carboxylated osteocalcin increases pancreatic insulin production and secretion is not clear. Second, if bone resorption is so critical to the posttranslational modification of osteocalcin and therefore insulin secretion, are individuals on antiresorptive therapy for osteoporosis more likely to be insulin resistant and glucose intolerant? In a small observational study, Kaji et al. (2009) report that fasting glucose levels are higher in women treated for 2 years with alendronate, the most commonly used treatment for osteoporosis. However, fat mass does not differ in these patients, and the statistical relationship between bone density and fasting glucose disappears when corrected for changes in lean mass. In addition, previous human investigations clearly demonstrate that an oral glucose load that increases insulin secretion suppresses markers of bone resorption by 50% (Clowes et al., 2003). Most importantly, and yet much more difficult to address, is whether the relationship between bone and energy metabolism provides an evolutionary advantage and, if so, where skeletal control fits into the complex hierarchy of acute and chronic regulation of insulin secretion during physiologic states. Notwithstanding these unanswered questions, these two studies provide a strong framework for understanding the emerging role of the skeleton in metabolic homeostasis.

ACKNOWLEDGMENTS

This work was supported by a grant from NIAMS: AR45433 and NIDDK-DK 84970.

REFERENCES

Clowes, J.A., Allen, H.C., Prentis, D.M., Eastell, R., and Blumsohn, A. (2003). J. Clin. Endocrinol. Metab. *88*, 4867–4873.

Ducy, P., Amling, M., Takeda, S., Priemel, M., Schilling, A.F., Beil, F.T., Shen, J., Vinson, C., Rueger, J.M., and Karsenty, G. (2000). Cell *100*, 197–207.

Ferron, M., Hinoi, E., Karsenty, G., and Ducy, P. (2008). Proc. Natl. Acad. Sci. USA *105*, 5266–5270.

Ferron, M., Wei, J., Yoshizawa, T., Del Fattore, A., DePinho, R.A., Teti, A., Ducy, P., and Karsenty, G. (2010). Cell, this issue.

Fulzele, K., Riddle, R.C., DiGirolamo, D.J., Cao, X., Wan, C., Chen, D., Faugere, M.-C., Aja, S., Hussain, M.A., Brüning, J.C., and Clemens, T.L. (2010). Cell, this issue.

Hinoi, E., Gao, N., Jung, D.Y., Yadav, V., Yoshizawa, T., Myers, M.G., Jr., Chua, S.C., Jr., Kim, J.K., Kaestner, K.H., and Karsenty, G. (2008). J. Cell Biol. *183*, 1235–1242.

Kaji, H., Hisa, I., Inoue, Y., Naito, J., Sugimoto, T., and Kasuga, M. (2009). J. Bone Miner. Metab. *27*, 76–82.

Lee, N.K., Sowa, H., Hinoi, E., Ferron, M., Ahn, J.D., Confavreux, C., Dacquin, R., Mee, P.J., McKee, M.D., Jung, D.Y., et al. (2007). Cell *130*, 456–469.

Yoshizawa, T., Hinoi, E., Jung, D.Y., Kajimura, D., Ferron, M., Seo, J., Graff, J.M., Kim, J.K., and Karsenty, G. (2009). J. Clin. Invest. *119*, 2807–2817.

Calcium and Energy: Making the Cake and Eating It too?

Douglas R. Green[1,*] and Ruoning Wang[1]

[1]Department of Immunology, St. Jude Children's Research Hospital, Memphis, TN 92105, USA

*Correspondence: douglas.green@stjude.org

DOI 10.1016/j.cell.2010.07.007

Mitochondrial calcium ions promote a number of events that sustain ATP levels in the cell. Cardenas et al. (2010) now demonstrate that the inositol 1,4,5-triphosphate receptor at the endoplasmic reticulum constitutively provides calcium for mitochondria. In the absence of this calcium transfer, cells use autophagy to sustain survival.

Life is all about capturing energy and putting it to use seeking more energy, avoiding being someone else's energy, building and repairing parts, and making more life. In eukaryotic cells, the mitochondria are the processing plants where the major forms of energy currency, such as ATP, are generated by catabolism of nutrients and consumption of oxygen. Much of mitochondrial research of the last 50 years has focused on how this flow and conversion of energy is controlled. But it is only in the last few years that we have realized that this process is an important node in the complex signal transduction machinery that extends throughout the cell and connects it to the external environment. In a sense, we are seeking the links between information and energy in the cell. Now, a paper in this issue provides one such link: Cardenas et al. (2010) report that calcium constitutively released from the endoplasmic reticulum (ER) by the inositol 1,4,5-triphosphate receptor (IP_3R) is taken up by mitochondria where it is required for efficient oxygen consumption and ATP production.

When eukaryotic cells are starved of nutrients, they engage the process of autophagy to catabolize themselves to survive, and this is generally triggered by

the inhibition of mTOR, which otherwise inhibits autophagy by phosphorylation of two of the initiating factors (Hosokawa et al., 2009). A link between ER calcium efflux and autophagy was discovered when it was noted that pharmacologic inhibition of the IP_3R induces autophagy under conditions of nutrient sufficiency (Vincencio et al., 2009). Cardenas and colleagues confirm this and extend the findings to DT40 cells (a chicken lymphoma line) in which all three forms of IP_3R have been knocked out (Sugawara et al., 1997). Other transformed cells, as well as primary hepatocytes and smooth muscle cells, similarly undergo autophagy upon IP_3R inhibition, and this depends on the activation of adeonsine monophosphate-activated protein kinase (AMPK), a sensor of energy insufficiency (Hardie, 2007). Surprisingly, this appears to be independent of mTOR function, at least in the transformed cells, a point to which we will return. These cells continue to consume glucose but nevertheless engage autophagy, which is required for their survival. Thus the energy requirements of the cell were not being met due to a defect in mitochondrial oxidative phosphorylation. Although introduction of wild-type IP_3R into the knockout cells restores energy production and inhibits autophagy, a mutant IP_3R incapable of inducing calcium efflux from the ER does not, and thus calcium is identified as the relevant signal. Indeed, elegant imaging studies confirm that in smooth muscle cells, constitutive low-level calcium efflux occurs in an IP_3R-dependent manner.

ER and mitochondria are closely associated in cells, and calcium released from the ER is efficiently taken up into the mitochondrial matrix, where it enhances the activities of several key enzymes, including pyruvate dehydrogenase (PDH), two enzymes in the tricarboxylic acid (TCA) cycle (ketoglutarate dehydrogenase and isocitrate dehydrogenase), and the F0F1 ATPase (Balaban, 2009). PDH is inhibited by phosphorylation, and calcium activates one of the PDH phosphatases (PDPs) that remove the inhibitory phosphate groups from PDH and thereby activate it. Cardenas et al. show that the regulation of PDH is a key step; inhibitors of the PDH kinase (either a pharmacologic inhibitor or pyruvate itself) restore oxygen consumption and energy generation. This is probably not a general effect, as hepatocytes express a specific PDP isoform that is calcium independent (Sugden and Holmes, 2003), and thus the other calcium targets mentioned above may predominate in these cells.

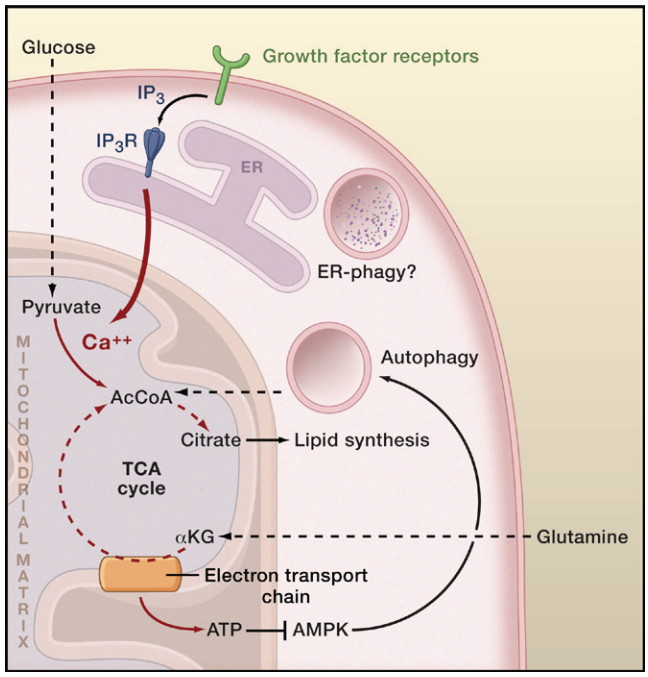

Figure 1. ER-to-Mitochondria Transfer of Calcium Ions

Proposed role for calcium ions in the control of metabolic flux and autophagy in transformed cells. Steady-state growth factor receptor signaling generates inositol 1,4,5-triphosphate (IP_3), which binds to the IP_3 receptor (IP_3) to trigger calcium efflux. Calcium (shown in red) is taken up into the mitochondrial matrix, where it promotes several activities (shown in red): the conversion of pyruvate to acetyl-CoA (AcCoA), the tricarboxylic acid (TCA) cycle, and the generation of ATP by the electron transport chain. In transformed cells, much of the pyruvate (from glycolysis) is converted to lipid via export of citrate, and the TCA cycle is sustained by glutamine uptake and conversion to α-ketoglutarate (αKG). When calcium efflux is limited, ATP production declines, and AMPK is activated. This in turn activates autophagy, which can generate energy via catabolism, providing the raw materials for generation of AcCoA (for example), and sustaining energetics. An alternative possibility is that the induced autophagy is involved in removal of damaged endoplasmic reticulum (ER-phagy). It is unlikely that the synthetic and autophagic pathways coexist at the same time in dividing cells, as this would represent a severe net energy loss, likely to impede cell proliferation.

The picture that emerges, then, is that constitutive signaling from cell surface growth factor receptors generates IP_3, which triggers calcium efflux from the ER, and this promotes the conversion of pyruvate into acetyl-coA in the mitochondria, TCA cycle activity, and production of ATP by the electron transport chain. When the IP_3R is not induced, energy levels drop, AMPK is activated, and autophagy is engaged to preserve energy and cell survival.

However, there is a fundamental problem with this view. Transformed cells, including DT40, utilize glucose predominantly to generate nucleotides, amino acids, and lipids. The latter requires that citrate is shuttled from the matrix to the cytosol, disrupting the TCA cycle. To compensate, glutamine is taken up and converted to glutamate and then to α-ketoglutarate in the matrix to restore the cycle and thereby maintain energy generation (DeBerardinis et al., 2007). Indeed, glutamine consumption in both the wild-type and IP_3R-deficient DT40 cells is highly active (and, if anything, the latter show higher levels) (Cardenas et al., 2010). However, this process is incompatible with autophagy: catabolism of cytosolic components (such as proteins and lipids) that are generated in the cell represents a net loss in energy. Yet, the DT40 cells lacking IP_3R proliferate without high levels of apoptosis (Sugawara et al., 1997). And indeed, mTOR, which promotes protein production and other cell-cycle requirements, is active in these cells (Cardenas et al., 2010). The scheme, with some of the possible roles for autophagy (discussed below), is shown in Figure 1.

One possibility is that the autophagy observed when IP_3R is blocked or absent is not for the purpose of energy generation, per se. Autophagy also functions to remove damaged organelles, and it is conceivable that cal-

cium imbalance in the ER triggers autophagic removal of ER that has become dysfunctional. Vincencio et al. (2009) suggest that disruption of an interaction between IP_3R and Beclin-1, a protein involved in autophagy targeting and initiation, is responsible for the induction of autophagy when IP_3R is inhibited. However, Cardenas et al. find that a mutant IP_3R that is capable of binding Beclin-1 but deficient in calcium efflux function fails to block autophagy in the knockout cells. This does not preclude the possibility that the observed autophagy is, in effect, restricted to "ER-phagy" but does not explain why AMPK activation is necessary for the observed autophagy.

Alternatively, it may be that the autophagy observed is indeed for energy production, but that it is cyclic. DT40 cells deficient in IP_3R display a marked increase in cells in S phase (Sugawara et al., 1997). This effect has not been commented upon, and it is not clear that this is observed for other cells in which IP_3R activity is inhibited. Might it be that in such cells, S phase is "stalled" due to low energy, and autophagy is engaged at this point to provide enough raw materials to bypass the block at PDH, providing acetyl-coA via β-oxidation of lipids? Although increased autophagy is observed, it is only seen in about 15% of cells. It would be interesting to know if these cells are in a delayed S phase. But if so, can we be sure that the pathway from AMPK to autophagy is truly independent of mTOR? It may be that the majority of the cells (that are not "stalled") may represent those with functional mTOR, as this is a known target of AMPK (Laplante and Sabatini, 2009)? Although intriguing, at this point it may be too early to say whether there is an AMPK-dependent, mTOR-independent pathway for autophagy. But then again maybe not, as a recent survey of the human autophagy machinery has indeed found an interaction of AMPK with the serine/threonine kinase ULK1, which acts at the earliest steps of autophagy (Behrends et al., 2010).

REFERENCES

Balaban, R.S. (2009). Biochim. Biophys. Acta *1787*, 1334–1341.

Behrends, C., Sowa, M.E., Gygi, S.P., and Harper, J.W. (2010). Nature *466*, 68–76.

Cardenas, C., Miller, R.A., Smith, I., Bui, T., Molgó, J., Müller, M., Vais, H., Cheung, K.-H., Yang, J., Parker, I., et al. (2010). Cell, this issue.

DeBerardinis, R.J., Mancuso, A., Daikhin, E., Nissim, H., Yudkoff, M., Wehrli, S., and Thompson, C.B. (2007). Proc. Natl. Acad. Sci. USA *149*, 19345–19350.

Hardie, D.G. (2007). Nat. Rev. Mol. Cell Biol. *8*, 774–785.

Hosokawa, N., Hara, T., Kaizuka, T., Kishi, C., Takamura, A., Miura, Y., Iemura, S., Natsume, T., Takehana, K., Yamada, N., et al. (2009). Mol. Biol. Cell *20*, 1981–1991.

Laplante, M., and Sabatini, D.M. (2009). J. Cell Sci. *122*, 3589–3594.

Sugawara, H., Kurosaki, M., Takata, M., and Kurosaki, T. (1997). EMBO J. *16*, 3078–3088.

Sugden, M.C., and Holmes, M.J. (2003). Am. J. Physiol. Endocrinol. Metab. *284*, 855–862.

Vincencio, J.M., Ortiz, C., Criollo, A., Jones, A.W.E., Kepp, O., Galluzzi, L., Joza, N., Vitale, I., Morselli, E., Tailler, M., et al. (2009). Cell Death Differ. *20*, 1006–1017.