a. mPPARγ2: Tissue-specific regulator of an adipocyte enhancer. *Genes & Dev.* 8: 1224–1234.

Tontonoz, P., Hu, E., and Spiegelman, B.M. 1994b. Stimulation of adipogenesis in fibroblasts by PPARγ2, a lipid-activated transcription factor. *Cell* 79: 1147–1156.

Wang, N.D., Finegold, M.J., Bradley, A., Ou, C.N., Abdelsayed, S.V., Wilde, M.D., Taylor, L.R., Wilson, D.R., and Darling, G.J. 1995. Impaired energy homeostasis in C/EBPα knockout mice. *Science* 269: 1108–1112.

Weiss, M.J., Yu, C., and Orkin, S.H. 1997. Erythroid-cell-specific properties of transcription factor GATA-1 revealed by phenotypic rescue of a gene-targeted cell line. *Mol. Cell. Biol.* 17: 1642–1651.

Werman, A., Hollenberg, A., Solanes, G., Bjorbaek, C., Vidal-Puig, A.J., and Flier, J.S. 1997. Ligand-independent activation domain in the N terminus of PPARγ. Differential activity of PPARγ1 and 2 isoforms and influence of insulin. *J. Biol. Chem.* 272: 20230–20235.

Wu, Z., Bucher, N.L.R., and Farmer, S.R. 1995. Conditional ecotropic expression of C/EBPβ in NIH-3T3 cells induces PPARγ and stimulates adipogenesis. *Genes & Dev.* 9: 2350–2363.

Wu, Z., Rosen, E.D., Brun, R., Hauser, S., Adelmant, G., Troy, A.E., McKeon, C., Darling, G.J., and Spiegelman, B.M. 1999. Cross-regulation of C/EBPα and PPARγ controls the transcriptional pathway of adipogenesis and insulin sensitivity. *Mol. Cell* 3: 151–158.

Xue, J.C., Schwarz, E.J., Chawla, A., and Lazar, M.A. 1996. Distinct stages in adipogenesis revealed by retinoid inhibition of differentiation after induction of PPARγ. *Mol. Cell. Biol.* 16: 1567–1575.

Yeh, W.-C., Cao, Z., Classon, M., and McKnight, S.L. 1995. Cascade regulation of terminal adipocyte differentiation by three members of the C/EBP family of leucine zipper proteins. *Genes & Dev.* 9: 168–181.
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