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E4F1 controls a transcriptional program essential for pyruvate dehydrogenase activity

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The mitochondrial pyruvate dehydrogenase (PDH) complex (PDC) acts as a central metabolic node that mediates pyruvate oxidation and fuels the tricarboxylic acid cycle to meet energy demand. Here, we reveal another level of regulation of the pyruvate oxidation pathway in mammals implicating the E4 transcription factor 1 (E4F1). E4F1 controls a set of four genes (dihydrolipoamide acetyltransferase (Dlat), dihydrolipoyl dehydrogenase (Dld), mitochondrial pyruvate carrier 1 (Mpc1), and solute carrier family 25 member 19 (Slc25a19)) in pyruvate oxidation and reported to be individually mutated in human metabolic syndromes. E4F1 dysfunction results in 80% decrease of PDH activity and alterations of pyruvate metabolism. Genetic inactivation of murine E4f1 in striated muscles results in viable animals that show low muscle PDH activity, severe endurance defects, and chronic lactic acidemia, recapitulating some clinical symptoms described in PDC-deficient patients. These phenotypes were attenuated by pharmacological stimulation of PDH or by a ketogenic diet, two treatments used for PDH deficiencies. Taken together, these data identify E4F1 as a master regulator of the PDC.

E4F1 | PDH | pyruvate | muscle | endurance

The pyruvate dehydrogenase (PDH) complex (PDC) is a mitochondrial multimeric complex that catalyzes the oxidative decarboxylation of pyruvate into acetyl-CoA (AcCoA), thus linking pyruvate metabolism to the tricarboxylic acid (TCA) cycle. Localized in the mitochondrial matrix, the core PDC is composed of multiple copies of three catalytic enzymes: PDHA1/E1, dihydrolipoamide transacetylase (DLAT)/E2, and dihydrolipoyl dehydrogenase (DLD)/E3 (1). To fuel the PDC, pyruvate translocates across the inner mitochondrial membrane through the heterodimeric pyruvate transporter mitochondrial pyruvate carrier 1 (MPC1)/MPC2 (2, 3). The activity of the PDC depends on several cofactors, including lipote, CoEnzymeA (CoA), FAD+, NAD+, and thiamine pyrophosphate, the latter being imported in the mitochondria by the SLCA25A19 transporter (4). So far, fine-tuning of PDC activity has been mainly attributed to post-translational modifications of its subunits (5, 6), including the extensively studied phosphorylation of PDHA1/E1 modulated by PDH kinases (PDK1–4) and phosphatases (PDP1–2). However, in lower organisms, such as Escherichia coli and Candida albicans, PDC is also controlled at the transcriptional level by the coordinated regulation of genes encoding its components and regulators (7, 8). The importance of such transcriptional regulation of the PDC in mammals remains elusive. Physiological regulation of PDC plays a pivotal role in metabolic flexibility to adjust energetic metabolism and biosynthesis to nutrient availability and energy demand (9), such as in skeletal muscles during exercise (10). PDH activity is altered in several human metabolic syndromes associated with chronic lactate acidosis, progressive neurological degeneration, and muscular atonia (11). Genetic mitochondrial disorders associated with PDH deficiency mainly result from hypomorphic mutations in genes encoding subunits or regulators of the PDC, including in PDHA1, DLAT, DLD, PDPI, MCP1, and SLCA25A19 (3, 11–13). The diverse clinical manifestations of PDC-deficient patients are significantly, but only partly, improved by ketogenic diets that provide alternative energetic substrates or by treatment with PDK inhibitors, such as dichloroacetate (DCA). Thiamine/lipoic acid supplementations that favor optimal PDH activity, or bicarbonate treatment that buffers lactate acidosis, have also been tested, although with moderate efficiency (14, 15). The design of new and more efficient therapeutic approaches will require a better understanding of PDH regulation and the development of clinically relevant animal models.

Here we reveal another level of regulation of the pyruvate oxidation pathway in mammals that implicates the E4 transcription factor.

Significance

Pyruvate dehydrogenase (PDH) deficiency is the cause of several human metabolic diseases. In mammals, the transcriptional control of PDH complex components and its impact on pathophysiology remain poorly understood. We show that E4 transcription factor 1 (E4F1) controls a transcriptional program essential for PDH activity that involves genes linked to human metabolic syndromes. Genetic inactivation of murine E4f1 results in a strong decrease of PDH activity and severe perturbations of pyruvate metabolism. In concordance with the work of Legati et al., we show that striated muscle-specific E4f1 KO animals display phenotypes that recapitulate these clinical symptoms, providing an exciting clinical perspective to the present work.


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1 (E4F1). Initially identified as a cellular target of the E1A viral oncoprotein (16), E4F1 was then described as a physical interactor of several tumor suppressors that gate cell division and survival in proliferating cells, including pRB, RASSF1A, p14ARF, and p53 (17–21). E4F1 is essential for early embryonic mouse development (22), and for either proliferation or survival of actively dividing mammalian cells (23–25). In proliferating cells, we have recently shown that E4F1 controls genes implicated in cell-cycle checkpoints and genome surveillance, but also unexpectedly, a transcriptional program involved in mitochondria functions (24, 26). Here we further characterized this mitochondria-associated program and found that E4F1 coordinates the transcription of a set of genes involved in PDH-mediated pyruvate oxidation. Accordingly, tissue-specific inactivation of murine E4f1 in the postmitotic and differentiated compartment of striated muscles resulted in a strong reduction of muscular PDH activity. Surprisingly, this constitutively low PDH activity did not compromise animal viability, although these animals displayed chronic lactic acidemia and endurance defects that recapitulate some clinical symptoms described in PDC-deficient patients.

Results

E4F1 Controls Genes Involved in PDH Activity. We recently identified by ChIP, combined with deep sequencing (ChIP-seq), the repertoire of endogenous target DNA sites bound by E4F1 in primary and Ha-Ras(12) transformed mouse embryonic fibroblasts (tMEFs) (24, 26). We completed this gene list by performing additional E4F1 ChIP-seq analyses in murine embryonic stem (ES) cells and defined a common set of promoter regions that were bound by E4F1 in these two cell types (Fig. 1A) (GSE57221 and GSE57228). Gene ontology analysis of E4F1 target genes revealed an unexpected enrichment for nuclear genes encoding mitochondrial proteins (24). Surprisingly, a closer analysis of this subprogram identified four genes, located on distinct chromosomes, which are directly involved in PDH function. These genes encode the E2 and E3 subunits of the PDH core enzyme (Dlat, Dld), the mitochondrial pyruvate transporter MPC1 (Brp44l), and the mitochondrial transporter of the PDH cofactor thiamine pyrophosphate (Slc25a19/DNC). A fifth gene, encoding the negative regulator of the PDH phosphatases (Pdpr) (27) was also identified as an E4F1 target gene by ChIP-seq in transformed fibroblasts, but not in ES cells (data not shown).

Fig. 1. E4F1 directly controls a transcriptional program involved in PDH activity in mammals. (A) E4F1 ChIP-Seq read densities in tMEFs and mouse ES cells (mES) at the Dlat, Dld, Mpc1/Brp44l, Slc25a19/DNC (deoxyribonucleotide carrier) genes, and at the Pdha1 gene as a representative control locus to which E4F1 does not bind. The E4F1 consensus motif determined by MEME is shown and arrows indicate the genes orientation. (B) Validation of these E4F1 target genes by ChIP-qPCR assays performed in E4f1 KO and control (CTR) tMEFs, 3 d after transduction with a self-excising Cre-retrovirus. A gene-poor noncoding region of chromosome 8 (NC1) and the Pdha1 promoter region (TSS) were used as controls. Enrichments are represented as percentages of input (mean value ± SEM, n = 3). (C) Protein levels of E4F1, and of the loading control TATA binding protein (TBP), determined by immunoblotting of total protein extracts prepared from E4f1 KO, and control tMEFs. (D) mRNA levels of Dlat, Dld, Slc25a19, Brp44l/Mpc1, Pdpr, and of two control genes (CS and Pdha1) determined by RT-qPCR analysis in E4f1 KO and control tMEFs. Histobars represent the mean value ± SEM (n = 6). ***p < 0.001; **p < 0.01; *p < 0.05; ns, not significant.

Fig. 2. Impaired PDH activity and deregulation of the pyruvate pathway in E4f1 KO cells. (A) Protein levels of E4F1, DLAT, DLL, lipoylated proteins (DLAT and DLST), PDH-E1a, MPC1/BRP44L, and Tubulin (loading control) determined by immunoblotting of total cell extracts prepared from E4f1 KO and control (CTR) tMEFs. (B) PDH enzymatic activity measured in E4f1 KO and CTR tMEFs. (C) Schematic representation of the pyruvate–AcCoA pathway. (D) Relative levels of several metabolites linked to the pyruvate pathway in E4f1 KO and CTR tMEFs. Histobars represent the mean value ± SEM (n = 5). (E) Relative abundance of Met-12 isomers of AcCoA and citrate that derive from pyruvate oxidation as determined by LC-MS in E4f1 KO and match CTR tMEFs cultured in α[1-13C]glucose for 30 min or 6 h (mean ± SD, performed in triplicate). (F) Relative levels of FAO measured upon incubation of E4f1 KO and CTR tMEFs with H-palmitate (mean value ± SEM, n = 3). ***p < 0.01; *p < 0.05; ns, not significant.
Sequence analyses revealed that these genes contained one or two bona fide E4F1 binding sites nearby their transcription start site (TSS) (Fig. 1A). These E4F1 direct target genes were further validated by ChIP-quantitative PCR (qPCR) experiments performed upon Cre-mediated inactivation of E4F1 in E4f1−/− KO MEFs (hereafter referred to as E4f1ko) (Fig. 1B and C). Consistent with a role for E4F1 as a bona fide transcriptional activator for these PDH-related genes, the mRNA levels of Dlat, Bp44l/Mpc1, Dld, Slc25a19, and Pdpr decreased in E4f1ko cells, although to various extents (Fig. 1D). In contrast, the transcript levels of another PDH core component, Pdhb1, and of the mitochondrial enzyme citrate synthase (CS), which were not identified as E4F1 direct target genes, did not vary upon acute E4f1 inactivation (Fig. 1D). At the protein level, a strong down-regulation of DLAT and BRP44L/MPC1, and a moderate decrease of DLD were observed in E4f1ko cells (Fig. 2A). Of note, siRNA-mediated depletion of E4F1 in fibroblasts also resulted in the down-regulation of DLAT, BRP44L/MPC1 and DLD proteins (Fig. S1A), confirming the role of E4F1 in the control of these genes. Taken together, our data highlight a previously undescribed function of E4F1 in the transcriptional control of genes involved in PDH activity in mammals.

**E4f1 Inactivation Results in Reduced PDH Activity and Metabolic Reprogramming.** As a direct consequence of decreased expression of PDH proteins, E4f1−/− fibroblasts and E4F1 siRNA-treated cells exhibited a marked decrease of PDH enzymatic activity (Fig. 2B and Fig. S1B). In E4F1-deficient cells, this reduced PDH activity should impact on pyruvate-derived mitochondrial AcCoA production, leading to accumulation of glycolytic intermediates, and induce the redirection of the glycolytic flux. We addressed this notion by performing comparative nontargeted gas chromatography/liquid chromatography-mass spectrometry (GC/LC-MS) metabolomic analyses in control and E4f1ko fibroblasts. As predicted, these analyses showed an accumulation of intracellular pyruvate and of its upstream precursor 2/3-phosphoglycerate (2/3PG) in E4f1ko cells (Fig. 2C and D). E4F1-deficient cells also exhibited increased level of extracellular lactate in their culture medium (Fig. 2E). To further assess the PDH-dependent pyruvate oxidation pathway, we next performed stable isotope tracing experiments in control and E4f1ko fibroblasts cultured in presence of uniformly labeled [U-13C]glucose. Comparative LC-MS analyses of intracellular metabolites clearly showed a strong decrease of 13C incorporation into AcCoA (M+2 isotopomer) and in its downstream metabolite (M+2 isotopomer), in E4f1ko cells (Fig. 2F and Fig. S2A). Of note, the relative 13C enrichment in the first glycolytic intermediates (glucose-6-phosphate, fructose-6-phosphate, fructose-1,6-biphosphate, 2/3-PG) was unaffected, suggesting that both control and E4f1ko fibroblasts display comparable glycolytic fluxes. Taken together, these analyses indicate that E4F1-deficiency impairs PDH activity with impacts on the pyruvate oxidation pathway (Fig. S2A).

Of note, we also assessed mitochondrial protein lipoylation as both a direct readout of DLAT expression and of an indirect readout of defective AcCoA production by PDH in E4f1ko cells (2, 28). Indeed, the precursor of lipoic acid, octanoic acid, is synthesized from mitochondrial AcCoA through fatty acid biosynthesis. Lipoic acid is then covalently attached to few proteins, for which it serves as a cofactor. At first glance, total protein lipoylation seemed to be strongly reduced in E4f1ko cells, as revealed by immunofluorescence (Fig. S2B) using an antibody that recognizes all lipoylated proteins. Although this strong decrease of total protein lipoylation likely reflects mainly DLAT protein down-regulation (the most abundant lipoylated protein), we also observed a moderate down-regulation of the lipoylation of dihydrolipoamide-S-succinyl transferase (DLST) by immunoblotting (Fig. 2A and Fig. S2C), suggesting that the AcCoA-dependent lipoylation pathway is also partly affected in E4f1ko cells, and could also contribute to the phenotype of E4f1ko. Finally, despite their low PDH activity, E4f1ko cells showed a moderate, but significant, decrease of intracellular ATP, as described in our previous report (see also ref. 24) (Fig. S2D). This moderate alteration of ATP levels suggests that alternative energetic pathways were activated in E4f1ko cells. Indeed, E4f1ko cells show signs of adaptive metabolic responses, as illustrated by increased fatty acid oxidation (FAO) (Fig. 2G). Accordingly, these cells were highly sensitive to the FAO inhibitor Etomoxir (Fig. S2E). Taken together, these data show that PDH activity and the mitochondrial pyruvate pathway are impaired in E4f1ko cells.

**E4f1 KO in Striated Skeletal Muscles Results in PDH Dysfunction.** We next assessed the in vivo relevance of E4f1-mediated control of this PDC transcriptional program in striated muscle, a tissue in which physiological function relies on high PDH activity during exercise (10). First, we confirmed by ChIP-qPCR the recruitment of E4F1 on Dlat, Dld, Slc25a19, and Bp44l/Mpc1 promoters in adult tibialis and
gastrocnemius muscles of resting mice, indicating that E4F1-PDH program also exists in adult muscle cells (Fig. S4 and Fig. S3A). Then, we inactivated E4f1 in vivo in striated muscles by crossing E4f1 KO mice with Acta1-Cre transgenic (Tg) mice that express the Cre recombineur under the control of the skeletal α-actin promoter [hereafter referred to as Tg(Acta1-Cre)] (Fig. 3B) (29). We verified the efficiency and the tissue specificity of Cre-driven recombination of the E4f1 [floxed] allele in E4f1 [floxed], Tg(Acta1-Cre) and E4f1 [floxed], Tg(Acta1-Cre) control littermates [hereafter referred to as CTL(ACTA) and E4f1 KO(ACTA), respectively]. Cre-driven inactivation of the E4f1 [floxed] allele was largely restricted to striated skeletal muscles, as shown by the strong reduction of E4f1 mRNA and protein levels in gastrocnemius of adult E4f1 KO(ACTA) mice (Fig. 3 D and E). Although limited Cre-mediated recombination (20% efficiency) was also detected in heart (Fig. 3C), this did not impair significantly the cardiac mRNA level of E4f1 when assessed at the whole tissue level (Fig. S3B). E4f1 KO(ACTA) mice were healthy and viable, and detailed anatomopathological analyses of skeletal muscles at 16 wk of age revealed neither major histological alterations nor significant differences in the number and size of muscle fibers compared with control littermates (Fig. S4 A, B, and D). Accordingly, mRNA levels of muscular differentiation markers and inducers such as Mf20, MF2c, MyoD, and Myogenin were similar in adult striated muscles of 16-wk-old E4f1 KO(ACTA) and control mice (Fig. S4E). However, alterations resembling degenerative to necrotizing and diffuse myopathy were gradually detected in older animals. Thus, H&E staining of striated muscle sections prepared from 18-mo-old animals showed that E4f1 KO led to myophagocytosis, hypercontracted fibers, centralized regenerative fibers, immune cell infiltration, and the presence of adipocytes (Fig. S4F). These data suggest that in the long term, E4f1 deficiency results in skeletal muscle disorganization and histological alterations.

Next, we evaluated the consequences of E4f1 inactivation in vivo on this PDH transcriptional program. In skeletal, but not in cardiac E4f1 KO muscles, mRNA, and protein levels of Dlat were strongly altered, whereas the mRNA level of Pdh1a, used as control, remained unchanged (Fig. 3E and Fig. S3D). Expression of Bnap44l, Slc25a19, and Dld was also slightly decreased at the mRNA level (Fig. 3D), although to a lesser extent than Dlat. As in E4f1 KO fibroblasts (tMEFs) in culture, protein lipoylation was also markedly decreased in E4f1 KO muscles, as shown by immunoblotting on proteins extracts and immunostaining of tissue sections (Fig. 3E and F). Impaired expression of these PDC components in E4f1 KO muscles resulted in 80–90% reduction of PDH enzymatic activity in gastrocnemius, as measured by two independent methods (Fig. 4 A and B and Fig. S3D). E4f1 KO muscles also exhibited increased level of circulating ketone bodies, suggesting that E4f1 KO mice activated FAO as in E4f1 KO tMEFs (Fig. 4C). Of note, DLAT expression and PDH activity were also strongly down-regulated in extensor digitorum longus and soleus striated muscles isolated from E4f1 KO(ACTA) mice, indicating that both red and white muscle fibers are equally affected by E4f1-deficiency (Fig. S5 A and B). Consistent with the absence of depletion of E4f1 mRNA in cardiac tissue in this animal model (Fig. S3B), no significant difference in PDH activity was detected in the heart of E4f1 KO(ACTA) mice (Fig. S3C). These data show that the E4f1–PDH connection is critical for the pyruvate–AcCoA metabolic pathway in adult striated skeletal muscles, confirming its biological relevance in vivo.

**E4f1 Inactivation in Skeletal Muscles Results in Lactate Acidosis and Muscular Endurance Defects.** Although E4f1-deficient muscles display a strong reduction of basal PDH activity, 16-wk-old E4f1 KO(ACTA) mice did not show spontaneous locomotor deficiency in normal housing conditions, as quantified by infrared light beam interruption in cages (Fig. S4C). This surprising result indicates that a low muscular PDH activity (10–20% of normal levels) (Fig. 4 A and B) is sufficient to sustain basal locomotor activity and viability. PDH activity has been documented to increase in skeletal muscles during high-intensity exercise and to contribute to muscular endurance (10).

**Fig. 4.** E4f1 inactivation in skeletal muscles results in reduced PDH activity, chronic lactate acidosis and reduced muscular endurance. (A and B) PDH activity measured in protein extracts prepared from gastrocnemius of resting 16-wk-old E4f1 KO(ACTA) animals and CTL(ACTA) littermates using two different methods, DipStick Assay (A) and [14C]-Pyruvate oxidation assay (B). CS enzymatic activity that does not vary (Fig. S3D) was used to normalize PDH activity in B. Histobars represent the mean value ± SEM of n = 5 (A) and n = 2 (B) independent experiments. (C) Ketone bodies level in serum of E4f1 KO(ACTA) and CTL(ACTA) males. (D) Schematic representation of the experimental design to measure the impact of E4f1 inactivation on physical endurance. A shift from a chow to a ketogenic (KETO) diet or addition of DCA in drinking water was done 2 wk before the first training session. (E) Locomotor performance (running distance before exhaustion) of 16-wk-old E4f1 KO(ACTA) and CTL(ACTA) animals under chow or KETO diets, or after administration of DCA, was evaluated using forced treadmill running. Histobars represent the mean value ± SEM of three independent measurements for each animal (n = 8 males per group). (F) Lactate level (serum) of E4f1 KO(ACTA) and CTL(ACTA) males under chow or ketogenic diets, or after administration of DCA, measured after running. Histobars represent the mean value ± SEM (n = 8 males per group). (G) PDH activity was measured after running in protein extracts from striated muscles of E4f1 KO(ACTA) and CTL(ACTA) males, in the presence or absence of DCA. Histobars represent the mean value ± SEM (n = 5). (H) Phosphorylation level of serine 300 of PDH-E1 assessed by immunoblotting of total protein extracts from gastrocnemius of E4f1 KO(ACTA) and CTL(ACTA) animals, in the absence or presence of DCA. HSP70 protein was used as loading control. **P < 0.001; *P < 0.01; *P < 0.05; ns, not significant.

Therefore, we hypothesized that the residual PDH activity in muscles of E4f1 KO(ACTA) mice might not be sufficient to support the energetic demand that occurs during an acute and high exercise workload. Locomotor activity of control and E4f1 KO(ACTA) adult mice was assessed upon forced treadmill running (Fig. 4D). Although PDH activity increased in all animals in this experimental setting, it remained much lower in E4f1 KO(ACTA) mice relative to control littermates (Fig. 4G). Accordingly, E4f1 KO(ACTA) animals displayed a marked decrease of their physical endurance, as documented by a twofold reduction of their running performance [total running distance (Fig. 4E) and time to exhaustion (Fig. S6A)].

Because PDH deficiency results in chronic lactic acidemia in patients, we measured lactate levels in the serum of E4f1 KO(ACTA) and control mice. E4f1 KO(ACTA) animals exhibited increased lactate levels relative to controls under normal housing conditions and regular chow diet (Fig. S6B). Lactic acidemia was further exacerbated upon acute exercise (Fig. 4F). Importantly, 16-wk-old E4f1 KO(ACTA) mice showed no apparent alterations of glucose homeostasis, as assessed by insulin- and glucose-tolerance tests (Fig. S6 C and D), glucose uptake, and expression of the glucose transporter GLUT1 (Fig. S6 E and F). Collectively, our data show that E4f1 KO(ACTA) mice display phenotypes that recapitulate some clinical symptoms observed in PDC-deficient patients, including lactic acidemia and exercise intolerance (11, 30).

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**PHYSIOLOGY**
Discussion
Here, we describe a level of regulation of PDH in mammals implicating the E4F1 transcription factor. To characterize its role in PDH-regulation, we developed a skeletal muscle-specific E4f1 KO mouse model, the phenotypes of which recapitulate some clinical symptoms observed in PDC-deficient patients. In mammals, the control of PDH activity has been mainly attributed to posttranslational modifications of its subunits by PDKs and PDPs. Its control at the transcriptional level has been far less investigated and mainly concerns the regulation of individual Pdk genes by multiple transcription factors, including Foxo1/3a (33), Hif1a (34), or p53 (35). Nevertheless, in more primitive organisms, it was demonstrated that genes coding for other regulators, but also for core PDC components, are coregulated and organized in regulatory units or operons. Thus, in E. coli, the ace/E1, the aceF/E2, and IpdA/E3 genes form a single operon that also includes the transcriptional regulator of this operon PdpR (7). In C. albicans, the Gal4p transcriptional regulator controls the expression of the five main components of the PDC complex, including the Pda1/E1, Pdh1/E1, Ipd1/E2, Lpd1/E3, and Pdc1 subunits (8). Our data reveal that such a coordinated transcriptional program, important for PDH-mediated pyruvate oxidation, also exists in mammals. Composed of at least four genes—Dlat/E2, Dld/E3, Bpp44/MPC1, and Slc25a19—this program is controlled by E4F1, a sequence-specific transcription factor bound nearby the TSS of these genes.

This E4F1-controlled transcription program is a main contributor of the total PDH activity, as demonstrated by the impact of conditional gene targeting of E4f1 in proliferating cells and in postmitotic differentiated muscular cells that resulted in a 80–90% reduction of the basal PDH activity. Surprisingly, we show that, despite their very weak muscular PDH activity, animals lacking E4F1 in their striated muscles were viable and displayed normal skeletal locomotor activity, at least in normal housing conditions. Nevertheless, these animals exhibited lactic acidemia and severe exercise intolerance that were partly rescued by the pharmacological reactivation of the remaining pool of PDH by DCA, or by shunting the need for PDH activity by promoting FAO using a ketogenic diet. Our histological analyses indicate that although E4f1 inactivation did not result in major disorganization of this tissue in young animals, long-term PDH-deficiency led to a degenerative muscular myopathy in older animals. So far, such clinical symptoms have not been described in PDC patients, likely because most of these patients do not live long enough to develop myopathies. On the other hand, it is commonly described that these patients often exhibit epileptic seizures and microcephalies. These symptoms were not observed in our muscle-specific E4f1KO mice, despite these animals displayed chronic lactic acidemia. This finding questions the origin of the neurological manifestations observed in PDC patients and suggests that the latter symptoms do not result solely from chronic systemic lactic acidemia, but could also arise from multiple brain-specific metabolic alterations. Tissue-specific inactivation of E4f1 in the central nervous system may provide a definitive answer to this clinically relevant question.

In conclusion, recently designed strategies to identify new mutated genes involved in unsolved cases of primary mitochondrial human disorders, led to the identification of a homozygous nonsynonymous mutation in the E4f1 gene of a patient showing reduced PDH complex activity, muscular defects, and lactic acidemia (36). This first indication that the E4f1-controlled program could be deregulated in a pathologival situation provides an exciting clinical perspective to the present work. Indeed, our E4f1KO mouse models display phenotypes that recapitulate some clinical symptoms observed in this PDC-deficient patient. Thus, these animals could represent potential models for preclinical studies aiming at testing new therapeutic strategies to improve the consequences of PDH deficiency.

Furthermore, it should be noted that missense mutations in the E4f1-target genes Dlat, Dld, Bpp44, and Slc25a19 have been identified in several congenital metabolic disorders associated with reduced PDH activity and alteration of the pyruvate oxidation pathway. These disorders include PDH deficiency, lipoamide dehydrogenase deficiency, or Amish lethal microcephaly syndromes (3, 11, 13). It is also worth noting that complete KO mouse models for Pdha1, Dld, Slc25a19, as well as for E4f1, all show severe developmental defects and lethality during early embryonic development (4, 22, 37–39). This clearly the importance of investigating the impact of E4f1-controlled PDH-program during embryogenesis and beyond, about the poorly characterized metabolic rewiring of the pyruvate pathway that may occur during development.

Altogether, our data highlight the role of E4F1 in PDH-dependent metabolic homeostasis and pave the way for new studies on the physiological rewiring of the pyruvate pathway. This work should also stimulate new research aiming at exploring the role of nuclear transcription factors in unsolved cases of mitochondrial disorders.

Experimental Procedures
Accession Numbers. The full series of data, including expression arrays and ChiP-Seq data reported in this paper were deposited on the Gene Expression Omnibus (GEO) database repository (see GEO SuperSeries GSE57242 and GSE57221) (24, 26). E4F1 binding regions were defined by combining bioinformatic tools.
provided by CiGenome and Qseq software systems. Detailed protocols, bio-informatic tools and primers used for Chip-seq and ChiP-qPCR validations were as previously described (24, 26) and detailed in SI Experimental Procedures.

Mouse Models and Experimental Treatment. E4f1+/-, E4f1lox/lox and E4f1floxflox mice (22, 40) were intercrossed with ActaCre mice to obtain E4f1lox/lox, ActaCre and E4f1floxflox, ActaCre compound mice on a mixed 129sv/IlbDBA/C57Bl6 background. Phenotypic characterization of compound E4f1lox/loxActaCre and CtlActa mice was performed on 16-wk-old or 18-mo-old littersmates. Mice were housed in a pathogen-free barrier facility in accordance with the ethic Committees for Animal Welfare (Comité d’Éthique en Experimentation Animal - Languedoc Rossillon, CEEA-LR-12116). Mice were maintained under Chow diet (A03; Safe; i.e., 22 kcal% protein, 65 kcal% carbohydrate, and 13 kcal% fat) in the presence or absence of DCA in the drinking water (added to the drinking water for 2 wk at a final concentration of 2 g/l), or were fed with a ketogenic diet (F3666, Bio-Serv; 5kcal% protein, 2kcal% carbohydrate, and 93kcal% fat) for 2 wk before assessing their physical appearances, as detailed in SI Experimental Procedures.

PDH Activity and Lactate Measurements, Metabolomics. Two different protocols were used in parallel to measure PDH activity in protein extracts prepared from cells or muscles (gastrocnemius and heart). PDH activity DiPstick Assay Kit (ab109882, Abcam) was used on cell (25 µg) or muscle (5 µg) extracts prepared according to the manufacturer’s protocol, and quantified by enzymatic assay. Activity was also assessed by measuring the release of [14C] pyruvate after incubation of protein extracts (1 mg of protein per milliliter) with [1-14C] pyruvate, as previously described and detailed in SI Experimental Procedures (41). To determine the concentration of glucose, 2,3PG, citrate, and succinate in E4f1 WT and KO