Inhibition of adipogenesis: a new job for the ER Ca^{2+} pool

Jacopo Meldolesi

Vita-Salute San Raffaele University and San Raffaele Institute, 20132 Milan, Italy

Adipogenesis is the process of differentiation of adipocytes from mesenchymal multipotent cells through adipocyte precursors. In this issue, a study by the groups of Opas and Michalak (Szabo, E., Y. Qiu, S. Baksh, M. Michalak, and M. Opas. 2008. *J. Cell. Biol.* 182:103–116) demonstrates that this process is repressed by increasing intracellular Ca^{2+}, which, in turn, is dependent on the expression of calreticulin, the major Ca^{2+}-binding protein of the endoplasmic reticulum lumen.

The rise of the adipose mass, which takes place throughout animal life, is caused not only by the increase of fat cell volume but also by the differentiation of multipotent mesenchymal stem cells. So far, however, this adipogenetic process has been investigated primarily in its terminal phase, the conversion of preadipocytes into mature adipocytes. The determination phase, when stem cells make their initial choice, has been deciphered only in general terms (Rosen and MacDougald, 2006; Gesta et al., 2007). Similar to other differentiation processes, adipogenesis depends on the expression of specific genes governed by both stimulatory (primarily PPARγ and CCAAT enhancer–binding protein [C/EBP]) and repressive (the GATA family) transcription factors activated by various extracellular signals, such as TGFβ, bone morphogenic protein, Notch, and others. An increase in the cellular Ca^{2+} level induced by events such as incubation in high Ca^{2+} media (Jensen et al., 2004) and activation of receptors or channels (Liu and Clipstone, 2007; Zhang et al., 2007) has been reported to inhibit the differentiation of 3T3-L1 preadipocytes. However, these results are often overlooked, possibly because they appear incompatible with the general role most often attributed to Ca^{2+}, the stimulation of rapid (or very rapid) events such as excitation, neurosecretion, and contraction.

In the study by Szabo et al. (see p. 103 of this issue), the role of Ca^{2+} in adipogenesis was reinvestigated by focusing on the general homeostasis of the cation and, in particular, on calreticulin, the major Ca^{2+}-binding protein of the ER lumen. Because of its large capacity (~50 mol/mole) and low affinity (Kd of ~0.5 mM) binding, calreticulin contributes the majority of the rapidly exchanging Ca^{2+} pool in most cells (Bastianutto et al., 1995; Nakamura et al., 2001). Previous studies by the Opas and Michalak groups had shown calreticulin to be needed for development of the heart (Lynch et al., 2006). The new experiments on adipogenesis in embryonic stem (ES) cells have yielded just the opposite result. An astonishing increase of the adipogenic potential (30-fold) and of the number of adipocyte colonies (ninefold) was induced not by the expression but by the down-regulation of calreticulin. Because calreticulin is both a binding Ca^{2+} protein and a chaperone, the authors went on to confirm that the effects on adipogenesis were indeed caused by a decrease in the Ca^{2+} concentration of the ER lumen and cytosol. Expression of either full-length calreticulin or the C-terminal Ca^{2+}-binding region of the protein (Nakamura et al., 2001) in the calreticulin down-regulated ES cells abolished the increase in adipogenic potential. Conversely, expression of the N-terminus chaperoning domain of the protein had no effect. Moreover, duplication of the adipogenetic results in wild-type calreticulin-expressing ES cells loaded with a Ca^{2+} chelator, BAPTA, revealed that the Ca^{2+} decrease is critical at an early checkpoint, within the first 3 d of differentiation. Finally, induction of adipogenesis in 3T3-L1 preadipocytes by exposure to retinoic acid resulted in a down-regulation of calreticulin. Therefore, Ca^{2+} and calreticulin appear to play a key role in the whole adipogenetic process, from the early commitment of stem cells to the late conversion of preadipocytes.

The most unexpected result of the paper by Szabo et al. (2008) dealt with the relationship between calreticulin and PPARγ_2, the master transcription factor of adipogenesis. Binding of PPARγ_2 to two specific sites in the calreticulin gene was found not to inhibit but to stimulate the expression of calreticulin. In addition, PPARγ_2 was found to be down-regulated in cells overexpressing calreticulin. Therefore, there appears to be a negative feedback loop in which PPARγ_2 stimulates the expression of calreticulin, which, in turn, inhibits the activity and expression of PPARγ_2. As a consequence, as long as the stem cells are not stimulated, their high calreticulin prevents their commitment to adipocyte differentiation. The enzyme that appears to mediate the inhibitory action of calreticulin is calcineurin, a well-known Ca^{2+}-dependent protein phosphatase that in other cell types is known to govern the translocation of specific transcription factors to the nucleus (Tomida et al., 2003; Colella et al., 2008). The differentiation of ES cells is also governed by a well-known Ca^{2+}-dependent enzyme, Ca^{2+}/calmodulin-dependent

Correspondence to Jacopo Meldolesi: meldolesi.jacopo@hsr.it

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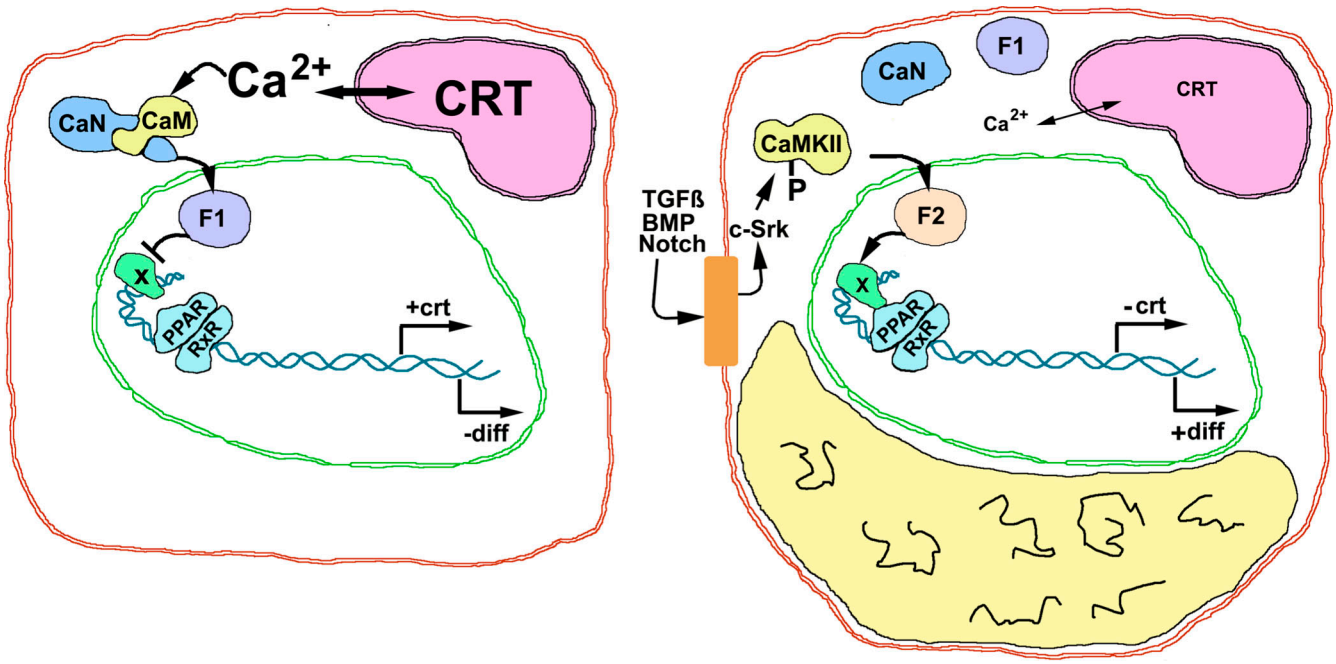

Figure 1. **Comparison of the events controlling adipogenesis in ES cells expressing high and low calreticulin.** In the stem cell to the left, the large pink-stained membrane-bound profile is an ER cisterna rich in calreticulin (CRT), which keeps the concentration of Ca^{2+} in the cytosol (Ca^{2+}) high. The ensuing activation of calcineurin (CaN), which is induced by Ca^{2+} binding to its calmodulin subunit (CaM), delivers a specific signal to the nuclear compartment, possibly by inducing the cytoplasmic–nuclear transport of a transcription factor (F1) that blocks at X the activation of a complex including RXR–PPARγ_2. As a consequence, transcription of genes involved in adipogenesis is blocked (−diff), whereas transcription of the calreticulin gene is stimulated (+crt). This may explain the high level of the protein and its accumulation within the ER. In the stem cell to the right, stimulation by one (or more) of the extracellular agents listed to the left induces the activation of c-Srk, with ensuing tyrosine phosphorylation of CaMKII. In spite of the low concentration of calreticulin (CRT) in the ER and the ensuing low cytosolic concentration of Ca^{2+}, the classic activator of the enzyme, this phosphorylation may be enough to induce the activation of CaMKII. A possible consequence is the nuclear transport of another factor (F2) that promotes the activation of a complex including X and RXR–PPARγ_2, with ensuing block of transcription at the calreticulin gene (−crt) and activation at the genes (+diff) that induce adipogenesis. The ultimate consequences are changes of the cell phenotype (i.e., accumulation of triglycerides in the cytoplasm [yellow mass] and swelling). Notice that in the differentiating cell, calcineurin and F1 are inactive and remain in the cytoplasm.

protein kinase II (CaMKII). In this case, however, CaMKII appears to be activated not by Ca^{2+} but by c-Srk. Phosphorylation by CaMKII results in the activation of PPARγ_2 and cAMP response element binding, a Ca^{2+}-independent transcription factor necessary for activation of the other key regulator of adipogenesis, C/EBPα. Thus, in this case, CaMKII appears to provide a Ca^{2+}-independent pathway for stimulating adipogenesis.

The results by Szabo et al. (2008) provide a comprehensive picture of Ca^{2+} and adipogenesis, revealing a variety of cellular aspects that so far have remained undefined (Fig. 1). In view of the properties of stem cells and adipocytes, the key role of the ER Ca^{2+} store and, therefore, of calreticulin is not surprising. More unexpected was the involvement of Ca^{2+} in the control of the whole differentiation process, from multipotent mesenchymal cells to adipocytes, and the complexity of its connection with the classic transcription factors. These results reveal a new long-term repressive role for calreticulin that is necessary to prevent the commitment of mesenchymal stem cells to adipocyte differentiation. The inhibitory role of Ca^{2+} in adipogenesis is also surprising. Ca^{2+} is known to participate in the regulation of a huge number of processes that are rapid and also slow, such as cell growth. In most of these cases, however, the role of Ca^{2+} is stimulatory. Also unexpected are the opposite roles of the two Ca^{2+}-dependent enzymes, calcineurin and CaMKII; the first role in working to mediate the inhibition of adipogenesis, and the second role as a key activator. Although the activation of CaMKII by Srk-induced tyrosine phosphorylation of calmodulin was known (Corti et al., 1999), to my knowledge, the stimulation by CaMKII of a Ca^{2+}-inhibited process is new.

Inevitably, quite a number of questions remain open. These include understanding the process by which retinoic acid induces the differentiation of wild-type stem cells that are rich in calreticulin and poor in PPARγ_2; deciphering the opposite roles of calreticulin and Ca^{2+} on differentiation of the heart (stimulatory) and adipocytes (inhibitory); and finding the substrates of CaMKII necessary for differentiation and the mechanisms of action of calcineurin (possibly via translocation of transcription factors).

Adipose tissue has long been one of the least investigated systems of the body. Interest has increased considerably since its identification as the largest endocrine system, participating in the control of processes as diverse as blood pressure, immune function, angiogenesis, and energy balance. Concomitantly, the epidemic surge of obesity and type 2 diabetes, with their fallout of morbidity and mortality, has called attention to adipogenesis. The identification of specific Ca^{2+}-dependent aspects of the process therefore appears timely. After all, the efficacy of Ca^{2+}-rich diets against obesity is known both in mouse and man (Shi et al., 2001; Zemel et al., 2004). The results of Szabo et al. (2008) could now help to find specific targets to which new investigative and therapeutic tools could be addressed.

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