A Nuclear Pyruvate Dehydrogenase Complex Is Important for the Generation of Acetyl-CoA and Histone Acetylation

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SUMMARY

DNA transcription, replication, and repair are regulated by histone acetylation, a process that requires the generation of acetyl-coenzyme A (CoA). Here, we show that all the subunits of the mitochondrial pyruvate dehydrogenase complex (PDC) are also present and functional in the nucleus of mammalian cells. We found that knockdown of nuclear PDC in isolated functional nuclei decreased the de novo synthesis of acetyl-CoA and acetylation of core histones. Nuclear PDC levels increased in a cell-cycle-dependent manner and in response to serum, epidermal growth factor, or mitochondrial stress; this was accompanied by a corresponding decrease in mitochondrial PDC levels, suggesting a translocation from the mitochondria to the nucleus. Inhibition of nuclear PDC decreased acetylation of specific lysine residues on histones important for G1-S phase progression and expression of S phase markers. Dynamic translocation of mitochondrial PDC to the nucleus provides a pathway for nuclear acetyl-CoA synthesis required for histone acetylation and epigenetic regulation.

INTRODUCTION

Epigenetic regulation of gene expression is essential for embryonic development and differentiation, protection against viruses, and progression of cancer (Jaenisch and Bird, 2003). It includes modifications of core histones by either methylation or acetylation. By neutralizing the positive charges of lysine residues in histones, acetylation promotes the relaxation of DNA, necessary for replication and transcription (Vogelauer et al., 2002). A recent high-resolution mass spectrometry analysis identified a large number of nuclear proteins with multiple acetylation sites, suggesting that the role of acetylation extends beyond histones (Choudhary et al., 2009). In eukaryotes, the biosynthesis of acetyl-coenzyme A (CoA) is thought to occur in the subcellular compartment where it is required, because it is membrane impermeable and very unstable, due to the high-energy thioester bond that joins the acetyl and CoA groups. Our understanding of how the nucleus generates acetyl-CoA in metazoan cells is incomplete. Acetyl-CoA synthetase (AceCS1) and ATP-citrate lyase (ACL) are both present in the nucleus (Wellen et al., 2009). ACL is the predominant pathway for nuclear acetyl-CoA generation in mammalian cells because it utilizes glucose-oxidation-derived mitochondrial citrate as its substrate, as opposed to AceCS1, which uses acetate, a fuel that is not an important energy source for mammalian cells (Wellen et al., 2009). However, in cells with decreased citrate levels due to mitochondrial suppression by Bcl-xL overexpression, levels of acetyl-CoA and N-α-acetylated proteins decreased in the cytoplasm with no apparent decrease in histone acetylation (Yi et al., 2011). This suggested the presence of a yet unidentified mitochondria-independent mechanism for histone acetylation in the nucleus.

Primitive protozoan cells that lack mitochondria, like Entamoeba histolytica, utilize pyruvate:ferrodoxin oxidoreductase (PFO) to generate acetyl-CoA (Rodrı´ guez et al., 1998). Mammalian cells do not express PFO but generate acetyl-CoA in mitochondria with a similar complex of nucleus-encoded proteins known as pyruvate dehydrogenase complex (PDC) (Horner et al., 1999). The generation of acetyl-CoA from both PFO and PDC is dependent on the glycolysis-derived product pyruvate.
Intact and functional PDC can translocate across mitochondrial membranes from the matrix to the outer mitochondrial membrane (Hitosugi et al., 2011). Thus, it would be possible for a chaperone protein to bind PDC from the outer mitochondrial membrane and bring it to the nucleus under conditions that stimulate S phase entry and cell-cycle progression, where histone acetylation is critical.

We provide evidence that supports the hypothesis that functional PDC can translocate from the mitochondria to the nucleus during cell-cycle progression, generating a nuclear pool of acetyl-CoA from pyruvate and increasing the acetylation of core histones important for S phase entry. The mitochondrial pyruvate dehydrogenase kinase (PDK), which phosphorylates and inhibits mitochondrial PDC, was not detectable in the nucleus, suggesting that nuclear PDC may be constitutively active and regulated differently than mitochondrial PDC. The nuclear translocation of PDC was triggered by growth signals, like serum and epidermal growth factor, or mitochondrial inhibitors, like rotenone. Nuclear PDC provides a means for the nucleus to generate acetyl-CoA in an autonomous fashion. The implications of this work extend to many conditions where nuclear acetylation is altered, like development, cancer, neurodegeneration, or cardiovascular disease.

RESULTS

All Components of PDC Are Present in the Nucleus

PDC is comprised of subunits from three catalytic enzymes: pyruvate dehydrogenase (E1), dihydrolipoamide transacetylase (E2), and dihydrolipoamide dehydrogenase (E3), as well as the tethering protein, E3-binding protein (E3BP) (Behal et al., 1993). We first identified the nuclear presence of PDC-E1 (a subunit) in human spermatozoa, which compartmentalize the nucleus (in the head) away from mitochondria (in the midsection), allowing for more clear visualization of nuclear versus mitochondrial PDC (Figure S1A available online). To confirm the nuclear presence of PDC-E1 in a terminally differentiated primary cell line, we isolated primary fibroblasts from human lungs and detected its nuclear presence (Figure 1A). We then performed a more-detailed assessment for the presence of nuclear PDC using microscopy in two commercially available cell lines, normal small airway epithelial cells (SAEC) and A549 cells (non-small-cell lung cancer cells). In intact SAEC and A549 cells cotransfected with an antibody against PDC-E1 and the mitochondrial marker MitoTracker Red, colocalization of the two signals was evident in the cytoplasm as expected, but PDC-E1 was also evident within the nucleus (marked by DAPI) without any mitochondrial signal (Figures 1B and 1C). For our imaging, we generated 25 separate images in each intact cell, systematically scanning in the z axis at 0.2 μm in depth for each image. We used these stacked images to generate 3D videos that show the presence of PDC-E1 throughout the nucleus (Movies S1 and S2). We selected specific planes that “cut through the nucleus” from the z stacked images, as shown in the top image of Figure 1D. Then, in the XY axis, we quantified the nuclear PDC-E1 and MitoTracker Red signals by measuring the fluorescence intensity. As we move from left to right in the image plane, an area of mitochondria is followed by an area where only the nucleus is present. PDC-E1 is highly detected in the mitochondria and colocalizes with the MitoTracker Red signal. However, whereas the MitoTracker Red signal intensity is absent in the nucleus, the PDC-E1 signal is present, though with lesser intensity than the mitochondrial PDC-E1 signal (Figure 1D). Furthermore, nuclear PDC-E1 followed a similar signal pattern to the nuclear protein histone 3, confirming the specificity of the nuclear PDC-E1 signal (Figure S1B). In addition, a clear nuclear signal of PDC-E1 was also detected in the nucleus using electron microscopy (Figure S1C). These data show that the nuclear PDC-E1 signal is not a “contamination” from overlapping mitochondria.

To address the possibility that our PDC-E1 antibody nonspecifically binds to other nuclear proteins, we transfected A549 and SAEC with a plasmid encoding for cloned human E1α subunit of PDC in frame with enhanced GFP (EGFP). Based on EGFP fluorescence, we detected the presence of the fused EGFP-E1 protein in mitochondria as well as the nucleus of both cell types (Figures 1E and S1D). We then identified the nuclear presence for other subunits of the PDC complex, including PDC-E2, PDC-E3, and the ancillary subunit PDC-E3BP in intact cells using immunofluorescence (Figures 1F–1H). For all confocal and electron microscopy experiments, a “secondary antibody-only” control staining was performed, which in all experiments showed no signal (Figure S1E).

Nuclear PDC Is Functional and Can Generate Acetyl-CoA from Pyruvate

To assess if nuclear PDC is functional, we isolated nuclei using a nuclei-specific, high-sucrose gradient centrifugation protocol. As even a small amount of mitochondrial membranes could be a confounding factor, we took several steps to ensure that our nuclei were not contaminated with any mitochondria. We showed that nuclear membranes were intact by the lamin-staining pattern and that our nuclear preparations were free of mitochondrial membranes, as assessed by nonyl-acridine orange (NAO) and MitoTracker Red imaging (Figures 2A and 2B), as well as free of cytoplasmic, mitochondrial matrix, and membrane proteins, as assessed by immunoblots with antibodies against α-tubulin, citrate synthase (CS), isocitrate dehydrogenase 2 (IDH2), succinyl-CoA synthetase (SCS), and succinate dehydrogenase (SDH) (Figure 2C). These vigorous purity indices were used in all of our experiments with isolated nuclear preparations throughout this work. In these pure nuclei preparations, we confirmed the presence of PDC-E1 in nuclei from the EGFP-transfected cells, as well as from intact nuclei from A549 and SAEC cells, using immunofluorescence, with absence of any detectable signal in secondary-only antibody staining (Figure S2A, upper panels). In addition to the α subunit of the PDC-E1, all the subunits of the PDC complex were present in isolated nuclei (Figure S2A, bottom panels). We then used immunoblots and showed the nuclear presence of all PDC subunits in isolated nuclei from A549 cells (Figure 2D) as well as a separate cancer cell line, 786-O renal cell

(Gao et al., 2012; Yang et al., 2012). However, the fate of pyruvate in the nucleus and whether this is related to the acetyl-CoA used in the H3K9 acetylation remain unknown.
carcinoma cells, in the absence of cytoplasmic or mitochondrial contamination (Figure 2E). As NAD(H) is abundant in the nucleus, our finding that PDC-E2 appears to be lipoylated (implying the nuclear presence of lipoic acid; not present in our media), suggests that nuclear PDC has all the necessary cofactors to be functional (Figure S2B).

To study the function of nuclear PDC without interference from mitochondrial PDC, we performed our initial experiments on isolated nuclei. We detected many glycolytic intermediates, including PEP, pyruvate, and acetyl-CoA, using collision-induced dissociation mass spectrometry (Figure 2F). We then designed an experiment to study whether nuclear PDC is functional in terms of the de novo biosynthesis of acetyl-CoA.

We first isolated intact and pure nuclei from either A549 or 786-O cells previously treated with scrambled versus PDC-E1 small interfering RNA (siRNA). We obtained an efficient knockdown of PDC-E1, resulting in no detectable immunoblot signal (Figure 2G, top). This was in keeping with the absence of any PDC activity in nuclei lacking PDC, measured by a standard dipstick assay that measures the NADH produced by the immuno-captured enzyme (Figure 2G, bottom).

We then treated these nuclei with an isotope-labeled form of pyruvate \(^{13}C_2\)-pyruvate) for 8 hr and measured the production of labeled acetyl-CoA \(^{13}C_2\)-acetyl-CoA; Figure 2H). Because the only way to synthesize acetyl-CoA from pyruvate is by PDC, the detection of labeled acetyl-CoA in our nuclear...
preparations reflects the activity of nuclear PDC. We detected a significant decrease in the levels of $^{13}$C$_1$-labeled acetyl-CoA in nuclei from PDC-E1 siRNA-treated cells compared to those from scrambled siRNA, using two separate mass spectrometry acquisition modes in A549 and 786-O nuclei (Figures 2I and S2C). We also detected increasing levels of labeled acetyl-CoA in response to increasing levels of labeled pyruvate, suggesting the presence of nonlimiting amounts of functional PDC in the isolated nuclei (Figures 2I and S2C).

Nuclear PDC Is Important for Histone Acetylation

We then assessed whether the acetyl-CoA generated from nuclear PDC is important for histone acetylation. Because histone acetylation depends on glucose and not free fatty acid metabolism (Wellen et al., 2009), we first deprived the A549 cells of glucose for 24 hr in order to synchronize acetylation, in the presence of scrambled or PDC-E1 siRNA. Next, we isolated functional nuclei from scrambled and PDC-E1 siRNA-treated cells, exposed them to pyruvate (10 mM) for 8 hr, and measured
histone acetylation (Figure 3A). Prior to the addition of pyruvate to our isolated nuclei (time = 0), there were no differences in acetyl-lysine of proteins within the molecular weight of acetylated histones (n = 3 experiments) between nuclei isolated from PDC-E1 siRNA or scramble-treated cells.

(C) Nuclei lacking PDC-E1 exposed to 10 mM pyruvate for 8 hr had decreased levels of acetylated H2B, H3, and H4, compared to control. Lamin and Ponceau S were loading controls. Representative immunoblots are shown to the left, and quantified mean data normalized to lamin are shown to the right (n = 3 experiments; *p < 0.05).

(D) PDC-E1 and ACL siRNA-treated A549 cells had almost complete knockdown of PDC-E1 and ACL, respectively, compared to scrambled siRNA-treated cells (top). Extracted histones from PDC-E1 and ACL siRNA-treated A549 cells exposed to 10 mM glucose had decreased levels of acetylated H2B, H3, and H4 compared to scrambled siRNA control (bottom). Quantified mean data (to total H3) are shown on the right (n = 3 experiments).

Error bars represent SEM. See also Figure S3.

Figure 3. PDC Is Important for Histone Acetylation

(A) Experimental design for acetylation experiments in isolated nuclei. (B) In the absence of pyruvate at time = 0, there were no differences in acetyl-lysine of proteins within the molecular weight of acetylated histones (n = 3 experiments) between nuclei isolated from PDC-E1 siRNA or scramble-treated cells.
source of acetyl-CoA may play a role in which target is acetylated.

**Pyruvate Dehydrogenase Kinase Is Not Present in the Nucleus**

Mitochondrial PDC-E1 is tonically inhibited by PDKs and activated by PDC phosphatases (PDPs). PDKs phosphorylate serine-293 of the \( \alpha \) subunit of PDC-E1, resulting in its inactivation (Behal et al., 1993). Whereas PDK I and II are ubiquitously expressed, PDK III and IV are only expressed in the testis and under starvation conditions in muscle, respectively (Bowker-Kinley et al., 1998). Hypoxia-inducible factor \( \alpha \) (HIF1\( \alpha \)) is activated in many cancers and can suppress mitochondrial PDC by inducing PDK expression (Kim et al., 2006). We detected PDKI and II in mitochondria, but not in isolated nuclei of A549 (which express both isoforms) and 786-O cancer cells (which only express PDKI; Figures 4A and 4B). On the other hand, we detected the presence of PDPI (but not PDPII) in nuclei of A549 and 786-O cells (Figures 4A and 4B). We found higher levels of PDC-E1 serine-293 phosphorylation in A549 mitochondria compared to normal cells (Figure 4C), in keeping with the finding that cancer cells have higher levels of PDK (Michelakis et al., 2010). Furthermore, PDC-E1 phosphorylated serine-293 was present in mitochondria, but not in nuclei (Figure 4A), in keeping with the absence of PDK. This suggested that, in certain conditions (like in cancer or hypoxia, where HIF1\( \alpha \) is activated), whereas mitochondrial PDC can be relatively inactive, nuclear PDC could remain constitutively active.

We then treated isolated nuclei from A549 and 786-O cancer cells with the small-molecule PDK inhibitor dichloroacetate (DCA). Error bars represent SEM. See also Figure S4.
(DCA), which primarily inhibits PDK I and II (Bonnet et al., 2007; Michelakis et al., 2010) and showed no differences in Ac-H3 levels (Figure 4D). However, DCA treatment of whole A549 cells resulted in both increased Ac-H3 (nuclear acetylation) and Ac-tubulin (cytoplasmic acetylation; Figures 4E, S4A, and S4B). The increase in acetylation by DCA in whole cells may be due to acetyl-CoA biosynthesis by cytoplasmic and nuclear ACL, which produces acetyl-CoA using citrate as substrate (Figure 4F). We confirmed the expected DCA-induced increase in citrate, along with another Krebs cycle intermediate (succinate) and the expected decrease in lactate (Figure S4C). Thus, the differential effects of DCA (which has known anticancer properties; Bonnet et al., 2007; Dhar and Lippard, 2009; Michelakis et al., 2010) between mitochondrial and nuclear PDC reflect the differential expression of PDK in the two cellular compartments and suggest that factors that can increase or decrease PDK function (like HIF1α and DCA, respectively) can be used to exploit the functional significance of nuclear PDC, an idea that we explored later on.

**PDC Translocates from the Mitochondria to the Nucleus during S Phase**

To study whether histone acetylation occurs prior to DNA replication during S phase in our cells, we first synchronized cells to the G1 phase by serum starvation for 24 hr. We then introduced serum and serially measured Ac-H3 and cyclin A. Ac-H3 levels increased first, followed by cyclin A (Figure 5A). By isolating pure nuclei from these cells, we also showed that nuclear PDC-E1 levels followed a similar increase pattern to Ac-H3 and then began to decrease toward baseline, but only after Ac-H3 levels peaked (Figure S5A). Furthermore, using microscopy, we showed that both PDC-E1 and Ac-H3 were higher during late S phase compared to baseline in isolated nuclei (Figure S5B).

We then measured both nuclear and mitochondrial PDC-E1 levels upon serum stimulation after cell cycle synchronization, at the same time points. We found that the increase in nuclear PDC-E1 levels during cell-cycle progression was associated with a parallel decrease in mitochondrial PDC-E1 levels, before both PDC fractions returned toward their baseline levels (Figure 5B). The decrease in mitochondrial PDC was not due to enhanced degradation because PDC levels remained unaltered in whole cells in response to increasing concentrations of serum (Figure S5C), suggesting the decrease in mitochondrial PDC was due to its translocation to the nucleus.

To measure the relative distribution of PDC in the mitochondria and nucleus, we loaded on the same gel the same amount of protein from isolated nuclei and mitochondria at baseline and 3 hr postserum stimulation. Although PDC-E1 levels were clearly higher in mitochondria than the nuclei, the percent ratio of nuclear to mitochondrial PDC-E1 increased from 17% to 30% after 3 hr of serum stimulation (Figure S5D). A similar increase in the percent ratio of nuclear to mitochondrial PDC in response to serum stimulation was also observed with the E2 component using immunofluorescence, where we were able to measure mitochondrial PDC (overlap with MitoRed) and nuclear PDC (overlap with DAPI) within the same cell (Figure 5C).

We then performed a series of experiments to further support the fact that nuclear PDC translocates from the mitochondria. To exclude the possibility that the increase in nuclear PDC in response to serum stimulation is a newly translated product from the endoplasmic reticulum (ER), we inhibited ribosomal translation with cycloheximide (CHX) and measured nuclear PDC levels (see Figure 5D). Serum stimulation increased nuclear PDC (E1 and E2), and this was not altered by CHX, although the translation of c-myc (a protein with a short half-life, previously shown to be decreased within 2 hr of CHX treatment; Alarcon-Vargas et al., 2002) was decreased during this timeframe (Figure 5E).

Newly synthesized mitochondrial proteins from the ER contain an N terminus mitochondrial localization sequence (MLS), and upon entry into the matrix, the MLS is cleaved by the mitochondrial-processing peptidase (MPP; see Figure 5D). The cleavage of these (otherwise destabilizing) sequences (~15–50 amino acids) of mitochondrial proteins is required to prevent degradation and facilitate subsequent assembly of subunits (Chacinska et al., 2009). Only mature (cleaved MLS) PDC proteins are able to refold and form complexes in the mitochondria. Therefore, we hypothesized that, if nuclear PDC is directly translocated to the nucleus after its translation in ribosomes, we should be able to detect the precursor form in the nucleus. Using two separate siRNA transfection approaches for the β-catalytic subunit of MPP, we were able to inhibit the mRNA levels by 65% and 90%, respectively, resulting in the accumulation of increasing levels of the precursor form of PDC-E1 (Figure 5F, see arrowhead) along with the mature form. We then used MPP siRNA-treated whole cells and probed for PDC-E1 on the same membrane with isolated nuclei and mitochondrial protein from untreated cells. Whereas the precursor form was clearly seen in the MPP-silenced whole cells in the same gel, only the mature form of PDC was detected in isolated nuclei and mitochondria (Figure 5G, top). We also isolated nuclei from both scrambled and MPP siRNA-treated cells and could not detect the presence of the precursor; only mature PDC was present in the nucleus (Figure 5G, bottom). As MPP is a mitochondria-specific protease, our data suggest that nuclear PDC is processed in the mitochondria prior to translocation to the nucleus.

All of the subunits of PDC are translated in the ER and are transported separately to the mitochondria, where they are processed prior to complex formation. We hypothesized that, if we inhibit only one of the subunits and thus disturb the stoichiometry balance of the subunits within the complex, we may affect the nuclear levels of the other subunits. Indeed, knockdown of only PDC-E1 by siRNA resulted in decreased levels of all catalytic components of PDC in the nucleus, without changing their overall protein expression in whole cell preparations (Figure S5E). This suggests that PDC is translocated from the mitochondria to the nucleus as a functional complex.

**Signals Increasing Nuclear PDC Levels**

We then studied signals that may trigger the nuclear translocation of PDC. Epidermal growth factor (EGF) signaling is important for S phase entry and cell-cycle progression (Kato et al., 1999) and has been shown to increase the nuclear translocation
of PKM2 in cancer (Yang et al., 2012). We found that recombinant human EGF (rhEGF) increased the nuclear levels of PDC components, along with PKM2, in A549 cells (Figures 6A and S6A). The increase in nuclear levels was through translocation because rhEGF did not change the total cellular expression of PDC subunits, whereas it predictably increased tyrosine-204 phosphorylation of mitogen-activated protein kinase (MAPK) (Figure 6B). Similarly, gefitinib, an EGF receptor inhibitor, decreased nuclear (Figure 6C), but not whole cell PDC levels (Figure 6D). We then used confocal imaging and studied the relative distribution of PDC subunits within the same cell, in 786-O cells. Similar to A549 cells, rhEGF increased the relative

Figure 5. PDC Is Translocated from the Mitochondria to the Nucleus

(A) Serum stimulation time course after cell-cycle synchronization in G1 phase showed an early increase in acetylation of H3, followed by an increase in the S phase marker cyclin A. Representative immunoblots are shown above. The rate of acetylation of H3 is faster than cyclin A expression within the first 4 hr after introduction of serum. Note the brake in the time scale, showing a later relative plateau in H3 acetylation, whereas cyclin A levels continue to increase (n = 3 experiments; *p < 0.001 for Ac-H3 compared to cyclin A slopes).

(B) Serum stimulation time course shows an increase in nuclear levels of PDC-E1 peaking at 3 or 4 hr, associated with a parallel decrease in mitochondrial PDC-E1. This was followed by a return toward baseline levels of both the mitochondrial and nuclear PDC levels. Representative immunoblots are shown above, and quantified data normalized to either lamin (nuclei) or CS (mitochondria) are shown below (n = 4 experiments).

(C) A549 cells were costained with PDC-E2 (green), MitoTracker Red (red), and DAPI (blue). The mitochondrial E2 signal was quantified by signal overlap with MitoTracker Red, whereas nuclear signal was quantified by signal overlap with DAPI in the same cell, and the ratio of the two for each cell was calculated. Serum stimulation increased the nuclear to mitochondrial ratio, suggesting nuclear translocation from mitochondria (n = 25 cells; *p < 0.05).

(D) Experimental design for the study of nuclear PDC translocation using cycloheximide (CHX) and gene silencing of MPP.

(E) In response to serum stimulation (4 hr), nuclear PDC-E1 and PDC-E2 increased, and this was not altered by CHX, which decreased the levels of c-myc.

(F) Using two siRNA transfection approaches to silence MPP, we found a clear signal of the unprocessed (precursor) form of PDC-E1 (arrowhead). Lamin was used as a loading control. Quantitative RT-PCR (qRT-PCR) shows the relative fold change for both MPP siRNA transfection approaches. Note that more-effective silencing (transfection 2) resulted in higher levels of the PDC-E1 precursor.

(G) The PDC-E1 precursor was not detected in protein from isolated nuclei or mitochondria loaded in the same gel with whole cell protein from MPP siRNA-treated cells, which clearly showed the presence of the precursor (top; arrowhead shows precursor form). The precursor PDC-E1 was not detectable in nuclei isolated from the MPP siRNA-treated cells (arrowhead represents where the precursor band would have been detected).
distribution of PDC-E1 and PDC-E2 from the mitochondria to the nucleus (Figure 6E).

We speculated that a mitochondrial stressor might trigger translocation of PDC from the mitochondria to the nucleus. We studied the electron transport chain (ETC) complex I inhibitor rotenone, because inhibition of ETC complexes is a well-known cause of mitochondrial stress (Durieux et al., 2011). We found that rotenone caused a significant translocation of PDC into the nucleus over and above serum and rhEGF (Figures 6E and S6B). Mitochondria adapt to stress by initiating the mitochondria unfolded protein response, which results in increased expression of mitochondrial import proteins, folding chaperones and heat shock proteins, amplifying the communication with the nucleus (Zhao et al., 2002). We speculated that a heat shock chaperone may be involved in the nuclear translocation of PDC.

Figure 6. Signals Increasing the Nuclear Translocation of PDC
(A and B) Isolated nuclei from rhEGF-treated A549 cells have increased levels of PDC-E1, E2, and E3 compared to the vehicle-treated cells (n = 3 experiments; *p < 0.05; A), without changing total cellular levels, whereas rhEGF treatment increased tyrosine-204 phosphorylation of MAPK (B). Lamin and actin were used as loading controls.

(C and D) Gefitinib decreased nuclear levels of all three PDC subunits, as shown by immunoblots, without changing the total cellular expression.

(E) 786-O cells were costained with PDC-E2 (green), MitoTracker Red (red), and DAPI (blue). Serum stimulation increased the nuclear to mitochondrial ratio, compared to serum-free treated cells; this was enhanced by rhEGF and further enhanced by the addition of rotenone (5 μM; n = 25 cells; *p < 0.05 compared to serum free; **p < 0.05 compared to vehicle; #p < 0.05 compared to rhEGF). A similar pattern was seen in PDC-E1 translocation (representative images shown in Figure S6B).

(F) Hsp70 coimmunoprecipitates with PDC-E1 and PDC-E2, but not with PDKI, in A549 cells. Input represents 2.5 μg of whole cell lysate. IP, immunoprecipitation.

(G) Serum-stimulated (4 hr) A549 cells pretreated with KNK437 (100 μM) show decreased nuclear levels of both Hsp70 and PDC-E1 compared to vehicle (DMSO)-treated cells. Mean data are normalized to Ponceau S (n = 3 experiments; *p > 0.05).

(H) The same cells as in (G) were costained with Hsp70 and PDC-E1, imaged with confocal microscopy, and the nuclear fluorescence intensity was measured. A Pearson product-moment correlation plot showed that nuclear levels of Hsp70 and PDC-E1 correlate positively (r = 0.66; p < 0.001; n = 50 cells/group). Representative images are shown in Figure S6H. AFU, arbitrary fluorescence units.

(I) Serum-stimulated (4 hr) A549 cells transfected with Hsp70 siRNA show decreased nuclear levels of both Hsp70 and PDC-E1, compared to scrambled-transfected cells. Error bars represent SEM.
We studied heat shock protein 70 (Hsp70) based on evidence that (1) induction of Hsp70 is cell cycle dependent, with its highest expression and nuclear localization observed during S phase (Milarski and Morimoto, 1986), (2) Hsp70 is involved in the nuclear translocation of several proteins (Shi and Thomas, 1992), and (3) Hsp70 binds to and activates mitochondrial PDC (Kiang et al., 2006). We confirmed that nuclear levels of Hsp70 increase in a cell-cycle-dependent manner, similarly to the increase in nuclear PDC-E1 following serum stimulation (Figure 6C). We performed immunoprecipitation studies on A549 cells and MRC-9 cells (fibroblasts) with Hsp70 and detected the presence of PDC-E1 and PDC-E2 (Figures 6F and S6D), suggesting that Hsp70 may bind to these components of the complex. Sequence and structural analysis of potential binding motifs for Hsp70 within the PDC complex showed a putative Hsp70-binding motif (i.e., hydrophobic peptide regions; Mayer and Bukau, 2005) within the PDK-binding site on PDC (Figure S6E). PDK did not coimmunoprecipitate with Hsp70 (Figures 6F and S6D), suggesting that Hsp70 and PDK may compete for similar binding domains within PDC.

We pretreated serum-starved A549 and 786-O cells with either vehicle (DMSO) or KNK437, an inhibitor of heat shock factor 1, which results in decreased expression of inducible Hsp70 (Koishi et al., 2001), prior to serum stimulation for 4 hr. KNK437 decreased mRNA expression (Figure S6F) and nuclear levels of both Hsp70 and PDC-E1, compared to vehicle (Figures 6G and S6G). Nuclear Hsp70 levels correlated positively with nuclear PDC-E1 levels in both vehicle and KNK437-treated A549 cells (Figures 6H and S6H). Similar to KNK437, a specific siRNA against Hsp70 also decreased nuclear levels of both Hsp70 and PDC-E1 in response to serum stimulation (Figure 6I).

Nuclear PDC Is Important for S Phase Entry and Cell-Cycle Progression

Our data on the dynamic translocation of PDC from the mitochondria to the nucleus suggest that it is the same enzymatic complex that is present in both compartments, rather than perhaps a different variant. This makes the study of the relative biological role of PDC on histone acetylation and cell-cycle progression in the two cellular compartments challenging, particularly in the intact cell setting. We took advantage of PDK’s localization in mitochondria, but not the nucleus, and designed experiments in an intact cell setting, in which by subtracting the effects of selective mitochondrial PDC inhibition (by the induction of PDK) from the effects of total cellular PDC inhibition (by PDC-E1 siRNA), we could expose the role of nuclear PDC.

Whereas PDK is already induced in cancer cells, we aimed to maximally inhibit mitochondrial PDC by two methods: first, inducing endogenous PDK by activation of HIF1α and, second, increasing exogenous PDKi via transfection with a PDKi plasmid (see Figure 7A). With either method, mitochondrial PDC should be maximally inhibited (condition 1). Then, by inhibiting total cellular PDC by siRNA gene silencing (condition 2), we could expose the effects of nuclear PDC on histone acetylation and cell-cycle progression by subtracting condition 1 from condition 2 (Figure 7A).

For the first experiment, we infected A549 cells with an adenovirus encoding for CA5 (AdCA5), a constitutively active mutant form of HIF1α (mHIF1α) (Manalio et al., 2005), avoiding the confounding effects of hypoxia. Compared to an adenovirus encoding for LacZ (AdLacZ), increased expression of mHIF1α by AdCA5 (Figures S7A and S7B) resulted in a significant increase in the expression of PDKi (Figure 7B) and phosphorylation of serine-293 on mitochondrial, but not nuclear, PDC-E1 (Figure 7C). Mutant HIF1α increased mitochondrial membrane potential (Figure S7C), further supporting the mitochondrial PDC suppression and its impact on mitochondrial function in our model, as we have previously described (Bonnet et al., 2007). We then treated AdCA5-infected cells with scrambled (condition 1) versus PDC-E1 siRNA (condition 2). We also gave scrambled siRNA to AdLacZ-infected cells (control). We synchronized all the groups to the G1 phase by serum starvation for 24 hr, followed by reintroduction of serum, and 24 hr later, we measured S phase markers and specific histone-3 acetylation sites that are involved in cell-cycle progression. AdCA5-infected cells treated with scrambled siRNA had increased acetylation of H3K9 and H3K18, both important for S phase progression (Cai et al., 2011); increased G1-S phase progression shown by increased levels of phosphorylated retinoblastoma (P-Rb); as well as elongation-2 factor (E2F), cyclin A, and cyclin-dependent kinase 2 (Cdk2), markers for S phase entry, compared to AdLacZ-infected cells treated with scrambled siRNA (Figure 7D). These effects (condition 1) are in agreement with the described effects of HIF1α on proliferation (Semenza, 2010) and the recruitment of histone acetyl transferases (Luo et al., 2011). In contrast, AdCA5-infected cells treated with PDC-E1 siRNA (condition 2) had significantly decreased acetylation of H3K9 and H3K18 and decreased levels of P-Rb, E2F, cyclin A, and Cdk2 compared to AdCA5-infected cells treated with scrambled siRNA (condition 1; Figure 7D). Because both conditions had activated HIF1α and inhibited mitochondrial PDC, subtraction of condition 2 (gray bars) from condition 1 (black bars) in Figure 7D shows that nuclear PDC inhibition decreases Ac-H3K9 and Ac-H3K18 (whereas total H3 levels remain relatively stable) as well as P-Rb and S phase regulators (E2F, cyclin A, and Cdk2).

For the second experiment, transfection with a PDKi plasmid increased the expression of PDKi (Figure S7D) and phosphorylation of PDC-E1 serine-293 (Figure 7E), compared to the empty vector control. PDKi overexpression did not change histone acetylation or cell-cycle progression (Figure 7E; compare white bars [control] to black bars [condition 1]), compatible with the fact that mitochondrial PDC is significantly inhibited in these cancer cells at baseline and further inhibition may not elicit any measurable effects. However, similar to the first experiment, PDC siRNA (condition 2), decreased H3K9 and H3K18 acetylation and decreased P-Rb, E2F, cyclin A, and Cdk2, compared to scrambled siRNA controls (condition 1; Figure 7E). Thus, two different approaches of inhibiting nuclear PDC support its role in S phase progression.

DISCUSSION

Here, we show that PDC is present and functional in the nucleus. Nuclear PDC can generate acetyl-CoA utilized for histone acetylation and S phase entry, providing a link between metabolism and epigenetic or cell-cycle regulation. This source of nuclear
acetyl-CoA may also be important in conditions where the availability of cytoplasmic citrate (which can cross the nuclear membrane and produce acetyl-CoA through ACL) is decreased due to suppressed production or shift toward lipid synthesis. Our work suggests that nuclear PDC has a mitochondrial origin because it lacks the MLS (which can only be cleaved in the mitochondria), and its nuclear increase in response to serum is not affected by inhibition of ribosomal translation. In addition to serum, nuclear PDC translocation increases under growth factors (EGF) or mitochondrial inhibitors (rotenone), suggesting a potential role in disease states with proliferative signals or mitochondrial dysfunction, like cancer.

It is intriguing that our data suggest translocation of PDC in the nucleus, given the large size of this enzymatic complex. High-resolution electron microscopy and structural analysis have recorded its size and diameter within the range of 8–10 MDa and 25–45 nm, respectively (Sumegi et al., 1987; Zhou et al., 2001a). However, there is evidence for size and conformational variability of PDC with identification of complexes as small as 1 MDa (Sumegi et al., 1987; Zhou et al., 2001b). Although the stoichiometry of the individual components in the complex (i.e., the relative amount of E1, E2, and E3 subunits) varies, in keeping with the reported variability in its size among tissues, only the interplay of all subunits within a functional complex can produce acetyl-CoA. The fact that we find de novo production of acetyl-CoA in isolated nuclei in response to pyruvate suggests that a functional complex is present. The fact that we can decrease the nuclear levels of all subunits by only silencing the gene for one subunit suggests that the complex travels as a whole from the mitochondria into the nucleus. PDC was recently identified

**Figure 7. Nuclear PDC Is Important for S Phase Entry**

(A) Experimental design for the study of nuclear PDC on S phase entry in whole cells (see Results section).

(B and C) AdCA5-treated cells had higher PDKI mRNA levels (n = 3 experiments; *p < 0.01) and higher PDKI protein levels and phosphorylated PDC-E1 serine-293 compared to AdLacZ-treated cells in isolated mitochondria, but no detectable levels of PDKI were seen in isolated nuclei from both groups compared to AdLacZ-treated cells (quantification of the immunoblots is shown to the right).

(D) A549 cells treated with AdCA5 (condition 1) followed by scrambled siRNA had increased levels of PDKI; phosphorylated PDC-E1; Ac-H3K9; Ac-H3K18; the G1-S phase progression marker P-Rb; and the S phase markers elongation-2 factor (E2F), cyclin A, and cyclin-dependent kinase 2 (Cdk2) compared to AdLacZ-treated scrambled siRNA cells. In contrast, A549 cells treated with AdCA5 followed by PDC-E1 siRNA (condition 2) had decreased levels of both PDC-E1 and phosphorylated PDC-E1; similar levels of PDKI; and decreased levels of Ac-H3K9, Ac-H3K18, P-Rb, E2F, cyclin A, and Cdk2 compared to AdCA5-treated scrambled siRNA cells (n = 3 experiments; *p < 0.05 versus AdLacZ scr. siRNA; #p < 0.05 versus AdCA5 scr. siRNA).

(E) Transfection with PDKI plasmid followed by PDC-E1 siRNA (condition 2) decreased the levels of both PDC-E1 and phosphorylated PDC-E1; Ac-H3K9, Ac-H3K18, P-Rb, E2F, cyclin A, and Cdk2 compared to transfection with PDKI plasmid but treated with scrambled siRNA (condition 1; n = 3 experiments; *p < 0.05 versus scr. siRNA).

(F) Proposed model for the translocation of PDC from mitochondria to the nucleus and its functional role (see Discussion). Error bars represent SEM. See also Figure S7.
as an intact functional complex on the outer mitochondrial membrane (Hitosugi et al., 2011), suggesting that it can translocate across mitochondrial membranes, a more complex process than translocation across the nuclear membrane. Because the nuclear pore complex can accommodate the entry of large complexes of similar diameter to PDC, like ribonucleoprotein complexes (Lodish et al., 2000) or intact nucleocapsids of viruses (Panté and Kann, 2002), it is possible that an intact PDC complex could translocate to the nucleus.

We provide evidence that Hsp70 may promote the nuclear translocation of a constitutively active form of PDC. By competing with PDK for binding to PDC, Hsp70 may allow PDC to remain active when translocated to the nucleus (Figure 7F). Kiang et al. (2006) showed that Hsp70 binds to and activates mitochondrial PDC, but did not show the mechanism of this activation. It may be that, by competing with PDK, Hsp70 inhibits the binding of this inhibitory kinase and thus activates PDC. It is possible that EGF can promote nuclear acetylation by a coordinated translocation of PKM2 and PDC, potentially by increasing Hsp70 levels (Miliarski and Morimoto, 1986; Figure 7F). This model may contribute to the recently described effects of nuclear PKM2 on tumor growth via histone acetylation, offering a source and mechanism for the nuclear acetyl-CoA generation used for the acetylation of H3K9 (Yang et al., 2012). It is appealing to consider that there is a mechanism by which several factors required for the nuclear response to proliferative stimuli (for example PKM2 and PDC) can be transferred simultaneously, increasing efficiency. Although our collective data support the existence of the translocation model shown in Figure 7F, we cannot rule out the possibility that individual subunits may be transported independently in the nucleus, where they could potentially be assembled in an intact complex with the help of a chaperone, like Hsp70.

Tyrosine phosphorylation by growth factor signaling, including EGFR, activates PDKI, providing a mechanism for suppression of mitochondrial PDC in cancer (Hitosugi et al., 2011), in addition to the induction of PDC expression by HIF1α (Kim et al., 2006). Therefore, in a “double hit” manner, EGF stimulation can activate PDK, suppressing mitochondrial PDC (which has been shown to inhibit mitochondria-dependent apoptosis in cancer; Bonnet et al., 2007) in tandem with Hsp70-mediated nuclear translocation of PDC, promoting histone acetylation and cell-cycle progression (Figure 7F). The inhibition of PDK by siRNA, DCA, or hybrid drugs (like metotatin, a drug that structurally combines cisplatin with DCA molecules; Dhar and Lippard, 2009) decreases cancer growth in animal models (Bonnet et al., 2007) and a small human trial (Michelakis et al., 2010) by activating glucose oxidation, reversing the Warburg effect and the resistance to apoptosis in cancer cells. Our work now suggests that the nuclear pool of PDC is “immune” to this strategy. It raises the possibility that, in response to these interventions, cancer cells may “escape” by promoting a transfer of PDC in the nucleus, where PDC may promote proliferation. It also suggests that anticancer strategies, in which PDK inhibition is used, may perhaps be strengthened by simultaneous inhibition of EGFR signaling or Hsp70 function. As PDC plays a prominent role in many metabolic disorders, it will be important for scientists to be aware that their efforts to target PDC may have direct and previously unrecognized effects on nuclear biology.

Our work suggests an alternative pathway to ACL for the nucleus to generate acetyl-CoA for histone acetylation. It is possible that, in specific tissues or disease states, the relative importance of ACL or nuclear PDC may be different. ACL has been shown to be important for histone acetylation during differentiation (Wellen et al., 2009), whereas our work suggests that histone acetylation by nuclear PDC may be important for cell-cycle progression. ACL and PDC should be studied together when assessing histone acetylation and epigenetic regulation in conditions in which both differentiation and proliferation are taking place, including development, cancer, and other proliferative conditions or stem cell biology.

**EXPERIMENTAL PROCEDURES**

For additional details, see Extended Experimental Procedures.

**Cell Culture**

Human A549 non-small-cell lung cancer cells, 786-O renal cell carcinoma, and MRC-9 fibroblasts were purchased from ATCC. A549 cells were maintained on F12K medium, whereas 786-O cells in RPMI-1640 media and MRC-9 cells in Eagle’s minimal essential medium (EMEM). Primary human fibroblasts were isolated from the lung of a transplant patient in accordance with the Human Ethics Committee at the University of Alberta and maintained in Dulbecco’s modified Eagle’s medium (DMEM). Human SAECs were purchased from ScienCell and maintained in SAECpCM, provided by the company. Media for all cell lines were supplemented with 10% fetal bovine serum (FBS) (unless stated otherwise) and 5% antibiotic and antifungic (Invitrogen-Gibco Canada).

**Confocal Microscopy**

Confocal microscopy was performed using a two-photon Zeiss LSM 510 NLO model (Carl Zeiss). All images were scanned in midplane of the cell in the z axis as shown in Figure 1D, using a 100 x numerical aperture (NA) 1.3 oil objective lens at 2x zoom, allowing for a pixel size of 0.04 x 0.04 x 0.2 μm. Fluorophore-conjugated secondary antibodies (Dako, Invitrogen, and Molecular Probes) and the nuclear stain DAPI (Molecular Probes) were used for immunofluorescence imaging with specific excitations of 488 nm (Fitc), 543 (Trtcl), 633 (Far Red), and 750 nm; two-photon (DAPI) and the corresponding emissions were detected with the following filter sets. Fitc: band pass (BP) 505–535; Trtcl: BP 565–615; Far red: high pass (LP) 650; and DAPI: BP 390–465. Overlap was eliminated between the emissions of any secondary antibodies, mitochondrial-specific dyes, EGFP, and nuclear stains by imaging each channel independently and sequentially with only one excitation wavelength active during each scan.

**Mass Spectrometry for 13C2-acetyl-CoA**

Isolated nuclei from scrambled and PDC-E1 siRNA-treated cells were exposed to 13C2-pyruvate (Cambridge Isotope Laboratories) for 8 hr, before the experiment was terminated with the addition of ice-cold storage buffer. Metabolites were extracted and 30 μl of samples was diluted to 120 μl with methanol and flow injected to the mass spectrometry (MS) using 4000 QTRAP mass spectrometer (AB Sciex) with either enhanced product ion (EPI; IonSpray voltage of –4,500 V) or enhanced MS (EMS; IonSpray voltage of 5,500 V).

**Immunoblotting**

Immunoblotting with standard SDS-PAGE was performed as previously described (Bonnet et al., 2007). Where required, SDS-PAGE of purified histones as well as nuclear, mitochondrial, and cellular protein was performed on 16.5% Tricine gels (Bio-Rad) followed by immunoblotting to low-pore-size (0.2 μm) nitrocellulose (Bio-Rad).
Statistical Analysis

Unpaired Student’s t test was used for statistical calculations when comparing the effects of treatment between two sample groups. Error bars indicate SEM.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Extended Experimental Procedures, seven figures, and two movies and can be found with this article online at http://dx.doi.org/10.1016/j.cell.2014.04.046.

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SUPPLEMENTAL INFORMATION

EXTENDED EXPERIMENTAL PROCEDURES

Cell Culture
Human A549 non-small cell lung cancer cells, 786-O renal cell carcinoma and MRC-9 fibroblasts were purchased from ATCC (Manassas, VA). A549 cells were maintained on F12K medium, while 786-O cells in RPMI-1640 media and MRC-9 cells in EMEM. Primary human fibroblasts were isolated from the lung of a transplant patient in accordance with the Human Ethics Committee at the University of Alberta and maintained in DMEM. Primary human fibroblasts were isolated from the lung using an enzymatic cocktail containing papain (1mg/ml), dithiothreitol (0.5mg/ml), collagenase (0.6mg/ml) and bovine serum albumin (0.6mg/ml) (Sigma-Aldrich) as previously described (Sutendra et al., 2011). Human small airway epithelial cells (SAECs) were purchased from ScienCell (Carisbad, CA). SAECs were maintained in SAEpCM, which was provided by the company. Media for all cell lines were supplemented with 10% FBS (unless stated otherwise) and 5% antibiotic and antifungal (Invitrogen-GIBCO Canada, Burlington, Canada).

Confocal Microscopy
Confocal microscopy imaging was performed using a Zeiss LSM 510 NLO confocal microscope with two-photon capability (Carl Zeiss Microscopy, Jena, Germany). PDC-E1, E2, PDK1, PDKII and Hsp70 antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA) and used at a 1:50 dilution. PDC-E1β, E3, E1BP, lamin and PKM2 antibodies were purchased from Abcam (Cambridge, MA) and used at a dilution of 1:100. Ac-H3 antibody (against all N-terminal acetyl-lysine residues) was purchased from Cell Signaling Technology and used at a dilution of 1:100. MitoTracker Red (250nM; Invitrogen-GIBCO) and Nonyl acridine orange (100nM; Invitrogen-Molecular Probes, Burlington, Canada) were used as mitochondrial membrane markers. Quantification of both the mitochondria and nuclear PDC signal was assessed using the Zeiss physiology option software, which can quantify fluorescence units along the plane (line) of a specified region, using the Profile setting. As depicted in Figure 1D, a plane that crosses through the mitochondria and nucleus was selected to directly compare the intensity of signal between the two organelles and to also show the purity of the nuclear signal for PDC as the signal for the mitochondrial marker Mitotracker Red was not detected in the portion of the plane that crosses the nucleus. Image J 64 software was used to measure the nuclear (signal present within the DAPI region of the cell) and mitochondrial (signal present within MitoRed region of the same cell) PDC signal (using the integrated density parameters) in response to serum stimulation, rhEGF or rotenone treatment. All images were scanned in mid-plane of the cell in the z-axis as shown in Figure 1D, using a 100X NA 1.3 oil objective lens at 2X zoom, allowing for an pixel size of 0.04 μm x 0.04 μm x 0.2 μm. This allows for the clear visualization of PDC in the mid-plane of the nucleus. Fluorophore conjugated secondary antibodies (DAKO, Carpinteria, CA and Invitrogen, Molecular Probes, Eugene OR) and the nuclear stain DAPI (Molecular Probes) were used for immunofluorescence imaging with specific excitations of 488 nm (Ftci), 543 (Tritc), 633 (Far Red) and 750 nm two photon (DAPI) and the corresponding emissions were detected with the following filter sets. Ftci: BP 505-535, Tritc: BP 565-615, Far red: LP 650 and DAPI: BP 390-465. Overlap was eliminated between the emissions of any secondary antibodies, mitochondrial-specific dyes, EGFP and nuclear stains by imaging each channel independently and sequentially with only one excitation wavelength active during each scan.

Reconstructed Video
Twenty-five stacked images consisting of 0.2 μm sections in depth were acquired using confocal microscopy. The frame size was 2048x2048 pixels. These stacked images covering an area of 46.1 μm (length) x 46.1 μm (width) x 5 μm (depth) generated the constructed video using the Zeiss 3D for LSM software. All experiments included negative control secondary antibody-only staining (Figure S1E, S2A, and S6A), which in all presented experiments showed no signal, supporting the specificity of the antibodies used.

EGFP-PDC-E1 Plasmid
EGFP-PDC-E1 plasmid was generated by OriGene Technologies (Rockville, MD). Briefly, cDNA ORF clone of Homo sapiens pyruvate dehydrogenase alpha 1 (PDHA1) (OriGene Technologies; Rockville, MD) was cloned into pEGFP-N1 vector (Clontech, Mountain View, CA) and transfected into A549 and SAECs using Xfect transfection agent (Clontech).

Spermatozoa Isolation
Human spermatozoa were isolated from semen by centrifugation for 10 min at 700 x g. Spermatozoa were then plated on slides coated with cell-tak (BD Biosciences, Mississauga, Canada) before immunofluorescence staining and confocal microscopy.

Immunogold Electron Microscopy
A549 cells were grown on coverslips, fixed in 3% freshly prepared paraformaldehyde plus 0.05% glutaraldehyde and permeabilized with 0.05% saponin. Following blocking, cells were incubated with a mouse monoclonal antibody against PDC-E1 α (Santa Cruz Biotechnology) for one hour and then with fluro-nanogold anti mouse Fab Alexa Fluor 488 (Nanoprobes, Yaphank, NY) secondary antibody over night at 4°C. Cells were further fixed in 2.5% glutaraldehyde in PBS plus 2% sucrose, and nanogold particles were gold-enhanced (Gold Enhance, Nanoprobes). Cells were then dehydrated and embedded in EMBed-812. Seventy-millimeter thin sections were prepared and observed using a Philips 410 electron microscope (Philips Research, Briarcliff Manor, NY). A Hitachi
H-7000 (Hitachi High Technologies America, Schaumburg, IL) Transmission Electron Microscopy (TEM) was used at 15,000x magnification and 80kV, in order to image isolated nuclei and confirm their purity.

**Functional Nuclear Isolation**

Isolation of nuclei was performed using the commercially available nuclei isolation kit: nuclei PURE prep from Sigma Aldrich. Briefly, adherent cells were washed with PBS and scraped from the plate in the presence of lysis buffer. Cells (in lysis media) were carefully placed on top of a 1.8M sucrose gradient and the resulting suspension was centrifuged at 30,000 x g for 45 min in a precooled swinging bucket ultracentrifuge. Nuclei were collected as a white pellet at the bottom of the centrifuge and washed with nuclei storage buffer (provided with the kit). Purity of nuclei was assessed by immunocytochemistry and immunoblot. For functional experiments, isolated nuclei were used immediately.

**PDC Activity Assay**

PDC activity was measured using the MitoProfile Dipstick Assay Kit (MitoSciences, Eugene, OR). Protein (50 μl of 1 μg/μL) was collected from isolated nuclei and placed in a 96-well dish and incubated with the dipstick containing the PDC antibody. The enzyme complex is immuno captured in its native form and activity is visualized by coupling PDC-dependent production of NADH to the reduction of NBT in the presence of excess diaphorase, forming an insoluble intensely colored precipitate at the capture line. PDC activity was measured by the intensity of band using a flat top scanner.

**siRNA Treatment**

PDC-E1, ACL, Hsp70 and scrambled-siRNA (Ambion, Austin, TX) were transfected at a final concentration of 20nM with CaCl₂. MPPβ was transfected at a final concentration of 200nM with CaCl₂. After 18 hr, media was changed and experiments were performed 48 hr later.

**Isolated Nuclei Experimental Protocol for Acetyl-CoA Measurement and Histone Acetylation**

A549 or 786-O cells transfected with scrambled or PDC-E1 siRNA were maintained in glucose free media 24 hr prior to nuclear isolation. Immediately following nuclear isolation, isolated nuclei were incubated with 10mM pyruvate in nuclear storage buffer (Sigma Aldrich) for 8 hr at 37°C. The experiments were terminated by the addition of ice-cold nuclear storage buffer and the nuclei were centrifuged at 500 x g before they were washed with ice-cold storage buffer to remove any excess pyruvate. Nuclei were then prepared for either metabolite extraction and acetyl-CoA measurements by mass spectrometry or protein extraction and histone acetylation by immunoblots.

**Metabolite Extraction**

Nuclei and cells were resuspended in 800 μl of ice-cold 80% methanol and 20% ddH₂O. Samples were vigorously vortexed and placed in liquid N₂ for 10 min to freeze. Samples were then thawed on ice for 10 min, before freeze-thaw cycle was repeated. Samples were centrifuged at 13,000 x g to pellet cell debris, lipids and proteins. Supernatant was evaporated and resulting metabolites were resuspended in HPLC-grade H₂O. Metabolites were normalized to protein concentration.

**Mass Spectrometry for 13C₁-Acetyl-CoA**

Isolated nuclei from scrambled and PDC-E1 siRNA-treated cells were exposed to 13C₂-pyruvate (Cambridge Isotope Laboratories, Andover, MA) for 8 hr, before the experiment was terminated with the addition of ice-cold storage buffer. Metabolites were extracted and 30 μl of samples was diluted to 120 μl with methanol and flow injected to the MS using 4000 QTRAP mass spectrometer (AB Sciex, Concord, Canada) with either enhanced product ion (EPI; IonSpray voltage of –4500V) or enhanced MS (EMS; IonSpray voltage of 5500V).

**Mass Spectrometry for Glycolytic Intermediates**

Seven microliters of sample was injected using a 4000 QTRAP mass spectrometer (AB Sciex) equipped with a UHPLC 1290 system (Agilent Technologies, Mississauga, Canada) via SRM for all 9 glycolytic intermediates and acetyl-CoA. Samples were delivered to the MS with mobile phases A (20mM NH₄OH, 20mM NH₄Ac in 95%/5% H₂O/CH₃CN) and B (98% CH₃CN, 2% H₂O) via a 2.0mm i.d. x 10cm HILIC Luna NH₄ column (Phenomenex, Torrance, CA) at 250 μl/min using negative ion LC/MS/MS analytical run. The dwell time was 5ms per SRM transition, and collision energy was optimized for each SRM transition. Total cycle time was 2.09 s.

**Histone Extraction**

Histone extraction was performed using the commercially available EpiQuik Global Histone Acetylation Assay kit (Epigentek, Brooklyn, NY). Briefly isolated nuclei and whole cells were lysed and proteins were precipitated with 25% trichloracetic acid. Extracted histone pellets were dissolved in HPLC-grade distilled water.
**1H-Nuclear Magnetic Resonance**

1H-NMR spectra was acquired on an 800-MHz Inova spectrometer (Agilent formerly Varian Inc, Palo Alto, CA) equipped with a HCN Z-axis gradient cold-probe. 1H-NMR spectra were acquired at 25°C using the first increment of a 2D-1H-1H-NOESY probe sequence, commonly referred to as the metnosey (i.e. 1D-1H-NOESY). Spectra were collected with 128 transients and 8 steady state scans using a 4 s acquisition time and a 990 ms presaturation, with saturation during the 100 ms-mixing period.

**Cycloheximide Experiments**

786-O cells were serum starved and pretreated with either vehicle (DMSO) or 100 µg/ml cycloheximide (Cell Signaling) for 24hrs prior to serum stimulation for 4 hr in the presence of either vehicle or cycloheximide. After nuclear isolation, protein was extracted and immunoblots were performed.

**Adenoviral Infection**

A549 cells were infected with AdCA5 or AdLacZ at a multiplicity of infection of 500 for 48 hr allowing an infection rate of ~100% (Figure S7A) as previously described (Manalo et al., 2005; Sutendra et al., 2013). After 48hrs cells were then transfected with scrambled versus PDC-E1 siRNA.

**PDKI Transfection**

PDKI plasmid was generated by OriGene Technologies (Rockville, MD) and 40 µg of plasmid was transfected into A549 cells using Xfect transfection agent (Clontech). After 48hrs cells were transfected with either scrambled or PDC-E1 siRNA.

**EGF Experiments**

A549 cells were treated with vehicle (PBS) or 500ng/mL rhEGF (Sigma Aldrich) or 10 µM Gefitinib (Cayman Chemicals) for 24 hr prior to nuclear isolation. After nuclear isolation, nuclei were prepared for either immunocytochemistry or immunoblots. 786-O cells were treated with either vehicle (DMSO) or 5 µM rotenone (Sigma Aldrich) in the presence of rhEGF for 24hrs prior to fixation in paraformaldehyde (4%), immunofluorescence staining and confocal imaging.

**KNK437 Experiments**

A549 or 786-O cells were serum starved and pretreated with either vehicle (DMSO) or 100 µM KNK437 (Santa Cruz Biotechnology) for 24hrs prior to serum stimulation for 4 hr in the presence of either vehicle or KNK437. After nuclear isolation, protein was extracted and immunoblots were performed.

**Immunoprecipitation**

The Dynabeads coimmunoprecipitation kit (Invitrogen Canada) was used as per manufacturer’s instructions. Immunoprecipitation was performed on conjugated beads with Hsp70 (mouse host; Santa Cruz Biotechnology). Immunoblots were then performed using a rabbit host to Hsp70 (abcam), PDC-E1 (abcam) and PDC-E2 (abcam) and a goat host to PDK I (Santa Cruz Biotechnology).

**Immunoblotting**

Standard SDS-PAGE and immunoblotting was performed as previously described (Bonnet et al., 2007) and with antibody dilutions as recommended by the manufacturer. Where required, SDS-PAGE of purified histones as well as nuclear, mitochondrial and cellular protein was performed on 16.5% Tricine gels (Bio-Rad, Montreal, Quebec) followed by immunoblotting to low-pore size (0.2 µm) nitrocellulose (Bio-Rad). Primary antibodies were the same as in confocal microscopy plus antibodies against: actin, Ac-H3K9, Ac-H3K18, ATP-citrate lyase, citrate synthase, Sestrin3-P-E1α, isocitrate dehydrogenase 2, lamin, lipoic acid, succinyl-CoA dehydrogenase, succinyl-CoA synthethase purchased from Abcam; HIF1α purchased from BD Biosciences; MAPK, Tyor242-P-MAPK, Ac-lysine (against all acetyl-lysine residues) and acetyl-p53 (against acetyl-lysine residue 382) were purchased from Cell Signaling Technology; Ac-H2B (against acetyl-lysine residues 5, 12, 15 and 20), Ac-H4 (against acetyl-lysine residues 5, 8, 12 and 16), Cdk2, cyclin A and E2F purchased from Millipore; PDPI & PDPII and α-tubulin & Ac-tubulin purchased from Sigma Aldrich; E3BP was purchased from GeneTex; Ac-Foxo1 (against acetyl-lysine residues 259, 262 and 271) was purchased from Santa Cruz Biotechnology. Data on PDC immunoblots were also confirmed with additional antibodies to PDC E1 (and its competing peptide) from Abgent. For all PDC antibodies (E1, E2 and E3), there was only one clear band at the correct molecular weight and the competing peptide to PDC-E1 eliminated the band of interest, confirming the specificity of the antibodies used.

**Statistical Analyses**

Unpaired Student’s t test was used for statistical calculations when comparing the effects of treatment between two sample groups. Error bars indicate standard error of the mean. For correlation studies, a Pearson Product-Moment Correlation Coefficient Test was used (Figure 6H).
SUPPLEMENTAL REFERENCES


Figure S1. PDC-E1 Is Localized in the Mitochondria and Nucleus, Related to Figure 1

(A) Spermatozoa were costained with an antibody to the α subunit of PDC-E1 (green), the mitochondrial marker MitoTracker Red (red) and the nuclear stain DAPI (blue) and imaged using confocal microscopy. PDC-E1 colocalizes with the mitochondria (yellow), shown in the merged panel, but also localizes in the nucleus, which lacks mitochondria. DIC shows morphology of a spermatozoon.

(B) An intact A549 cell costained with PDC-E1 (green), MitoTracker Red (red) and the nuclear marker histone 3 (purple) was systematically scanned at 0.2 μm increments along the z-axis using confocal microscopy (top image). Zeiss physiology option software was used to analyze the fluorescence intensity mid-plane in the cell at the XY axis. PDC-E1 and histone 3 have a similar signal pattern in the nucleus. The histone 3 fluorescence signal was not detected in the mitochondria, while the MitoTracker signal was not detected in the nucleus.

(C) A549 cells were labeled using immunogold staining with an antibody against the α subunit of PDC-E1 (black dots) and imaged using transmission electron microscopy. PDC-E1 was highly expressed in the nucleus within the nuclear membrane (Cyt = cytosol). PDC-E1 presence in the cytoplasm and the nuclear membrane is also compatible with the trafficking of PDC-E1 from the mitochondria to the nucleus as discussed in the text. The negative control (immunogold only) labeling is presented in Figure S1E.

(D) SAECs were transfected with a plasmid encoding for enhanced green fluorescent protein in-frame with the α subunit of PDC-E1 (EGFP-PDC; green) and costained with MitoTracker Red (red) and the nuclear stain DAPI (blue). EGFP-PDC-E1 colocalization with the mitochondria (yellow) is shown in the merged panel (left). EGFP-PDC-E1 is also localized in the nucleus as shown by the EGFP signal, obtained at a mid-plane level cutting through the nucleus.

(E) Secondary-only antibody to Ficoll did not provide a fluorescence signal, validating the specificity of the antibody used. MitoTracker Red (red) and the nuclear stain DAPI (blue) are also shown (top). The EGFP signal detected was not due to our transfection agent, as there was no detectable fluorescence signal with our transfection agent-only control sample. MitoTracker (red) and the nuclear stain DAPI (blue) are also shown (middle). Immunogold-only staining and electron microscopy shows the specificity of our immunogold labeling.
Figure S2. PDC Is Present in Isolated Highly Pure Intact Nuclei Free of Mitochondrial Membranes from A549 and SAEC Cells and Is Important for Nuclear Generation of Acetyl-CoA, Related to Figure 2

(A) Isolated nuclei from A549 cells transfected with EGFP-PDC-E1 plasmid (green) were intact as assessed by the nuclear membrane marker lamin (red). EGFP-PDC-E1 localization within the nuclei (stained blue with DAPI) is shown in the merged panel (upper left). Isolated nuclei from A549 and SAECs (upper middle) were costained with antibodies to the α subunit of PDC-E1 (red) and histone 3 (green) and the nuclear stain DAPI (blue). Both PDC-E1 and histone 3 were detected in isolated nuclei, using confocal microscopy. DIC shows morphology of isolated nuclei. Secondary only antibodies did not provide a fluorescence signal, validating the specificity of the antibodies used. The nuclear stain DAPI is in blue and DIC shows the morphology of our isolated nuclei (upper right). All four components of PDC (E1, E2, E3 and E3BP) are localized in isolated nuclei from A549 cells (red). In addition our nuclei had intact membranes as assessed by lamin (purple) and expressed histones (purple) in indicated panels (bottom).

(B) Isolated nuclei contain a lipoylated protein at the molecular weight of E2 (~70kDa) as assessed by an antibody to lipoic acid and immunoblots. The same lipoylated protein is also highly detected in isolated mitochondria. The purity of the nuclear samples are shown in Figure 2C.

(C) Nuclei from PDC-E1 siRNA-treated A549 and 786-O cells had decreased levels of 13C1-acetyl-CoA compared to scrambled siRNA controls, measured by mass spectrometry (EPI mode) and normalized to protein concentration. Furthermore, isolated nuclei had a dose-dependent increase of labeled acetyl-CoA from labeled pyruvate using EPI mode mass spectrometry.
Figure S3. Acetyl-CoA Generated from Nuclear PDC Is Not Required for Acetylation of the Tumor Suppressor Proteins p53 and FOXO1 in A549 Cells, Related to Figure 3

Isolated nuclei from PDC-E1 siRNA-treated cells exposed to 10mM pyruvate for 8hrs showed no differences in acetylated p53 and acetylated FOXO1, compared to nuclei from scrambled siRNA-treated cells. Total protein levels as measured by Ponceau S were unchanged between isolated nuclei from scrambled versus PDC-E1 siRNA-treated cells. Representative gels are shown.
Figure S4. The PDK Inhibitor DCA Increases Acetyl-H3 and Acetyl-Tubulin in Whole-Cell Preparations, Related to Figure 4

(A) A549 cells treated with DCA had increased levels of acetylated-H3 (green) compared to vehicle-treated controls, using immunofluorescence and confocal microscopy. Acetylated-H3 colocalized with the nuclear stain DAPI (blue). Representative images (left) and quantified mean data (right) are shown (n = 4 experiments, *p < 0.05).

(B) A549 cells treated with DCA had increased levels of acetylated-H3, using a commercially available H3 Elisa assay (n = 3 experiments, *p < 0.05).

(C) A549 cells treated with DCA had increased levels of succinate and citrate, and had decreased levels of lactate, using proton-nuclear magnetic resonance (1H-NMR; left). Representative image showing the peak for DCA (top) and levels of metabolites (below) are shown. A549 cells treated with DCA had increased levels of citrate, using a commercially available citrate assay kit (right; n = 3 experiments, *p < 0.05).

Error bars represent SEM.
Figure S5. Nuclear/Mito PDC-E1 Ratio Increases during Serum Stimulation, and PDC Translocates to the Nucleus as an Intact Unit, Related to Figure 5

(A) Nuclear PDC-E1 shows a similar increase to acetyl-H3 peaking (shown in Figure 5A) at 4 hrs post serum stimulation. Total protein levels as measured by Ponceau S remained similar between all time points.

(B) Isolated nuclei from A549 cells were costained with antibodies to Ac-H3 (red), the α subunit of PDC-E1 (green) and the nuclear stain DAPI (blue). Both PDC-E1 and Ac-H3 were increased in isolated nuclei during late S-phase. DIC shows morphology of isolated nuclei.

(C) Serum stimulation as low as 5% increases nuclear levels of all catalytic components of PDC, while serum stimulation, even as high as 25%, does not decrease overall PDC levels in whole cells. This suggests that the associated decrease in mitochondrial PDC components that we show earlier is not due to protein degradation, but it represents translocation to the nucleus.

(D) The same amount of protein (10 μg) from isolated nuclei and mitochondria preparations at baseline and 3 hrs post serum stimulation (as shown in Figure 5B) was loaded on the same immunoblot. Total protein levels were similar between the 0 and 3 hr groups, although there are apparent differences in the bands between the nuclear and mitochondria on Ponceau S, reflecting the different proteome in the two organelles.

(E) PDC translocates to the nucleus as an intact unit as knockdown of one specific component of PDC (i.e., gene silencing of the E1 gene by siRNA) results in decreased nuclear levels of other PDC components (E2 and E3), but does not alter overall levels of these components in whole cells. Nuclear purity was shown by absence of citrate synthase (CS) and tubulin in the nucleus. The ratios of PDC components to lamin are shown on the right.
Figure S6. rhEGF, Rotenone, and Hsp70 Can Increase Nuclear Localization of PDC, Related to Figure 6
(A) Isolated nuclei from recombinant human EGF (rhEGF)-treated A549 cells have increased nuclear levels of PDC (E1, E2 and E3), using immunofluorescence and confocal microscopy, rhEGF treatment increased nuclear localization of PDC-E1 (green; top panel) and Ac-H3 (red; top panel), PDC-E2 (green; middle panel), PDC-E3 (green; bottom panel) and PKM2 (red; bottom panel). All images included the nuclear stain DAPI (blue). The nuclei had intact membranes as indicated by lamin staining (red; middle panel). DIC shows the morphology and structural integrity of the isolated nuclei. Secondary only antibodies did not provide a fluorescence signal, validating the specificity of the antibodies used.

(B) 786-O cells were costained with PDC-E1 (green), the mitochondrial marker MitoTracker Red (red) and the nuclear stain DAPI (blue) and imaged using confocal microscopy. Mitochondrial E1 signal was quantified by signal overlap with MitoTracker Red, while nuclear signal was quantified by signal overlap with DAPI, using the Image J 64 processing software. These are representative images of the mean data shown in Figure 6E.

(C) Serum stimulation of A549 cells for 4hrs shows progressively increasing nuclear Hsp70 levels compared to baseline control cells. Hsp70 is shown in green, the mitochondrial marker MitoTracker Red is shown in red and the nuclear stain DAPI is shown in blue. Black arrows show Hsp70 colocalization with MitoTracker Red (yellow) and white arrows show nuclear Hsp70. Representative images are shown on the left and quantified mean data for nuclear Hsp70 are shown on the right (n = 50 cells per group).

(D) Hsp70 coimmunoprecipitates with PDC-E1 and PDC-E2, but not with PDKI in MRC-9 cells as shown by immunoblots. Input represents 2.5 μg of whole cell lysate.

(E) Sequence of residues 133-182 of PDC-E2 encoding for part of the lipoyl-domain 2 (LD2), reveals putative Hsp70 binding motifs (red) and known PDK interacting residues (highlighted by *). These include Leu 140 (L), Pro 142 (P), Lys 173 (K), Ala 174 (A) and Ile 176 (I) of LD2 (Kato et al., 2005; Roche et al., 2003) (top). Ribbon representation of putative Hsp70 binding motifs (red), shows a potential binding region within the PDK binding site for LD2. PDK is shown in purple and LD2 is shown in gray surface representation. Inset shows higher magnification of putative Hsp70 and PDK binding sites on LD2 of PDC-E2. PDB code 1Y8O (Kato et al., 2005) and the visual molecular dynamics program was used to generate structural images.

(F) Serum stimulated (4hrs) A549 cells, previously serum starved and pretreated with 100 μM of KNK437, show decreased mRNA levels of HSPA1A and HSPA1B, the two genes responsible for inducible Hsp70, compared to vehicle (DMSO)-treated cells (n = 3 experiments, *p < 0.05).

(G) Serum stimulated (4hrs) 786-O cells previously serum starved and pretreated with 100 μM of KNK437 (which inhibits induced Hsp70) show decreased nuclear levels of Hsp70, PDC-E1 and PDC-E2 compared to vehicle (DMSO)-treated cells, as shown by immunoblots. Lamin and Ponceau S show similar protein loading between vehicle and KNK437 treated cells.

(H) Serum stimulated (4hrs) A549 cells, previously serum starved and pretreated with 100 μM of KNK437 show decreased nuclear levels of Hsp70 (red) and PDC-E1 (green) compared to vehicle (DMSO)-treated cells as shown by immunofluorescence and confocal microscopy. These are representative images of the mean data shown in Figure 6H for a Pearson product-moment correlation plot between nuclear Hsp70 and nuclear PDC-E1. Error bars represent SEM.
Figure S7. AdCA5 Infection Increases Mitochondrial Membrane Potential, and PDKI Plasmid Increases PDKI Expression, Related to Figure 7

(A) Infection of A549 cells with AdCA5, which coexpresses green fluorescent protein (GFP) at a multiplicity of infection (MOI) of 500 results in almost 100% infection of cells as indicated by GFP (green). The nuclear stain DAPI is shown in blue. Two different images of the infected A549 cells are shown in the two rows.

(B) Isolated nuclei from AdLacZ and AdCA5-treated A549 cells shows that the CA5 mutant form of HIF1α, which has deletion of amino acids 392-520 and is present at a lower molecular weight than endogenous HIF1α (Manalo et al., 2005), (thus allowing its clear separation from endogenous HIF1α, is only present in the AdCA5-treated cells.

(C) Infection of A549 cells with AdCA5 (GFP; green) results in increased mitochondrial membrane potential (measured the mitochondria specific voltage-sensitive dye TMRM in red; i.e., the more the red the higher the membrane potential) compared to noninfected cells in the same image and AdLacZ infected A549 cells. The nuclei stain Hoechst is shown in blue. This shows that the mutant, constitutively active HIF1α has the expected effects on mitochondria, as we have previously published (see text).

(D) A549 cells transfected with a PDKI plasmid had higher PDKI mRNA levels compared to empty vector transfected cells, measured by qRT-PCR. Treatment with PDC-E1 siRNA of A549 cells transfected with the PDKI plasmid did not alter PDKI mRNA levels compared to scrambled siRNA-treated cells (n = 3 experiments, *p < 0.01 compared to empty control vector and scrambled siRNA).

Error bars represent SEM.
A Mitochondrial Expatriate: Nuclear Pyruvate Dehydrogenase

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The pyruvate dehydrogenase complex (PDC) catalyzes the conversion of pyruvate into acetyl-CoA, a critical step in metabolism. Sutendra et al. now demonstrate that PDC can translocate from the mitochondria to the nucleus to provide acetyl-CoA necessary for histone acetylation, suggesting a new pathway for mitochondrial-nuclear communication.

The pyruvate dehydrogenase complex (PDC) plays a central role in cellular metabolism by catalyzing the irreversible conversion of pyruvate into acetyl-CoA. The activity of PDC is therefore tightly controlled via reversible inactivating phosphorylation due to the activity of specific kinases (PDK1-4) and phosphatases (PDP1-2). The localization of PDC has been thought to be strictly mitochondrial. In this issue, Sutendra et al. (2014) reveal that PDC also resides in the cell nucleus, where it generates acetyl-CoA, a substrate for histone acetylation (Figure 1).

Histone posttranslational modifications (PTMs), including acetylation, methylation, and O-GlcNAcylation, form a histone code that regulates nucleosome dynamics and serves as a platform for epigenetic readers. Though a diverse set of protein families mediates the processing of this histone code, recent studies highlight an additional level of control of histone modification via the cosubstrates of PTM reactions, such as acetyl-CoA for acetylation, S-adenosylmethionine for methylation, and N-acetylglucosamine for GlcNAcylation. Levels and routing of these metabolites have been demonstrated to play a significant role in driving histone PTM dynamics (Kaelin and McKnight, 2013).

Acetyl-CoA is a central metabolite that interconnects multiple metabolic pathways (Figure 1). Although a major fate of acetyl-CoA is its oxidation in the citric acid cycle, it is also used for many other cellular processes. Acetyl-CoA is the obligatory acetyl donor for lysine acetylation reactions in mammalian cells, linking metabolic activity with epigenetics. ATP-citrate lyase (ACL) and acetyl-CoA synthetase (ACS) are acetyl-CoA-generating enzymes localized in the cytosol and the nucleus, providing acetyl units for nuclear histone acetylation (Figure 1). Importantly, ACL is critical for differentiation of 3T3-L1 preadipocytes into mature adipocytes via histone acetylation (Wellen et al., 2009), and ACS-dependent histone acetylation is necessary for cell-cycle progression in yeast (Cai et al., 2011). These findings demonstrate that delivery of nuclear acetyl-CoA contributes to the control of cellular proliferation and differentiation.

Sutendra et al. now provide further support of metabolic control of histone acetylation via the generation of acetyl-CoA from pyruvate in the nucleus by PDC. Nuclear PDC levels, as well as the level of acetylation of histone 3 (Ac-H3), increase upon stimulation of cells with serum or epidermal growth factor (EGF) that triggers cell-cycle progression, whereas inhibition of EGF signaling lowers the nuclear PDC level. Furthermore, knockdown of one PDC subunit decreases not only the nuclear levels of the entire complex, but also the levels of Ac-H3 and cell-cycle progression markers. These findings imply that acetyl-CoA, as an important substrate source for histone acetylation, can be generated inside of the nucleus from pyruvate and that its nuclear synthesis is controlled by a growth-factor-mediated regulation of nuclear PDC.

An intriguing aspect of this study is the localization of PDC inside of the nucleus and its apparent translocation from the mitochondria. Upon serum stimulation, mitochondrial and nuclear PDC levels change in the opposite direction. Experiments using cycloheximide further confirm that the increase in nuclear PDC does not depend on protein translation. Interestingly, PDK is not present in the nucleus, and consequently, nuclear PDC is not subject to phosphorylation regulation. The nuclear translocation of PDC is a unique pathway by which mitochondria can communicate with the nucleus. Other well-studied signals arising from mitochondria are mostly peptides generated by the mitochondrial unfolded protein response (Haynes et al., 2010), redox state, and small molecule intermediates such as α-ketoglutarate, succinate, fumarate, AMP, and ROS (Gut and Verdin, 2013).

Although this work reveals that PDC can localize to the nucleus, new questions arise. Importantly, a mechanism that can explain the specific export of the PDC across the mitochondrial membrane remains unknown. Although mitochondria can export peptides into the cytosol and mitochondrial-encoded respiratory chain
subunits into the mitochondrial inner membrane (Bauerschmitt et al., 2010; Haynes et al., 2010), these processes are unlikely to be responsible for the exit of one of the largest multienzyme complexes that has been known. The identification of this mechanism will be instrumental for further studying the regulation and specificity of this mode of mitochondrial-nuclear communication.

The study also raises the intriguing question of whether other mitochondrial matrix proteins are able to exit the mitochondria. In this respect, it is worth mentioning that ketolysis and fatty acid oxidation are additional sources of mitochondrial acetyl-CoA, especially during fasting (Figure 1). Fatty acid oxidation has recently been shown to be an important source for mitochondrial acetylation (Pougovkina et al., 2014). In addition, the newly discovered histone lysine crotonylation modification is likely to use crotonyl-CoA as the substrate (Tan et al., 2011), which is solely generated by oxidation of fatty acids and specific amino acids. Indeed, the finding showing that the ketone body β-hydroxybutyrate can inhibit histone deacetylases and consequently alter specific histone acetylation marks (Gut and Verdin, 2013) further supports a role for signals arising from fatty acid oxidation to contribute to histone epigenetic regulation.

Lastly, one may wonder whether the acetyl-CoA generated by nuclear PDC can be used for other biochemical processes such as lipid synthesis. Because proliferating tumor cells critically rely on cytosolic acetyl-CoA for membrane generation (Lunt and Vander Heiden, 2011), it will be interesting to further explore this hypothesis.

In summary, the work by Sutendra et al. reveals a new pathway for mitochondrial-nuclear signaling and intriguingly demonstrates that protein complexes, like many scientists, can become expatriates. Whether this particular expatriate would ever return to its organelle of origin and, if so, what the functional significance for that process would be, remain open questions for future investigation.

REFERENCES

**NEURONAL RECEPTORS**

**A lipid closes a loop**

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GABAergic synapses, characterized by the presence of GABA receptors (GABAARs), are involved in inhibition of neuronal signals. Gephyrin is a scaffold protein that regulates the clustering of GABAARs at synapses, which is important for postsynaptic currents, indicative of an increase in the GABAAR pool, whereas a GABAAR antagonist inhibited gephyrin palmitoylation. These results suggest that GABAAR-mediated increases in gephyrin palmitoylation promote postsynaptic clustering of the receptor itself. MB

**BIOSYNTHESIS**

**Redox not required**


Ketoreductase domains are responsible for reducing carbonyl groups during polyketide biosynthesis, thus determining the stereochemistry of these centers; some ketoreductases also function as epimerases to invert adjacent methyl-bearing stereocenters. Ketoreductases require NADPH, yet several multimodular polyketide synthases also contain enigmatic ‘redox-inactive’ homologs of ketoreductases that have lost the capacity to bind NADPH. Garg et al. suspected that these proteins might still perform epimerizations. To test this idea, the authors adapted a previously reported equilibrium isotope exchange assay used to determine whether individual ketoreductases are also epimerases to work as a tandem assay in which an NADPH-dependent, nonepimerizing ketoreductase (‘KR’ in image) is used to oxidize a representative substrate, and then epimerization of the transiently generated ketoester by the redox-active KR domain was detected by monitoring washout of deuterium from the reduced substrate. The authors observed that both redox-active KR domains tested were able to deplete deuterium from the substrate, and tests with a different NADPH-utilizing KR confirmed the generality of the assay. Finally, to explore the epimerization mechanism used by redox-active and redox-inactive ketoreductases, the authors introduced a series of mutations into an epimerase-active KR domain that disrupted NADPH binding, thereby abolishing the native redox activity. These mutants were still able to epimerize the deuterated substrate. These results define a function for this little-studied group of enzymes and rule out an existing NADPH-dependent mechanistic proposal for epimerization. CG

**PROTEIN DEGRADATION**

**Savior or executioner?**

Nature doi:10.1038/nature13527

Thalidomide and its derivatives lenalidomide and pomalidomide interact with cerebolen (CRBN), a substrate recognition component of the Cul4 ubiquitin E3 ligase complex, to promote the ubiquitin-dependent degradation of two members of the Ikaros family of transcription factors, IKZF1 and IKZF3. However, the location of the thalidomide-interacting site on CRBN was not known. Fischer et al. obtained crystal structures of a Cul4 complex component made up of the human DDB1–chicken CRBN co-complex with thalidomide and its derivatives. The S enantiomer of all three compounds bound a conserved pocket on the C-terminal domain of CRBN and required their glutarimide moiety for proper engagement. Thalidomide occupation on CRBN was known to be required for IKZF1 and IKZF3 degradation, but its effect on endogenous substrates was unknown. To identify these, the authors performed a biochemical screen and identified the homeobox transcription factor MEIS2, which is involved in brain and eye development, as a target for ubiquitination. In this case, ubiquitin-mediated degradation of MEIS2 was blocked upon treatment with lenalidomide. Multiple lines of evidence on the biochemical and cellular levels support the finding that lenalidomide and MEIS2 compete for the same binding site. The binding of thalidomide-like derivatives to CRBN prevents access to endogenous CRBN substrates such as MEIS2, resulting in their stabilization while promoting the degradation of IKZF1 and IKZF2. Overall, thalidomide-like derivatives can modulate both the stability and degradation of particular proteins. GM

**HISTONE ACETYLATION**

**On-demand production**

Cell 158, 84–97 (2014)

Histone acetylation, a post-translational modification and epigenetic regulator of gene expression in eukaryotes, is maintained by acetyltransferases that use acetyl-coenzyme A (Ac-CoA) as a substrate. The primary source of Ac-CoA in cells is the pyruvate dehydrogenase complex (PDC), a multiprotein enzyme that is typically localized in mitochondria. Because Ac-CoA contains a reactive thioester, it has remained unclear how the nucleus is able to obtain enough Ac-CoA to support histone modification. Sutendra et al. now show that PDC undergoes transport to the nucleus in a cell cycle–dependent manner where it generates Ac-CoA and facilitates histone acetylation. Using antibodies against all components of PDC, the authors visualized the complex in the nuclei of human sperm, normal fibroblasts and cancer cell lines. Immunoblotting identified assembled PDCs in highly purified nuclei preparations, and isotopic labeling and MS analysis revealed that the nuclear PDCs generate Ac-CoA in the presence of pyruvate. Nuclear synthesis of Ac-CoA is correlated with elevated histone acetylation, as validated by siRNA knockdown of a PDC component and selective inhibition of mitochondrial versus nuclear PDC. Cellular analysis showed that PDC from mitochondria is translocated to the nucleus at the S phase of the cell cycle in a process that is facilitated by the Hsp70 chaperone. The nuclear translocation of PDC is induced by signals including epidermal growth factor or mitochondrial stress, leading the authors to suggest that PDC-mediated nuclear production of Ac-CoA and histone acetylation is a key regulator of S phase cell-cycle entry. TLS