Novel role for the LKB1 pathway in controlling monocarboxylate fuel transporters

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A question preoccupying many researchers is how signal transduction pathways control metabolic processes and energy production. A study by Jang et al. (Jang, C., G. Lee, and J. Chung. 2008. *J. Cell Biol.* 183:11–17) provides evidence that in *Drosophila melanogaster* a signaling network controlled by the LKB1 tumor suppressor regulates trafficking of an Sln/dMCT1 monocarboxylate transporter to the plasma membrane. This enables cells to import additional energy sources such as lactate and butyrate, enhancing the repertoire of fuels they can use to power vital activities.

Loss-of-function mutations in the gene encoding for the LKB1 tumor suppressor protein kinase were discovered 10 yr ago to cause the rare inherited Peutz-Jeghers cancer syndrome, in which patients are predisposed to develop benign as well as malignant tumors in multiple tissues (Hemminki et al., 1998). This observation gave birth to a new industry of research aimed at understanding how LKB1 exerts its cancer-suppressive effects. Much is still not understood. What is becoming clear is that LKB1 is mutated in a significant number of sporadic cancers, most frequently in adenocarcinomas of the lung (Sanchez-Cespedes, 2007). At the molecular level, LKB1 is activated by forming a heterotrimeric complex with the STRAD pseudokinase and the armadillo repeat adaptor protein MO25 (Alessi et al., 2006). Most evidence points to LKB1 exerting its physiological effects by phosphorylating a group of 14 protein kinases that belong to the AMP-activated protein kinase (APMK) subfamily (Lizcano et al., 2004). The most studied members are AMPKα1 and AMPKα2. These are normally referred to as AMPK and are activated by LKB1 after rises in 5′-AMP levels in energy-stressed cells. They phosphorylate a plethora of proteins to restore energy levels and stimulate the transport of glucose and other nutrients into cells (Hardie and Sakamoto, 2006). AMPK activation also suppresses nonessential energy-consuming processes such as protein synthesis and growth. The 12 other LKB1-activated kinases are collectively termed AMPK-related kinases. These enzymes include isoforms of PAR1/MARK as well as SAD/BRSK and, unlike AMPK, are not stimulated by energy stress but have been implicated in controlling cell polarity (Alessi et al., 2006).

A paper published in this issue of the *Journal of Cell Biology* describes a new role for LKB1 in *Drosophila melanogaster* (Jang et al., 2008; see p. 11). The data that emerged from a forward genetic screen suggest that the LKB1 signaling pathway controls the trafficking of a *Drosophila* homologue of the monocarboxylate transporter-1 (MCT1) to the apical membrane of polarized wing cells. The MCT1 transporter was termed Silnoon (Sln), meaning narrow eyes in Korean, because of the phenotype that identified it in the screen. As there are many published studies on mammalian MCT1, dMCT1 is an intuitive alternative name for the *Drosophila* MCT1 transporter and we thus refer to it as Sln/dMCT1. Mammalian MCT1 and Sln/dMCT1 are predicted to function as integral membrane proteins containing 12 transmembrane-spanning segments with N- and C-terminal cytoplasmic domains. In *Drosophila*, there are 18 characterized MCT family members and 14 in humans. Most work has been done on mammalian forms of these enzymes. MCT1–4 function as symport cotransporters of protons and monocarboxylate such as lactate, butyrate, and pyruvate (for review see Enerson and Drewes, 2003; Pierre and Pellerin, 2005; for review see Morris and Felmlee, 2008). Other MCT family members are likely to carry other substrates, for example, MCT8 transports thyroid hormones and MCT6 transports prostaglandin F2α as well as several pharmaceutical drugs (for review see Morris and Felmlee, 2008).

Jang et al. (2008) identified Sln/dMCT1 as a gene that when overexpressed together with wild-type LKB1, but not kinase-inactive LKB1, induced *Drosophila* to develop narrow eyes and upwardly curved small wings. Moreover, overexpression of LKB1 and Sln/dMCT1 in *Drosophila* wing discs enhanced uptake of radiolabeled butyrate and lactate. This enhanced uptake was inhibited by mutation of an essential conserved MCT catalytic residue. The authors provide evidence that the narrow eyes and curved small wing phenotypes result from apoptosis triggered by the increased uptake of monocarboxylates. How enhanced uptake of monocarboxylates induces apoptosis is not established,

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but the authors postulate that inhibition of histone deacetylase by butyrate and/or activation of p53-dependent apoptosis might be involved based on previous work (for review see Gupta et al., 2006). Potentially, the apoptotic phenotypes observed in this study could have resulted from high levels of nonphysiological import of monocarboxylates caused by the overexpression of Sln/dMCT1 and LKB1. Another *Drosophila* MCT termed OUT, most related to human MCT13, was also recently shown to control p53-triggered apoptosis of primordial germ cells during development (Yamada et al., 2008).

A key question concerns how overexpression of LKB1 stimulates the monocarboxylate Sln/dMCT1-dependent uptake. Previous experiments on the regulation of MCT family transporters have focused on the control of these enzymes at the level of transcription and translation (for review see Morris and Felmlee, 2008). However, in the fly wing disc, LKB1 does not appear to influence Sln/dMCT1 expression, but instead induces a striking relocalization of Sln/dMCT1 from the basolateral to the apical membrane. Indeed, when kinase-deficient LKB1 was expressed or LKB1 expression was reduced by siRNA, Sln/dMCT1 was predominantly localized on the basolateral wing disc membrane. Incubation of wing discs overexpressing wild-type LKB1 and Sln/dMCT1 with butyrate (but not lactate) induced massive apoptosis, which was not observed in the absence of LKB1, i.e., when Sln/dMCT1 was at the basolateral membrane.

In future work it will be crucial to establish whether LKB1 also controls MCTs in mammalian cells. It will also be important to determine whether the effects of LKB1 on Sln/dMCT1 trafficking are mediated via an AMPK family member and, if so, identify which one is involved. An attractive model would be if an AMPK directly phosphorylated Sln/dMCT1/MCT, thus promoting its trafficking to the apical membrane. However, analysis of the Sln/dMCT1 sequence for putative AMPK family phosphorylation site motifs using Scansite database (http://scansite.mit.edu/) reveals only a weak "low stringency" site that is not conserved in human or mouse MCT1. No putative AMPK phosphorylation site motifs were revealed in analysis of mammalian MCT1 sequences. Membrane localization of mammalian MCT1 and MCT2 is also reported to be stabilized through their interaction with glycoproteins (CD147 for MCT1 and Gp70 for MCT2; for reviews see Enerson and Drewes, 2003; Morris and Felmlee, 2008). It will also be important to investigate whether the LKB1–AMPK pathway can influence the interaction of Sln/dMCT1/MCT with membrane-anchoring glycoproteins. Scansite analysis does not reveal obvious AMPK sequence consensus motifs in either CD147 or Gp70. Collectively, these observations suggest that the effects of LKB1 on Sln/dMCT1 trafficking, if indeed mediated via activation of an AMPK, would involve an intermediate substrate.

The proposed pathway by which LKB1 regulates the trafficking of MCTs has similarities to the mechanism by which exercise stimulates translocation of the glucose transporter GLUT4 from intracellular vesicles to the plasma membrane (Fig. 1). In contracting muscle, depletion of ATP leads to activation of AMPK, which phosphorylates and inhibits the Rab-GTPase–activating proteins termed AS160 and TBC1D1 (Sakamoto and Holman, 2008). This stimulates loading of GTP to Rab8A,

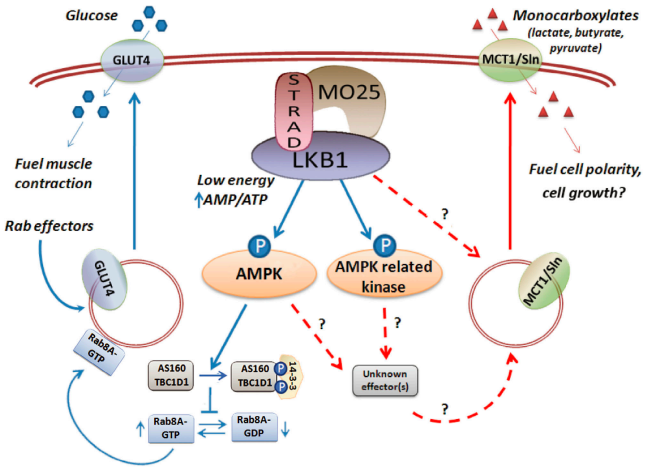

Figure 1. **Comparison of potential mechanisms by which LKB1 controls monocarboxylate and GLUT4 glucose transporters.** In contracting muscle, depletion of ATP leads to activation of AMPK, which phosphorylates and inhibits the Rab-GTPase–activating proteins termed AS160 and TBC1D1. This leads to an increase in the levels of active (GTP-bound) Rab8A that promotes relocalization of GLUT4 to the plasma membrane. AMPK or an AMPK-related kinase could phosphorylate an intermediate substrate to induce the translocation of MCT1/Sln to the plasma membrane.

which has been proposed to trigger relocalization of GLUT4-containing vesicles to the plasma membrane (Fig. 1).

Although the phenotypic data presented in Jang et al. (2008) are convincing, there are some noteworthy limitations. To observe apical localization of Sln/dMCT1, Jang et al. (2008) overexpressed wild-type LKB1. The assumption was that under these conditions, LKB1 would phosphorylate its physiological substrates. LKB1 needs to interact with its STRAD and MO25 regulators to be activated as well as to be localized in the cytoplasm rather than the nucleus (Alessi et al., 2006). Overexpression of LKB1 in *Drosophila*, without STRAD and MO25, might be expected to result in the accumulation of inactive LKB1 in the nucleus that cannot activate downstream pathways. Moreover, in mammalian cells, LKB1 is constitutively active and there is sufficient endogenous LKB1 to fully activate AMPK and AMPK-related kinases without the need to resort to LKB1 overexpression (Alessi et al., 2006). An over reliance on overexpressing LKB1 may have affected interpretations of previous analysis of LKB1 signaling in *Drosophila*. For example, earlier research concluded that the *Drosophila* Par1/MARK kinases phosphorylated and controlled LKB1 (Martin and St Johnston, 2003), when it is now clear that LKB1 regulates Par1/MARK kinases (Lizcano et al., 2004). Moreover, the authors of the Sln/dMCT1 study have reported that LKB1 regulated mitotic cell division and epithelial polarization via AMPK, directly phosphorylating the nonmuscle myosin regulatory light chain (MRLC; Lee et al., 2007). A recent study found that in LKB1-deficient pancreatic cells that lack AMPK activity phosphorylation of MRLC was enhanced rather than ablated (Hezel et al., 2008). Moreover, others have been unable to demonstrate that highly purified AMPK phosphorylates MRLC in vitro under conditions where it phosphorylates other established substrates. Further work is therefore required to understand how LKB1 regulates MRLC. It is also possible that there are differences between mammalian

and insect signaling networks regulating LKB1 and MRLC. AMPK has also been shown to phosphorylate and regulate myosin light chain kinase, which represents another mechanism by which the LKB1 pathway might affect MRLC phosphorylation (Horman et al., 2008). The authors of Jang et al. (2008) have also reported that LKB1 controls apoptosis by activating the JNK pathway (Lee et al., 2006), although this has not yet been confirmed in any subsequent study.

Monocarboxylates such as lactate have long been considered as waste products to be disposed of before harmful levels accumulate. However, these molecules also comprise vital energy substrates for brain and muscle under conditions where glucose or other nutrients are scarce (Pellerin, 2003; Pierre and Pellerin, 2005). Butyrate is also produced by colonic bacterial fermentation of dietary carbohydrates and serves as the major respiratory fuel for colonic epithelial cells (Cuff et al., 2005). How energy stress or other signals prompt cells to use monocarboxylates as a fuel source is unknown. Given the results presented in Jang et al. (2008), it is possible that relocalization of MCTs to the membrane, at least after energy stress, is triggered by LKB1-mediated activation of AMPK. Similarly, alteration of cellular structures during polarization must consume considerable energy. The ability of cells to import monocarboxylates may aide in providing additional injection of fuel to power polarization.

The Jang et al. (2008) findings suggest that loss of LKB1 would hinder the ability of cancer cells to use monocarboxylates as an energy source. At first glance, limiting the substrates that a cancer cell could exploit as a source of fuel seems detrimental. However, in colonic epithelia during transition to malignancy, a decline in MCT1 expression and a switch from using butyrate to glucose as an energy source has been observed (Lambert et al., 2002). Furthermore, a hallmark of loss of LKB1 expression in human and mice is a dramatic failure of colonic intestinal cells to polarize, resulting in polyp growth (Katajisto et al., 2008). Thus, disruption of monocarboxylate import pathways may promote tumor formation by interfering with energy supplies required to power polarization. Loss of monocarboxylate transport in cancer might also promote survival through decreased monocarboxylate-dependent apoptosis. It would indeed be interesting to explore in more depth the importance of monocarboxylate transport in controlling polarization and whether inability of LKB1-deficient cancer cells to import monocarboxylates represents a weakness in their armory that could be exploited therapeutically.

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