

Regulation of the pyruvate dehydrogenase complex

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Abstract

The PDC (pyruvate dehydrogenase complex) plays a central role in the maintenance of glucose homeostasis in mammals. The carbon flux through the PDC is meticulously controlled by elaborate mechanisms involving post-translational (short-term) phosphorylation/dephosphorylation and transcriptional (long-term) controls. The former regulatory mechanism involving multiple phosphorylation sites and tissue-specific distribution of the dedicated kinases and phosphatases is not only dependent on the interactions among the catalytic and regulatory components of the complex but also sensitive to the intramitochondrial redox state and metabolite levels as indicators of the energy status. Furthermore, differential transcriptional controls of the regulatory components of PDC further add to the complexity needed for long-term tuning of PDC activity for the maintenance of glucose homeostasis during normal and disease states.

Introduction

The mammalian PDC [PDH (pyruvate dehydrogenase) complex] catalyses the physiologically irreversible oxidative decarboxylation of pyruvate to acetyl-CoA, thereby linking the glycolytic pathway to the oxidative pathway of the tricarboxylic acid cycle. Acetyl-CoA generated by the PDC also serves as a precursor for lipid biosynthesis in certain tissues under the conditions of carbohydrate/energy excess (fed condition). Due to the fact that approx. 50% of daily calorie intake (as the carbon flux of pyruvate derived from all carbohydrate sources and about one-half of carbon skeletons of gluconeogenic amino acids) passes through PDC, it is no surprise that the flux through this reaction is tightly regulated to maintain glucose homeostasis during both the fed and fasting states. PDCs of higher eukaryotes are composed of multiple copies of three catalytic, one binding and two regulatory components: PDH (PDH designation used in this paper refers to PDH1 isoenzyme containing the α subunit, a product of the *PDHA1* gene localized on chromosome X in mammals and expressed in somatic cells), E2 (dihydrolipoamide acetyltransferase) and E3 (dihydrolipoamide dehydrogenase) plus E3BP (E3-binding protein) and PDHK (PDH kinase) and PDP (PDH phosphatase) [1,2]. The elaborate regulation of PDC is achieved by a combination of three major mechanisms, namely (i) reversible phosphorylation/dephosphorylation (short-term regulation); (ii) regulation of the activities of the regulatory components by the redox state and acetyl-CoA/CoA ratio

and (iii) transcriptional regulation of the regulatory component enzymes (long-term regulation). This review will highlight these regulatory properties only; detailed accounts can be found in several excellent recent reviews [3–6].

Multiple phosphorylation sites

The target of phosphorylation/dephosphorylation is the α subunit of PDH ($\alpha_2\beta_2$ tetramer), the first component of PDC which catalyses the rate-limiting reaction of the oxidative decarboxylation of pyruvic acid and reductive acetylation of the lipoyl moieties of E2. Mammalian PDH has three phosphorylation sites (numbered as per mature human sequence): Ser²⁶⁴, site 1; Ser²⁷¹, site 2; and Ser²⁰³, site 3 [7,8]. The presence of three phosphorylation sites is observed in many different species of the animalia (except nematodes, which have either two sites in *Caenorhabditis elegans* or three sites in *Ascaris suum* type II; Figure 1A). Plants have only one site, and the number of phosphorylation sites is quite variable in species from Protista and Fungi (Figure 1A). Archaea and bacteria show even fewer phosphorylation sites (Figure 1A). PDC was shown to be regulated by phosphorylation in mammals, aves, nematodes, plants and the fungus *Neurospora crassa* [9–12]. In yeast, *Saccharomyces cerevisiae*, phosphorylation was not detected; however, site 1 of this organism can be phosphorylated by bovine PDHK and dephosphorylated by bovine PDP [13]. It is apparent that the phosphorylation sites have evolved gradually, predating the appearance of the PDHK, and their functionality becomes evident with emergence of PDHK activity in higher organisms (Figure 1B; see also below). Since PDH is a heterotetramer ($\alpha_2\beta_2$), it contains six potential phosphorylation sites. Interestingly, phosphorylation of only one site renders this component inactive because of its half-of-the-sites-reactivity [7,14,15]. One then wonders: what is the evolutionary advantage of having three functional

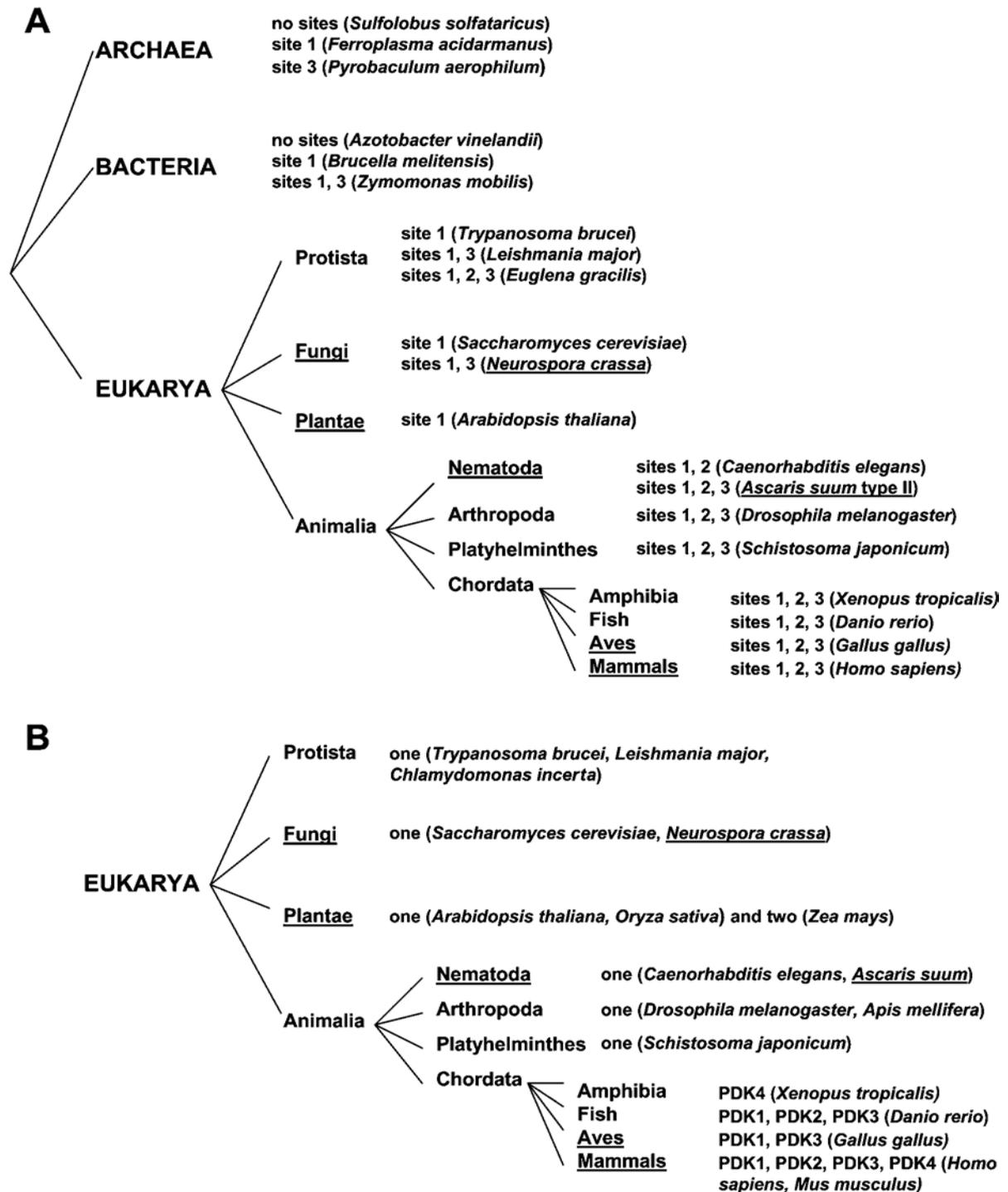
Key words: glucose homeostasis, hormonal regulation, multiple phosphorylation sites, phosphorylation/dephosphorylation, pyruvate dehydrogenase complex, pyruvate dehydrogenase kinase isoenzyme.

Abbreviations used: CBP, cAMP-response-element-binding-protein-binding protein; E2, dihydrolipoamide acetyltransferase; E3, dihydrolipoamide dehydrogenase; E3BP, E3-binding protein; FOXO, forkhead box O; PDH, pyruvate dehydrogenase; PDC, PDH complex; PDHK, PDH kinase; PDP, PDH phosphatase; TPP, thiamin pyrophosphate.

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Figure 1 | Presence of phosphorylation sites in the PDH sequence (A) and presence of PDHK (PDK) sequences (B) in different species

The presence of phosphorylation sites and PDHK sequences was determined by BLASTP analysis [48] based on human PDH1 α (PDH α is present in two isoforms: somatic-specific, PDH1 α ; testis-specific, PDH2 α) as a query sequence in (A) and human PDHK2 as a query sequence in (B). The analysis generated 502 BLAST hits on query sequence in (A) and 320 in (B). Sequences in (A) were analysed for the presence of serine residues in the positions corresponding to the positions of phosphorylation site 1 (Ser²⁶⁴ in human PDH α), site 2 (Ser²⁷¹) and site 3 (Ser²⁰³). The presence of serines in these positions does not necessarily mean the presence of the mechanism of phosphorylation. The organisms in which the presence of the mechanism of phosphorylation was confirmed are underlined. For PDHK sequences, number one or two indicates the number of isoenzymes of PDHK detected (not referring to PDHK1 or PDHK2). In Chordata, PDHK isoenzymes were named as PDHK1–PDHK4 as identified in the Figure (PDK1–PDK4). Only selected examples of the species are given in parentheses for each taxonomic group.



phosphorylation sites in mammalian PDH? Differential rates of phosphorylation and dephosphorylation may provide advantageous regulation as discussed below.

All three sites were found to be phosphorylated *in vivo* to a different extent, with the maximum phosphorylation of site 1 and less phosphorylation of sites 2 and 3 [16]. Mutagenic studies with double mutant human PDHs having only a single site (1, 2 or 3) available for phosphorylation revealed that each of the three phosphorylation sites can be phosphorylated independently, resulting in PDH inactivation in each case [14]. *A. suum* has two isoforms of PDH, an anaerobic isoform PDH-I with two phosphorylation sites and an aerobic isoform PDH-II with three phosphorylation sites [17]. Phosphorylation of each site resulted in inactivation of PDH-I and -II. Plants have mitochondrial PDC and plastid PDC, and only mitochondrial PDC is regulated by phosphorylation on a single site [6].

The molecular mechanism of inactivation of human PDH by phosphorylation is site-specific, and the three sites are localized differently in the structure of human PDH relative to the active site [15,18]. Phosphorylation of site 1 and substitution of glutamate, glutamine or aspartate for Ser²⁶⁴ only marginally affected the decarboxylation reaction but severely impaired the reductive acetylation reaction. PDH phosphorylated at site 1 and PDH site 1 mutants with glutamate and aspartate substitutions are inactive in the PDC reaction, and the glutamine mutant has only 3% of the activity of the wild-type enzyme. Since site 1 (Ser²⁶⁴) is positioned in the substrate channel leading to the PDH active site, its phosphorylation would introduce a negatively charged and bulkier group, and would prevent the lipoyl domain of E2 from interacting with the PDH active site [15]. A similar mechanism of inactivation was suggested for branched-chain α -oxo acid dehydrogenase based on the structure of its phosphorylated form and its site 1 mutants [19]. Phosphorylation was suggested to result in conformational disordering of the loop, with the phosphorylated serine near the active site which prevented the lipoyl domain binding [19]. Phosphorylation of sites 2 and 3 in PDH, which are not localized near the active site, probably affects PDH activity differently. The increased K_m value for TPP (thiamin pyrophosphate) for the glutamate substitution at site 3 (27-fold) and almost complete protection of site 3 from phosphorylation by high concentrations of TPP (200 μ M) indicate the effect of site 3 phosphorylation on TPP binding [18].

PDHK and PDP isoenzymes

In principle, a single PDHK with reactivity towards the three phosphorylation sites in PDH could have fulfilled its regulatory role. In mammals, however, four isoenzymes of PDHK are present [20,21]. The importance of multiple forms of PDHK is illustrated in PDC regulation, with differences in their specific activities towards the three sites, their regulation and specificity towards the lipoyl domains and their tissue-specific distribution (Table 1) [3,22–24]. Other species are found to have either two PDHKs (some plants) or one PDHK (some plants, nematodes and insects; Figure 1B). In Protista

and Fungi, sequences similar to that of human PDHK are detected with low sequence identity (25–40%). *N. crassa* was shown to have both PDHK and PDP functional activities towards its PDH [12]. Bacteria do not have sequences similar to PDHKs.

Mammalian PDC-E2 is composed of an outer lipoyl domain (L1), an inner lipoyl domain (L2), a subunit-binding domain (binding PDH) and an inner domain (forming the PDC core) (Figure 2). E3BP (E3-binding protein) has a similar domain structure with only one lipoyl domain but plays only a structural and not a catalytic role [2]. Forty-eight subunits of E2 and 12 subunits of E3BP are proposed to form the central core of PDC, having a pentagonal dodecahedron structure [25]. All other components of PDC bind non-covalently to the central core. PDH binds to the subunit-binding domain of E2. E3 binds to the subunit-binding domain of E3BP. PDHKs and PDP1 bind to the lipoyl domains of E2 and E3BP (Figure 2) [3]. The lipoyl domains of E2 are highly mobile and move between the active sites of PDH, E2 and E3, transferring substrates, products and reducing equivalents.

The four mammalian PDHKs are distributed differently in tissues (Table 1) [24]. PDHK2 is abundant in many tissues, PDHK1 is present mostly in heart, with lower levels in skeletal muscle, liver and pancreas, whereas PDHK3 is found mostly in testis and to a lesser extent in lung, brain and kidney. PDHK4 is highly expressed in skeletal muscle and heart, with variable levels in lung, liver and kidney [3,24]. Figure 2 lists the activities of the four PDHK isoenzymes towards the three phosphorylation sites of human PDH reconstituted in PDC [22]. PDH mutants having only one of the three phosphorylation sites available for phosphorylation, with alanine substitutions at the other two sites, were used in these experiments [22]. All four PDHKs can phosphorylate sites 1 and 2; however, site 3 is phosphorylated by PDHK1 only [22,23]. In phosphate buffer, PDHK2 has the highest activity for site 1 and PDHK3 has highest activity for site 2. PDHKs can also phosphorylate free PDH not bound in PDC. The site specificity remains the same, i.e. only PDHK1 can phosphorylate site 3 of free PDH. The activities of PDHKs are lower towards free PDH, especially for site 2. Only PDHK4 has high activity for both sites 1 and 2 towards free PDH [22].

Two isoforms of PDP were identified: PDP1 (a heterodimer consisting of a catalytic and a regulatory subunit) and PDP2 [3,26]. The recombinant catalytic subunit of PDP1 can catalyse dephosphorylation in the absence of the regulatory subunit [3]. PDP1 was detected mostly in heart and brain and PDP2 in liver, kidney, brain and heart (Table 1) [26]. Both PDP1 and PDP2 can dephosphorylate all three phosphorylation sites. The rate of dephosphorylation is in the order site 2 > site 3 > site 1, and the activity of PDP1 is higher than that of PDP2 (Figure 2) [27,28]. The physiological significance of this finding is not known. It is speculated that a higher rate of inactivation of site 1 may allow rapid inactivation of PDH, and a lower rate of dephosphorylation of site 1 may result in gradual activation of PDH.

Table 1 | Characteristics of mammalian PDHK isoenzymes and PDP isoenzymes

The information presented here is derived from [1,3,22–24,26,39–43]. Sk. muscle, skeletal muscle.

Properties	PDHK1	PDHK2	PDHK3	PDHK4	PDP1	PDP2
Phosphorylation sites	1, 2, 3	1, 2	1, 2	1, 2	1, 2, 3	1, 2, 3
Binding to lipoyl domains	L1, L2	L2 > L1	L2 > L1	L3 > L1	L2	
Activation by lipoyl domains (fold)						
1	1	1.7	6.6	1		
2	3.6	6.3	29	1.7		
3	3.2	–	–	–		
Stimulation by reduction and acetylation of lipoyl domains (fold)	1.5	2.4	1.2	2.0		
Tissue distribution						
Brain		+++	++		++++	++++
Heart	+++++	++++	+	++++	+++++	+++++
Sk. muscle	+++	++++		+++++	+	
Pancreas	+	+++				
Liver	++	+++		++		+++++
Kidney		+++	++	+		+++++
Testis		+++	++++		+++	
Spleen		+	+			+
Lung		+	+++	+++	+	
Post-translational regulation	Inhibited by ADP and pyruvate; activated by NADH and acetyl-CoA	Inhibited strongly by ADP and pyruvate; activated maximally by NADH and acetyl-CoA	Inhibited weakly by pyruvate	Inhibited by ADP and pyruvate; activated by NADH and acetyl-CoA	Activated by increases in Mg ²⁺ and Ca ²⁺	Activated by spermine
Transcriptional regulation		Up-regulation during: starvation (liver, kidney, mammary gland, fast-twitch muscle); diabetes (sk. muscle); and hyperthyroidism (fast-twitch muscle)		Up-regulation during: starvation (liver, kidney, muscle, mammary gland, heart); diabetes (heart, sk. muscle); and hyperthyroidism (heart, sk. muscle)	Down-regulated during diabetes (sk. muscle)	Down-regulated during starvation (heart, kidney) and diabetes (heart, kidney)

Regulation of phosphorylation/dephosphorylation

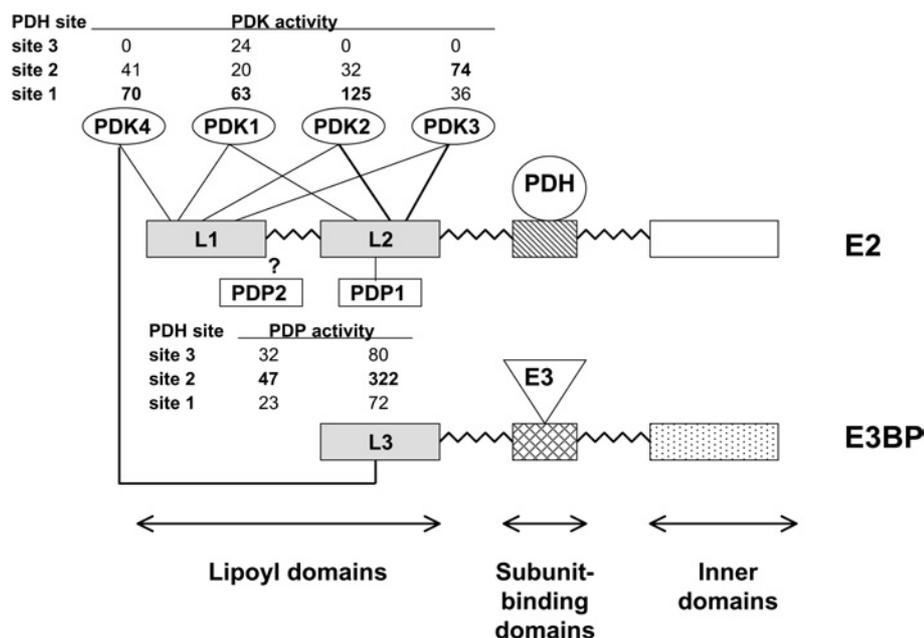
The lipoyl domains play an essential role in modulation of PDHK activities [29]. The four PDHK isoenzymes have different preferences towards the lipoyl domains of E2 and E3BP (Table 1) [3]. Binding to the lipoyl domains activates PDHKs to different extents (Table 1) and requires the lipoyl prosthetic group. The activation is suggested to result from the co-localization of PDHK and PDH on E2 and conformational changes in PDHK [30,31]. PDHK3 activity is enhanced to a greater extent by binding to E2 than the activities of the other PDHK isoenzymes [3,22]. The recently solved structure of PDHK3 bound to the L2 domain of

human E2 shows that the C-terminal tail of one subunit of PDHK3 interacts with the other subunit of PDHK3 in the lipoyl domain binding region [32]. Binding to L2 was proposed to change the conformation of PDHK3 in such a way as to induce a ‘crossover’ conformation of the C-terminal tails, resulting in the opening of the active site cleft and the release of ADP, the product of the reaction. The lipoyl domains also participate in the movement of PDHKs on the surface of PDC, possibly by a ‘hand-over-hand’ mechanism to phosphorylate multiple copies of PDH [33].

The activities of PDHKs are regulated by the concentrations of the physiological ligands pyruvate, ADP and P_i and by the NADH/NAD⁺ and acetyl-CoA/CoA ratios [3]. PDHK activity is inhibited by high levels of pyruvate and

Figure 2 | Binding of PDC components PDH, E3, PDHs (PDKs) and PDPs to the structural domains of E2 and E3BP

Thicker lines connecting PDHs to the lipoyl domains indicate higher binding. Activities of PDHs and PDPs (in m-units/mg of enzyme protein) towards the three phosphorylation sites of human PDH in PDC are shown based on [22] for PDHs and our results for PDPs (L.G. Korotchkina, S. Sidhu and M.S. Patel, unpublished work). PDH mutants having only one site (1, 2 or 3) available for phosphorylation and dephosphorylation, with alanine substitutions at the other two sites, were used.



a low energy state (high levels of ADP, NAD⁺, CoA and P_i) to increase PDC activity [34]. NADH and acetyl-CoA, the products of the PDC reaction, stimulate PDHK activity through reduction and acetylation of the lipoyl moieties of E2, which in turn allosterically affect PDHK bound to the lipoyl domain [34]. PDHK2 is the most sensitive isoenzyme to stimulation by the reduction and acetylation of the lipoyl domains of E2 and to inhibition by pyruvate (Table 1). Pyruvate binding further slows ADP dissociation, which is the rate-limiting step in PDHK catalysis [35].

PDP1 binds to the L2 domain of E2 with the participation of Ca²⁺ [36]. Binding of PDP2 to a lipoyl domain was not detected and PDP2 is not sensitive to Ca²⁺ concentration [27]. PDP1 and PDP2 have different sensitivities to Mg²⁺ [3]. PDP1 requires lower Mg²⁺ for catalysis. The regulatory subunit increases the concentration of Mg²⁺ necessary for PDP1 catalysis. Spermine and other polyamines reduce the *K_m* for Mg²⁺ by possible binding with the regulatory subunit of PDP1. Insulin was shown to increase the activity of PDP through protein kinase C δ that phosphorylates and activates PDP [37] and through the phosphoinositide 3-kinase and MEK1/2 [MAPK (mitogen-activated protein kinase)/ERK (extracellular-signal-regulated kinase) kinase 1/2] pathways [38].

In different nutritional and disease states, the activity of PDC is regulated by the levels of PDHs and PDPs determined at the transcriptional level [4,5]. Among the four PDHK isoenzymes, PDHK4 and to a lesser extent PDHK2 were shown to be up-regulated in several tissues in

starvation, diabetes and hyperthyroidism, and PDP1 and PDP2 were shown to be down-regulated in starvation and diabetes (Table 1) [39–43]. As a result, PDC activity is reduced for glucose conservation. Fatty acids regulate the expression of PDHK4 in liver and kidney and partly in heart through peroxisome-proliferator-activated receptor α [44,45]. Another mechanism of transcriptional regulation of PDHK4 was suggested recently in which glucocorticoids elevated in starvation and diabetes increase acetylation of histone by recruiting the p300/CBP (cAMP-response-element-binding-protein-binding protein) complex, leading to PDHK4 transcription [46]. FOXO (forkhead box O) factors interact with p300/CBP to fully activate PDHK4 transcription [46]. Insulin down-regulates PDHK4 expression by translocation of FOXO factors to the cytoplasm [46]. Expression of PDHK4 was also shown to be stimulated by peroxisome-proliferator-activated receptor γ co-activator [47].

Concluding remarks

The 'gatekeeper' role of PDC in the oxidation of carbohydrate and some amino acids in mammals is fulfilled by its elaborate mechanisms of regulation by phosphorylation/dephosphorylation involving protein–protein interactions among PDC components and sensing of the changes in the concentrations of intramitochondrial metabolites such as pyruvate, acetyl-CoA and NADH. Furthermore, tissue-specific regulation of PDC has been achieved by the evolutionary

development of multiple phosphorylation sites and the tissue-specific distribution of multiple isoenzymes of dedicated kinases and phosphatases. Hormonal regulation at the transcriptional level of these regulatory enzymes adds long-term regulation of PDC under normal nutritional and disease states. This multienzyme complex is therefore unique in the acquisition of multilayered mechanisms of regulation involving interactions among its component proteins and elaborate sensing of the changes in its substrates, products and intramitochondrial redox state (NAD⁺/NADH) to maintain glucose homeostasis in mammals.

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