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COMMENTARY

Trials, tribulations and finally, a transporter: the identification of the mitochondrial pyruvate transporter

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Pyruvate occupies a central role in energy homoeostasis, and dysregulation of its cellular disposition underlies many metabolic disturbances. Although the mitochondrial membrane pyruvate transporter has been characterized, its molecular identity has proved elusive. Recent work has now identified a single candidate protein for the mitochondrial pyruvate carrier in yeast, opening the way for further studies in mammalian systems, which may have important therapeutic applications within the context of metabolic disease.

Key words: mitochondrial transporter, pyruvate, pyruvate carrier, pyruvate metabolism.

Monocarboxylates, such as lactate and pyruvate, occupy a central role in cellular metabolism. Some 30 years ago, the discovery that cyanocinnamates inhibited both mitochondrial pyruvate transport and transport of lactate and pyruvate into the red blood cell provided the first definitive proof for the existence of specific, but distinct, lactate/pyruvate transporters in both plasma and mitochondrial membranes. Both transport processes were characterized extensively [1,2], and the quest to identify the proteins responsible for both processes began in the late 1970s. The plasma membrane transporter was successfully identified [3,4], subsequently cloned [5], and a whole family of monocarboxylate transporters with an evolutionary history reaching back to bacteria was characterized (for a review, see [6]). The identity of the mitochondrial transporter has proved harder to 'crack', and until now has remained elusive. In recent work by Halestrap's group, now published in the *Biochemical Journal* [7], new evidence has been acquired by assaying inhibitor-sensitive pyruvate transport directly in isolated mitochondria from mutant yeast strains with a single gene deletion. In studies reported in this key paper, mitochondria isolated from a single yeast strain ΔYIL006w exhibited specific loss of mitochondrial pyruvate transport, and the deleted gene of YIL006w was shown to encode a 41.9 kDa member of the mitochondrial carrier family.

The significance of regulated pyruvate transport in higher organisms arises because pyruvate is positioned at a 'crossroad' in metabolism: it can act as an oxidative fuel or as a precursor for other fuels. It is also involved in amino acid metabolism and, through its interconversion with lactate in the cytosol via lactate dehydrogenase, can regulate the cellular redox state. Transport of pyruvate across the mitochondrial membrane is vital for many of these functions. For example, transport of pyruvate into mitochondria is necessary for the oxidative decarboxylation of pyruvate to acetyl-CoA and is therefore fundamental to glucose oxidation and ATP production [7a]. In the fed state, transport of pyruvate into mitochondria is also essential for the use of pyruvate for fatty acid synthesis in liver and adipose tissue, whereas in the starved state, it is essential for gluconeogenesis in liver and kidney. Dysregulation of pyruvate handling can therefore underlie many metabolic disturbances, including hyperglycaemia, hypoglycaemia, hyperlipidaemia and inadequate ATP production. The mitochondrial pyruvate carrier is thought to be a member of the six-transmembrane-helix mitochondrial carrier family, but substantial progress in the understanding of mitochondrial-membrane pyruvate transport has been hindered because the mammalian transporter has not yet been cloned and sequenced. This new paper opens the way for this.

The route to the new findings described by Hildyard and Halestrap has been tortuous. Initial labelling studies aimed at identifying the mitochondrial pyruvate transporter used the cyanocinnamate derivative UK5099 to protect a reactive cysteine on the carrier from labelling with *N*-phenylmaleimide, and tentatively identified a 15 kDa protein as the carrier [8]. This was unexpected, since other members of the mitochondrial transporter family were all approx. 30–40 kDa, and recent studies from the same group have subsequently identified this protein to be a subunit of complex 4 of the respiratory chain (COXIV) that presumably also binds UK5099. Later studies employed a cyanocinnamate affinity column in an attempt to bind the carrier selectively. Although this provided a single-step purification of a 55 kDa protein, it was found not to be the mitochondrial pyruvate carrier, but mitochondrial matrix aldehyde dehydrogenase [9]. Selective labelling experiments with a wide range of biotinylated reagents has continued, but none enabled identification of the mitochondrial pyruvate carrier in mammalian mitochondria. The other standard approach used to identify mitochondrial carrier proteins is purification and reconstitution of the Triton-solubilized, inner-membrane proteins that fail to stick to hydroxyapatite columns. This is not really appropriate for the pyruvate carrier, since pyruvate is capable of diffusing across the phospholipid membrane at an appreciable rate, even in the absence of a transporter. Thus assaying for enrichment in specific protein-mediated transport is extremely difficult.

Sequencing of the genome of baker's yeast, *Saccharomyces cerevisiae*, led to the identification of 35 members of the mitochondrial carrier family. Having exhausted many possible approaches to identify the mitochondrial pyruvate carrier in mammalian mitochondria, the Halestrap group isolated mitochondria from mutant yeast strains with a deletion in one of the

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mitochondrial carrier family and assayed inhibitor (UK5099)-sensitive pyruvate transport directly. As reported in this key paper, mitochondria isolated from a single yeast strain ΔYIL006w showed specific loss of mitochondrial pyruvate transport and revealed a single candidate protein. In the context of the higher organism, the way ahead now is to see whether the closest mammalian homologue identified in the database is the mammalian mitochondrial pyruvate transporter. The searches revealed that the most closely related proteins of approx. 30 % identity are the mitochondrial folate carrier and two other mitochondrial carriers of as-yet-unknown function (MGC4399 on chromosome 1 and NP_060625). The human carrier proteins of unknown function are approx. 35 kDa, which is smaller than the pyruvate carrier in yeast (41.9 kDa), with the best alignment giving an approximately 66-amino-acid-shorter N-terminal extramitochondrial domain. This is not uncommon when comparisons are made between mammalian mitochondrial transporters and their yeast counterparts. Hopefully, the putative mammalian pyruvate transporter(s) can be expressed in an active form in yeast mitochondria lacking their endogenous transporter, although this is not always a straightforward task.

This study opens up an entirely new area of research and important questions remain. Within the physiological context of mammalian fuel homoeostasis, key investigations will be to see whether the transporter is regulated, for example during the fed-to-starved transition in liver, during development or upon aging. Within the pathological context, regulation of the expression of the mitochondrial pyruvate transporter may be important in disease states, such as diabetes mellitus, where it has been demonstrated that there are changes in functional levels of the mitochondrial pyruvate transporter [10]. Similarly, the future identification of isoforms with unique properties or regulation whose expression varies within different pathophysiological states, or which differ in tissue distribution, may have important implications for targeted therapies aimed at correcting abnormalities associated with pyruvate handling. For example, two diverse but specialized roles for pyruvate are first, its participation in glucose-stimulated insulin secretion, and secondly, its possible function as an antioxidant in the prevention of programmed cell death. In the endocrine pancreas, the β cells of the islets of Langerhans are unusual in that they express little, if any, lactate dehydrogenase activity. This is believed to reflect the importance of anaplerotic entrance of pyruvate into the tricarboxylic acid cycle in coupling glucose metabolism to insulin secretion [11,12]. Antioxidant pyruvate prevents programmed cell death, and such protection is mediated, at least in part, by the mitochondrial matrix compartment [13,14]. Clearly, these key functions of mitochondrial pyruvate will be influenced by the efficacy of mitochondrial pyruvate transport. Finally, one might also speculate that one particular isoform might be specific for the oxo acids derived from the branched-chain keto acids or for the ketone bodies acetoacetate and hydroxybutyrate, important metabolic fuels that can also use the pyruvate carrier to enter or leave mitochondria. Within the context of human disease, key questions are whether there are patients with mutations in the transporter, and if so, what are their characteristics? Watch this space!

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