Charles University in Prague

Faculty of Science

Study program: Biology

Field of study: Biology

Róbert Zach

Connections between intermediary metabolism and acetylation of histones

Spojitosti medzi intermediárnym metabolizmom a acetyláciou histonov

Bachelor's thesis

Supervisor: RNDr. Martin Převorovský, Ph.D.

Prague, 2015

Acknowledgements

I would like to thank my supervisor, Martin Převorovský, for his constructive suggestions and professional approach. Special thanks go to all members of the Laboratory of Gene Expression Regulation, with who I am allowed to work.

Prehlásenie

Prehlasujem, že som záverečnú prácu spracoval samostatne a že som uviedol všetky použité informačné zdroje a literatúru. Táto práca ani jej podstatná časť nebola predložená k získaniu iného alebo rovnakého akademického titulu.

V Prahe, 10.05.2015

Podpis:

Abstract

Acetylation of histone proteins affects chromatin structure and functions as a coactivating signal for transcription. Acetylation of histone lysine residues is mediated by histone acetyltransferases, which utilize molecule of Ac-CoA as a donor of acetyl group. Ac-CoA is located in the centre of intermediary carbon metabolism, where it fuels citric acid cycle and fatty acid synthesis. Level of intracellular Ac-CoA fluctuates in response to changes in availability of utilizable carbon sources and metabolic activity of the cell. Since changes in intracellular concentration of Ac-CoA positively correlate with histone acetylation level, Ac-CoA might contribute to transcriptional modulation in response to nutritional stress. Moreover, Ac-CoA takes part in process of differentiation and seems to be important for cell cycle regulation.

Key words: Ac-CoA, histone acetylation, nutrition, intermediary metabolism, regulation of transcription, cell cycle, glucose

Abstrakt

Acetylácia histonov ovplyvňuje štruktúru chromatínu a pôsobí ako koaktivačný signál pre transkripciu. Acetylácia lyzínových zvyškov na histonoch je zprostredkovaná histon acetyltransferázami, ktoré využívajú molekulu Ac-CoA ako donor acetylovej skupiny. Ac-CoA sa nachádza v strede intermediárneho metabolizmu, kde zásobuje cyklus kyseliny citrónovej a syntézu mastných kyselín. Hladina intracelulárneho Ac-CoA fluktuuje v dôsledku zmien v dostupnosti spracovateľných zdrojov uhlíku a metabolickej aktivity bunky. Keďže zmeny v intracelulárnej koncentrácii pozitívne korelujú so stupňom acetylácie histonov, Ac-CoA by mohol prispievať k modulácii transkripcie vyvolanej nutričným stresom. Ac-CoA sa navyše účastní procesu diferenciácie a zdá sa byť dôležitý v regulácii bunkového cyklu.

Kľúčové slová: Ac-CoA, acetylácia histonov, výživa, intermediárny metabolizmus, regulácia transkripcie, bunkový cyklus, glukóza

List of Abbreviations

2-DG	2-deoxy-D-glucose
Ac-CoA	Acetyl coenzyme A
AceCS1	Acetyl coenzyme A synthetase 1
ACL	ATP-citrate lyase
DAPI	4′,6-diamidino-2-phenylindole
E2F	Elongation factor 2
HAT	Histone acetyl transferase
HDAC	Histone deacetylase
KAT	Lysine acetyl transferase
KDAC	Lysin deacetylase
MLS	Mitochondrial localisation signal
NAD+	Nicotinamide adenin dinucleotide (oxidized)
NADH	Nicotinamide adenin dinucleotide (reduced)
OGD	Oxygen and glucose deprivation
Ox	Oxidative
PDC	Pyruvate dehydrogenase complex
P-RB	Phosphorylated retinoblastoma
PSCs	Pluripotent stem cells
R/B	Reductive binding
R/C	Reductive charging
rDNA	DNA encoding ribosomal RNAs
siRNA	small interfering ribonucleic acid
TCA	tricarboxylic acid
YMC	Yeast metabolic cycle
YPGE	Yeast extract-peptone-glycerol-ethanol

List of Genes

gene	protein	organism	function
ACLY	ACLY	*Homo sapiens* (Human)	ATP-citrate lyase
ACSS2	ACSS2	*Homo sapiens* (Human)	Acetyl coenzyme A synthetase
CDK2	CDK2	*Homo sapiens* (Human)	Serine/threonine-protein kinase, cell cycle regulation
FOXO1	FOXO1	*Homo sapiens* (Human)	Transcription factor
HK1	HK1	*Homo sapiens* (Human)	Hexokinase
P53	P53	*Homo sapiens* (Human)	Tumor supressor, Cell cycle regulator, DNA-damage response
PKM	PKM	*Homo sapiens* (Human)	Pyruvate kinase
RB1	RB1	*Homo sapiens* (Human)	Cell division regulator, phosphorylated upon S-phase entry
SIRT1	SIRT1	*Homo sapiens* (Human)	NAD+ dependent lysine deacetylase
Ap2	AP2	*Mus musculus* (Mouse)	Fatty acid-binding protein, adipocyte protein
Glut4	GLUT4	*Mus musculus* (Mouse)	Glucose transporter
Hk2	HK2	*Mus musculus* (Mouse)	Hexokinase 2
Ldh-A	LDH-A	*Mus musculus* (Mouse)	L-lactate dehydrogenase
Pfk-1	PFK-1	*Mus musculus* (Mouse)	6-phosphofructokinase
Gcn5	GCN5	*Rattus norvegicus* (Rat)	Histone acetyltransferase
Sglt1	SGLT1	*Rattus norvegicus* (Rat)	Sodium dependent glucose transporter.
Si	SI	*Rattus norvegicus* (Rat)	Sucrase isomaltase
GCN5	Gcn5p	*Saccharomyces cerevisae* (budding yeast)	Histone acetyltransferase, histone H3 acetylation
ACC1	Acc1p	*Saccharomyces cerevisae* (budding yeast)	Acetyl coenzyme A carboxylase
ACS1	Acs1p	*Saccharomyces cerevisae* (budding yeast)	Acetyl coenzyme A synthetase
ACS2	Acs2p	*Saccharomyces cerevisae* (budding yeast)	Acetyl coenzyme A synthetase
acs2-Ts1	Acs2-Ts1p	*Saccharomyces cerevisae* (budding yeast)	Acetyl coenzyme A synthetase Temperature-sensitive allele
ACH1	Ach1p	*Saccharomyces cerevisae* (budding yeast)	Acetyl coenzyme A hydrolase
ESA1	Esa1p	*Saccharomyces cerevisae* (budding yeast)	Histone acetyltransferase, histone H4 acetylation
PDA1	Pda1p	*Saccharomyces cerevisae* (budding yeast)	Pyruvate dehydrogenase complex E1 α subunit
POT1	Pot1p	*Saccharomyces cerevisae* (budding yeast)	Peroxisomal oxoacyl thiolase
YAT2	Yat2p	*Saccharomyces cerevisae* (budding yeast)	Carnitine acetyltransferase
cut6	Cut6	*Schizosaccharomyces pombe* (Fission yeast)	Acetyl coenzyme A carboxylase
fas1	Fas1	*Schizosaccharomyces pombe* (Fission yeast)	Fatty acid synthetase
ppc1	Ppc1	*Schizosaccharomyces pombe* (Fission yeast)	Phosphopantothenoylcystein synthetase

Table of Contents

1. Historical perspective

Chromatin is indeed a complex and interesting structure. It is not surprising that its composition and architecture have been investigated for several decades. Many questions have been answered and now we are able to picture this structure in great detail. However, the real complexity of chromatin lies in dynamics of its modifications among which the acetylation plays a crucial role.

- 1962, histone proteins complexed with DNA act like barrier for DNA-dependent RNA synthesis (Huang and Bonner, 1962).
- 1964, acetylation of histone lysines diminishes their ability to inhibit the RNA synthesis (Allfrey *et al.*, 1964).
- 1991, Deletion of histone H4 N-terminal region causes reduction in activation of GAL1 and PHO5 promoters, suggesting that H4 N-terminal domain is essential for proper initiation of transcription (Durrin *et al.*, 1991).
- 1992-1994, hyperacetylation of histones H3, H4 and H2B is characteristic for transcriptionally active and poised chromatin states of erythrocyte β-globin encoding genes (Hebbes *et al.*, 1992, 1994).
- 1998, acetylation of nucleosomal arrays coincides with elevation of transcription rate *in vitro* (Tse *et al.*, 1998).

From all these and many other findings of twentieth century, it is clear that acetylation of histone N-terminal regions is an important co-activating signal for transcription. Since donor of acetylation mark (Acetyl-Coenzyme A) originates in the intermediary carbon metabolism, it is possible that fundamental biochemical pathways directly participate in regulation of histone acetylation. This work reviews recent knowledge of histone acetylation dynamics in relation to nutritional availability and summarizes best characterized biochemical players contributing to this issue. Additionally, epigenetically-regulated adjustments of transcription and cell-cycle related processes are discussed.

2. Fundamental concepts

2.1. Chromatin

Eukaryotic DNA exists in a form of nucleoprotein complex also known as chromatin. Chromatin could be considered a platform where balancing between structural needs and realization of genetic information takes place. According to the recent model, fundamental chromatin structure consists of primary building blocks called nucleosome core particles. Each nucleosome core particle comprises of 147 base pair long DNA segment wrapped around the protein core. This protein core is composed of two copies of each of standard histones (H2A, H2B, H3 and H4), which are assembled into the octameric complex. These basal chromatin units are linked together by linker DNA segments, which length may vary from 10 to 90 base pairs and usually associate with histone H1. Moreover, the standard chromatin fiber forms higher order three-dimensional structures, in which two fundamental domains can be recognized: transcriptionally active (euchromatin) and transcriptionally silenced (heterochromatin). Both chromatin states are characterized by distinct modifications, which form a possible informational storage independent on DNA sequence alterations (Venkatesh and Workman, 2015).

2.2. Histone acetylation and its mediators

Protein acetylation is one of the major post-translational modifications participating in cell signaling. The introduction of acetyl group to the lysine residues is catalyzed by lysine acetyl transferases (KATs). The reaction is ATP independent and utilizes energy rich molecule of acetyl-coenzyme A as an acetyl group donor. The members of KAT subgroup predominantly participating in histone acetylation are known as histone acetyl transferases (HATs). Removal of acetyl group is mediated by lysine/histone deacetylases (KDAC, HDAC). The review by C. Choudhary and colleagues from 2014 reports identification of twenty-two members and three main groups (GNAT, MYST, p300/CBP) of KATs in human and mouse cells. As for KDACs, eleven Zn^{2+} dependent histone deacetylases (HDAC1-11) and seven NAD+ dependent sirtuins (SIRT1-7) have been reported (Choudhary *et al.*, 2014). Lysine acetylation takes place at terminal amino-group of the lysine side chains, which are positively charged *in vivo*. Introduction of acetyl group neutralizes the charge of lysine residues resulting in alteration of protein structure and function. The special case of this post-translational modification is acetylation of histone N-terminal domains, also described as histone tails. These

domains are rich in lysine residues, whose positive charge enables them to interact with negatively charged DNA backbone. As lysine acetylation leads to neutralization of positive charge of lysine terminal amines, it weakens DNA-protein interactions and therefore is characteristic for less condensed chromatin state. Histone acetylation marks also function as important docking sites for bromodomain containing transcription activators and chromatin remodelers (Dhalluin *et al.*, 1999; Bell *et al.*, 2011). Moreover, it has been reported, that acetylation of lysine H3K27 causes acceleration of RNA polymerase II transition from phase of initiation to elongation (Stasevich *et al.*, 2014). Therefore, histone acetylation directly participates in chromatin structure maintenance and regulation of gene expression.

2.3. Acetyl-Coenzyme A: metabolic perspective

As mentioned above, KAT mediated acetylation of histone and non-histone proteins uses Ac-CoA as a donor of acetyl group. However, that is not the only function of this molecule. Ac-CoA is considered to be one of the most important cellular metabolites. Primary biochemical sources of Ac-CoA are beta oxidation of fatty acids (Lazarow, 1978) and reaction mediated by pyruvate dehydrogenase complex (Pronk *et al.*, 1996). In general, these productions supply the mitochondrial and peroxisomal Ac-CoA pools. While Ac-CoA cannot pass through mitochondrial and peroxisomal membrane, it can freely diffuse between cytoplasm and nucleus. As a consequence, mitochondrial/peroxisomal and nucleocytosolic pools are established (Takahashi *et al.*, 2006). Extra-mitochondrial Ac-CoA is essential for lipogenesis and acetylation processes. Mammalian cells furnish this demand by two systems. Cytosolic acetyl coenzyme A synthetase generates Ac-CoA via ATP-dependent coupling of acetate and coenzyme A. The second system uses malate-citrate antiporter, which transports tricarboxylic acid cycle (TCA)-derived mitochondrial citrate to the cytosol, where it is turned into Ac-CoA and oxaloacetate by ATP citrate lyase (ACL) in an ATP and CoA dependent manner. The most vigorously studied yeast organism (*Saccharomyces cerevisiae*) lacks ACL, but utilizes two homologs of acetyl-CoA synthetases (*ACS1*, *ACS2*), which sufficiently satisfy the cellular needs for lipid biosynthesis and signaling based on post-translational protein acetylation (Van den Berg *et al.*, 1996).

3. Changes in histone acetylation and transcription induced by altered nurture

In some way, the environment we live in could be pictured as a complex system of signals that are processed by our signal integration machinery. This system is characterized by stochastic behavior and instability, which are represented by extensive fluctuations. Adaptation to these environmental changes concerns every living organism from the simplest ones to those with complex architectures. One of the suboptimal conditions, to which organisms must respond, is nutritional stress. Nutritional stress is caused by inappropriate availability or dosage of essential nutrients. One of the several possibilities to overcome the states of insufficient nurture is epigenetic-mediated transcriptional reprogramming, during which the alteration of histone-modification pattern results in modulation of gene expression (Kim and Choi, 2011).

3.1. Physiological consequences of altered glucose availability

Even if laboratory conditions are kept strictly constant, some similarities with the natural environment can still be found. Yeast cells cultivated in liquid culture experience temporal changes in nutrient availability. When the concentration of glucose as a main carbon and energy source drops to the threshold level, cells stop dividing and enter stationary phase. Stationary phase cells suffering from nutritional and energetic deficiency function in an energy saving mode. It has been reported, that these cells are characterized by significantly decreased level of transcription (Jona *et al.*, 2000) and protein synthesis (Fuge *et al.*, 1994), which are accompanied by less acetylated chromatin state (Ramaswamy *et al.*, 2003). After single step refeeding of stationary phase *Saccharomyces cerevisiae* cells by glucose, they show hyper-acetylated histones H3 and H4 relative to the cells under glucose starvation. H4 hyperacetylation signal peaks 4 hours after refeeding. At this point it starts to decline and after 24 hours it is completely lost. Glucose-induced H3/H4 acetylation has been proved to be dependent on the activity of histone acetyltransferases Gcn5p and Esa1p that catalyze acetylation of histones H3 and H4, respectively. The addition of glucose into the stationary phase culture also increases the intracellular levels of ATP, ADP, acetate and ethanol (Friis *et al.*, 2009). All these molecules are directly connected to the yeast intermediary carbon metabolism and provide a means of Ac-CoA production. Alcohol dehydrogenase converts ethanol to acetaldehyde, which can be subsequently oxidized to acetate by aldehyde dehydrogenase. Acetate together with ATP is essential for Acs1/2 catalyzed

generation of Ac-CoA. As correlation between glucose availability and intracellular Ac-CoA level has already been reported in different yeast organism, *Schizosaccharomyces pombe* (Pluskal *et al.*, 2011), possible glucose-induced elevation of Ac-CoA concentration could lead to activation of Gcn5p and Esa1p and hyperacetylated state of chromatin, which is typical for exponentially growing cells (Weinert *et al.*, 2014). The fact, that exponentially growing cells exhibit elevated rate of transcription (Jona *et al.*, 2000) and relatively high levels of intracellular Ac-CoA, which gradually decreases as the cells enter the stationary phase (Cai *et al.*, 2011) indicates, that nutrient availability induced fluctuations of distinct cellular metabolites, such as Ac-CoA could directly affect the epigenetic state of chromatin and alter gene expression pattern.

The similar phenomenon has been observed in rodents. The mRNA and protein levels of intestinal *Si* and *Sglt1* genes, encoding intestinal sucrase isomaltase and sodium dependent glucose transporter, respecitevely, are elevated in rats nourished with high-starch/low-fat (HS) diet (Inoue *et al.*, 2011). Carbohydrate-rich diet has no effect on amount nor binding activity of transcription factors responsible for the regulation of *Si* and *Sglt1*, but increases occupancy of GCN5 acetyltransferase in both loci (Inoue *et al.*, 2011). As a possible consequence, HS diet leads to hyperacetylation of H3 K9/14 and H4 K5/8/12/16 histone lysines (Honma *et al.*, 2009; Inoue *et al.*, 2011). Other results discussing availability of glucose in connection with epigenetics have emerged from the studies of cerebral ischemia, which is simulated by oxygen and glucose deprivation (OGD) *in vitro*. Exposure of cortical neurons to OGD induces global deacetylation of histones H3 and H4 (Yildirim *et al.*, 2014). It has been shown, that H4K16 specific deacetylation progresses in time, reaching minimum (20-30% of initial value) after 5-7 hours after OGD exposure. Re-perfusion of glucose and oxygen suppresses this effect. As HDACs function antagonistically to HATs, their inhibition by trichostatin A (Yoshida *et al.*, 1990) also diminishes OGD-induced deacetylation (Dmitriev and Papkovsky, 2014).

3.2. Yeast metabolic cycle

Direct connection between epigenetics, regulation of transcription and intracellular Ac-CoA level has emerged from the study of yeast cultures under continual nutritional supplementation. Reintroduction of glucose into the briefly starved yeast culture causes metabolic synchronization represented by repeating pattern of oxygen consumption (Figure 1. A) (Tu *et al.*, 2005, 2007), though it has been reported that addition and subsequent continuous supplementation of phosphate leads to similar phenomenon

(Slavov *et al.*, 2011). Based on oxygen consumption pattern, two standard phases of 6 hours-long yeast metabolic cycle (YMC) have been defined: oxidative, which is characteristic by rapid oxygen consumption and reductive, during which respiration is absent (Tu *et al.*, 2005, 2007). Consistently with the length of YMC, microarray data has revealed, that more than a half of *S. cerevisiae* genes are transcribed periodically in approximately 6 hours-long intervals. As expression of different genes peaks in different time points, three super clusters of periodically transcribed genes have been characterized: oxidative (Ox), reductive/building (R/B) and reductive/charging (R/C), defining actual three YMC phases (Figure 1. B). Gene-products of the Ox super cluster participate in ATP consuming proteosynthesis. Namely, these transcripts are linked to processes such as initiation of translation, amino acid synthesis, RNA processing and sulfur metabolism. Genes specific for R/B and R/C super clusters mainly encode proteins related to cell division, non-respiratory metabolism and cellular-maintenance (Tu *et al.*, 2005).

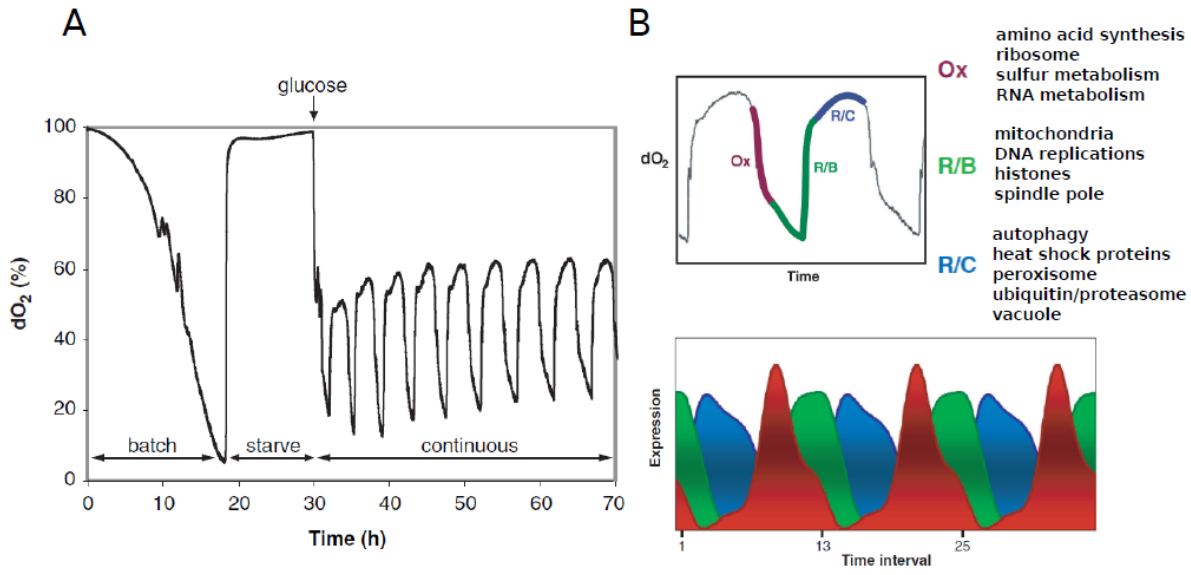

Figure 1. – Metabolic and expression cycling in *Saccharomyces cerevisiae.*

(A) Continuous glucose supplementation after brief period of starvation results in metabolic synchronization represented by repeating pattern of oxygen consumption. (B) Three phases of YMC are characterized based on periodic transcription of genes related to distinct cellular processes. Adapted from (Tu *et al.*, 2005).

Ox phase and start of the R/B phase are accompanied by elevation of Ac-CoA concentration. This increment coincides with hyperacetylation of various lysine residues of histones H3 and H4 (Figure 2. A) (Cai *et al.*, 2011). It has been also observed, that several components of Gcn5 containing SAGA acetyltransferase complex (Grant *et al.*, 1997) are acetylated. The acetylation states of SAGA complex subunits has been found to be dependent on the Gcn5p activity and their level positively correlates with the Ac-CoA concentration *in vitro* (Cai *et al.*, 2011). Although, there is no evidence that these modifications alter the catalytic properties of this complex, it has been shown that SAGA predominantly binds to the target genes of the Ox super cluster during the Oxidative phase and target genes of the R/C super cluster during reductive phase (Figure 2. B). The fact, that significant change in histone H3 acetylation occurs only at growth related genes of the Ox super cluster (Figure 2. B) implicates, that sufficient concentration of Ac-CoA is essential for transcriptional activation of genes related to proteosynthesis and thereby growth. This is supported by the fact, that addition of acetate (substance utilizable for Ac-CoA production) into the metabolically synchronous culture during the R/C phase causes increase in Ac-CoA concentration and instantly drives the cells into the Oxidative-growth phase. Considering, that *gcn5Δ* cells cultured in previously described chemostat exhibit no cycling in oxygen consumption (Cai *et al.*, 2011), histone acetyltransferase activity of SAGA complex, whose function is already known to be associated with transcription (Lee *et al.*, 2000), plays a crucial role in induction of oxidative phase.

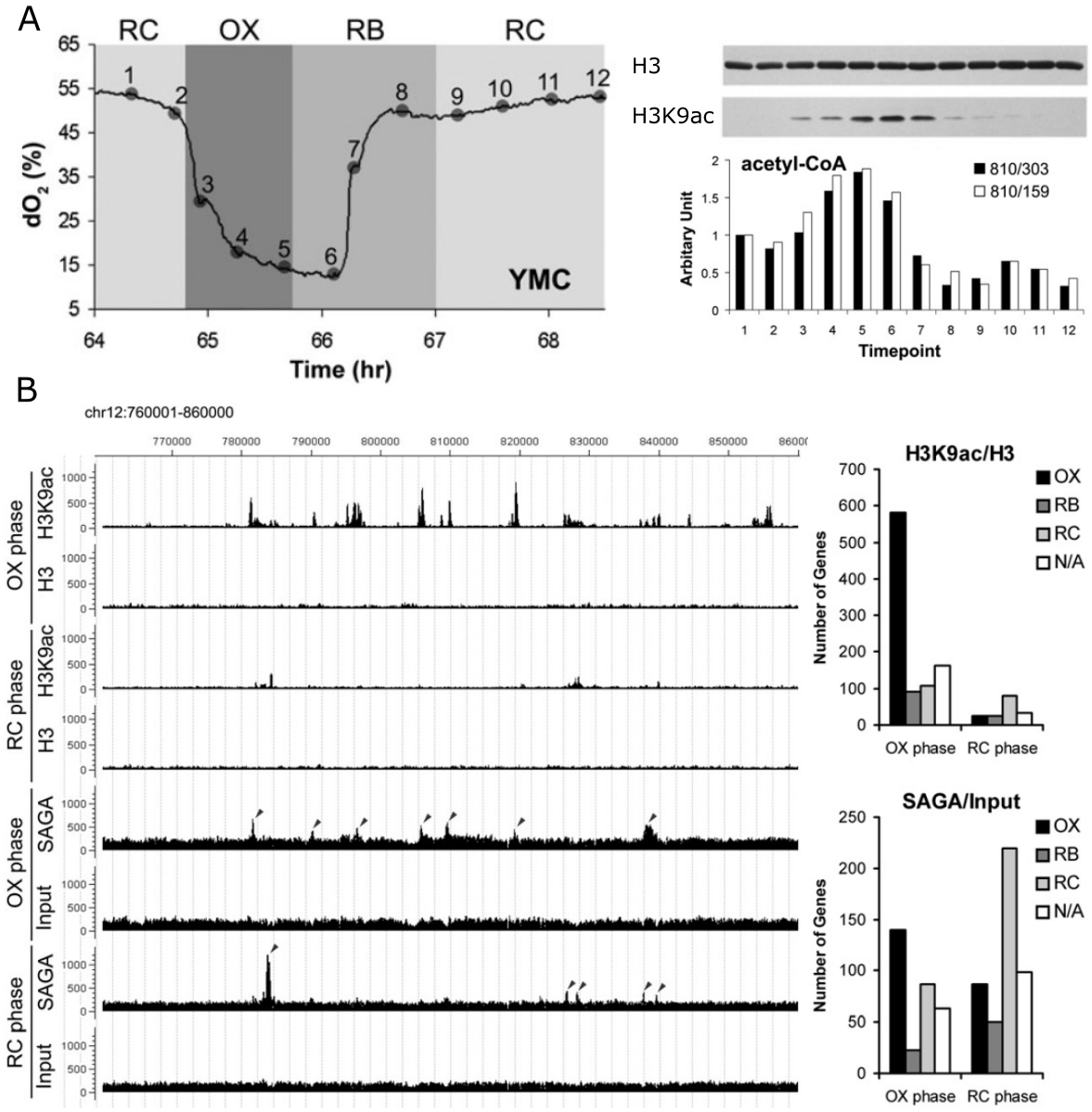

Figure 2. – Metabolic cycle related changes in levels of Ac-CoA, histone acetylation and DNA-occupancy of SAGA complex

(A) Metabolic cycling is accompanied by fluctuation of intracellular Ac-CoA level. Elevation of Ac-CoA level coincides with hyperacetylation of various lysine residues of histones H3 and H4. H3K9 has been chosen as a representative modification. (B) Chromatin immunoprecipitation followed by sequencing revealed that SAGA complex show differential DNA-occupancy dependent on actual YMC phase. Arrows point at the peaks referred to the gene loci, whose activation of transcription is specific for a given phase. Signal specific for SAGA binding during Ox phase correlates with signal specific for histone H3K9 acetylation. Adapted from (Cai *et al.*, 2011).

Another interesting fact is that DNA replication and mitosis are almost completely absent during Ox-phase. As already mentioned, transcription of cell division related genes show periodical character peaking during R/B phase (Tu *et al.*, 2005). Hence, it is possible that yeasts coordinate their cell division and metabolic cycles. It is discussed that DNA-replication and budding predominantly take place during Reductive phases of YMC (Figure 3. A and B) because of decreased possibility of DNA damage caused by free radicals created as a byproduct of respiration, which is absent during reductive phases. However, elevation of Ac-CoA correlates with activation of growth related genes and relatively high levels of Ac-CoA are still maintained at the start of the R/B phase (Figure 2. A). Therefore, Ac-CoA should be also considered as a direct contributor to the regulation of S-phase entry, mitosis and proliferation.

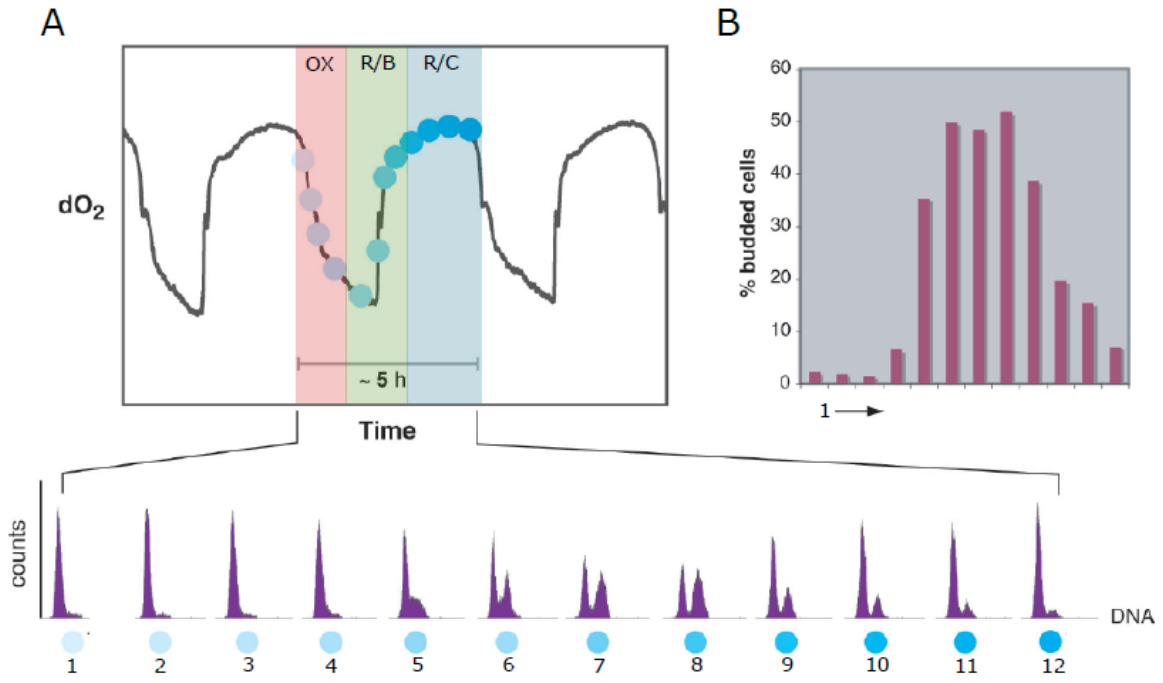

Figure 3. – Coordination of metabolic and cell division cycles.

(A) Fluorescence-activated cell sorting analysis of DNA content in *Saccharomyces cerevisiae* cells collected in different stages of YMC. The peak representing duplicated DNA (S-phase) is specific for Reductive phases. (B) Microscopy based analysis of cell budding events. Budding only takes place in Reductive phases of YMC. Adapted from (Tu *et al.*, 2005).

3.3. Metabolic reprogramming

Since availability of usable carbohydrates influences chromatin acetylation and transcription, glycolysis as a main glucose utilizing pathway should be considered as a possible contributor to this phenomenon. Cancer cells are remarkable for their metabolic reprogramming. They are characterized by production of lactate even in the presence of oxygen. This metabolic behavior has not been seen in differentiated tissues and from 1930 is known as Warburg effect (Vander Heiden *et al.*, 2009). Energy production of so called aerobic glycolysis is not as efficient as glycolysis followed by oxidative phosphorylation. This is compensated by elevated rate of glucose consumption, therefore living entities utilizing aerobic glycolysis represent an ideal model for study of connection between rate of glycolysis and histone acetylation. The molecule of 2-deoxy-D-gluose (2-DG) devoided of 2-hydroxyl group cannot enter glycolysis and competitively inhibits formation of glucose-6-phosphate as an initial step of glycolysis. Replacement of D-glucose by 2-DG as well as siRNA mediated silencing of *HK1* (hexokinase1) and *PKM* (pyruvate kinase) as rate limiting glycolytic enzymes results in loss of histone acetylation and heterochromatinisation in human lung carcinoma cells, A549 (Figure 4. A). These aberrancies induced by impairment of glycolysis are accompanied by decrement of intercellular Ac-CoA, lactate and pyruvate levels. Ac-CoA is essential for HAT activity and both lactate and pyruvate function as inhibitors of HDACs (Thangaraju *et al.*, 2006; Latham *et al.*, 2012; Liu *et al.*, 2015). Therefore, alteration in cellular metabolite levels may be responsible for unbalanced state of HAT and HDAC activities, which could result in previously described phenotypes. This is supported by report that panobinostat-mediated inhibition of HDACs (Sato *et al.*, 2014) leads to rescue of glycolysis impairment-induced histone acetylation loss and heterchromatinisation. Considering, that histone acetylation of 11 different types of human cells positively correlates with the glycolysis rate (Figure 4. B), nutrient-induced acceleration or moderation of glycolysis could directly regulate the epigenetics (Liu *et al.*, 2015). This hypothesis is supported by finding, that glucose deprivation induces rDNA histone hypoacetylation in HeLa cells (Murayama *et al.*, 2008). Murayama and colleagues showed that ribosomal DNA (rDNA) acetylation level positively correlates with dosage of glucose, which unavailability leads to reduced transcription of rDNA repetitions. Besides previously described disruption of pyruvate/lactate inhibition of HDACs, it is discussed that insufficient glucose catabolism

increases the NAD+/NADH ratio, which causes hyper activation of NAD+ dependent lysine deacetylase SIRT1, further potentiating hypoacetylation effect (Murayama *et al.*, 2008).

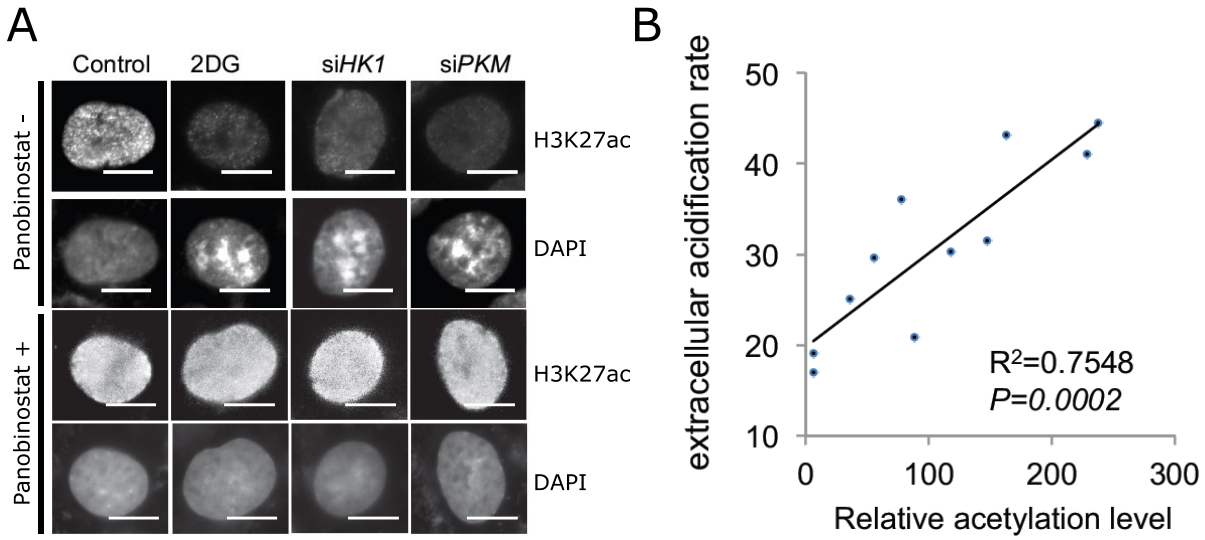

Figure 4. - Rate of glycolysis affects histone acetylation and chromatin structure in cancer cells. (A) Disruption of glycolysis in A549 cells leads to H3K27 acetylation loss (immunofluorescence staining) and heterochromatinisation (DAPI) staining. These effects are not present in cells treated with HDAC inhibitor panobinostat. (B) Relative histone acetylation level positively correlates with rate of glycolysis expressed as extracellular acidification rate. Adapted from (Liu *et al.*, 2015).

Cancer cells are not the only cell types performing aerobic glycolysis instead of glycolysis coupled to oxidative phosphorylation. The Warburg effect is also characteristic for pluripotent stem cells (PSCs), which are capable of differentiation into matured cells of all types. In addition to metabolic alteration, H3K9 hyperacetylation and decondensed chromatin state are distinctive for PSCs (Hezroni *et al.*, 2011). Additionally to the loss of pluripotency markers, differentiation process is connected with metabolic reprogramming. Consumption of glucose and intracellular levels of lactate and acetate are reduced 24 hours after induction of differentiation, which implicates the shift towards utilization of oxidative phosphorylation. This event is accompanied by downregulation of ATP citrate lyase (*ACLY*) and Ac-CoA synthetase 2 (*ACSS2*), which are responsible for Ac-CoA production. In this stage of early differentiation, level of intracellular Ac-CoA is decreased and histones H3 and H4 show hypoacetylated state in comparison to pluripotent state. Interestingly, addition of acetate into the medium does not impede the reduction of *ACLY* and *ACSS2* transcription, but prevents the Ac-CoA deprivation and loss of histone acetylation. Loss of PSC markers

is also diminished while acetate is available. Therefore, distinct levels of metabolites typical for aerobic glycolysis of PSCs might directly participate in maintenance of genomic and transcriptomic state characteristic for PSCs (Moussaieff *et al.*, 2015).

4. Enzymes involved in Ac-CoA metabolism

Other studies implying the importance of intracellular Ac-CoA in connection with epigenetics and transcription are based on characterization of enzymes involved in synthesis of this molecule.

For better orientation, schematic overview of Ac-CoA producing pathways (Figure 6.) is present at the end of the chapter, page 20.

4.1. Acetyl coenzyme A synthetases

There are 3 major pathways supplying Ac-CoA in *Saccharomyces cerevisiae*: peroxisomal production via fatty acid β oxidation, mitochondrial production via activity of pyruvate dehydrogenase complex and nucleocytosolic production via activity of Ac-CoA synthetases. Mitochondrial, peroxisomal and nucleocytosolic Ac-CoA pools are often refered as biochemicaly isolated sections. However, the idea of 100 % isolation is not accurate as intercompartmental flow of Ac-CoA based on Yat2, carnitine acetyltransferase (Swiegers *et al.*, 2001) and Ach1, mitochondrial Ac-CoA hydrolase (Buu *et al.*, 2003) has been described. TCA cycle and respiration are inhibited by glucose in *Saccharomyces cerevisiae*. Hence, glycolysis and subsequent fermentation are the major players in energy and carbon metabolism when fermentable sugar is available (Gombert *et al.*, 2001; Heyland *et al.*, 2009). While both PDC activity and β oxidation are tightly connected to TCA and respiration, it is possible that their activities are also reduced while glucose is abbundant. Consistently, deletions of *PDA1* and *POT1* genes, essential for pyruvate dehydrogenase activity (Wenzel *et al.*, 1993) and β oxidation of fatty acids (Igual *et al.*, 1992), respectively, do not impair the overall histone acetylation state, indicating that mitochondrial/peroxisomal Ac-CoA pools does not contribute to acetylation of histones while glucose is abundant. Consequently, the *Saccharomyces cerevisiae* Ac-CoA syntethase system is the only supplier of the Ac-CoA available for HATs while growing on glucose (Takahashi *et al.*, 2006). *Saccharomyces cerevisae* cells utilize two Ac-CoA synthetases: Acs1p and Acs2p. The Acs1p/Acs2p homologs are characterized by different K_M and mechanism of transcriptional regulation (Van den

Berg *et al.*, 1996). While *ACS2* is required for growth on glucose (Van den Berg and Steensma, 1995), the expression of *ACS1* is repressed by glucose and its activity is connected with the metabolism of non-fermentable carbon sources such as acetate, glycerol and ethanol (Schüller, 1995). As deletion of both ACS homologs results in synthetic lethality, the following experiments are based on thermosensitive mutant, *acs2-Ts1*. It has been reported, that exponentially growing *acs2-Ts1* cells utilizing glucose as a carbon source shifted to the restrictive temperature suffer from histone deacetylation. This effect is not further potentiated by *ACS1* deletion, which is consistent with the statement, that transcription of *ACS1* is repressed while glucose is present. When cells are grown on YPGE medium containing glycerol and ethanol but not glucose, either *ACS1* or *ACS2* alone is sufficient for histone acetylation as their single disruptions show no effect. As expected, under restrictive temperature the *acs1Δ acs2-Ts1* strain exhibits diminished ability to maintain the WT level of histone acetylation under non-fermentable nurture. Therefore, Acs1p and Acs2p functions are redundant under nonfermentative conditions (Takahashi *et al.*, 2006).

Although these findings might be intriguing, they say nothing about the causality of the system. Is the global histone acetylation in yeasts directly dependent on the catalytic activity of the Acs1p/Acs2p or the availability of nucleocytosolic Ac-CoA produced by Acs enzymes? The possible insight into this problem lies in the work of B. Kozak and his colleagues, who managed to successfully rescue the synthetic lethality of *ACS1*/*ACS2* double deletants by introduction of genes responsible for alternative bacterial Ac-CoA production pathways (Kozak *et al.*, 2014). Therefore, sufficient amount of Ac-CoA is most likely main requirement for activity of HATs and acetylation of histones.

4.2. ATP-citrate lyase

Additionally to Ac-CoA synthetase system, metazoans and some fungal species utilize cytoplasmic/nuclear ATP citrate lyase (ACL) (Wellen *et al.*, 2009). ACL participates in generation of nucleocytosolic Ac-CoA from the mitochondrial citrate transported to the cytosol via malate/citrate shuttle. It has been reported, that ATP-citrate lyase is essential for growth and sporulation of *Aspergilus nidullans* under glucose based nurture, but loss of ACL function can be rescued by activity of Ac-CoA syntethase when sufficient amount of acetate is available (Hynes and Murray, 2010). The siRNA mediated silencing experiments revealed, that decreased ACL level in human colon carcinoma cell (HCT116) significantly reduces global histone acetylation of all core histones under

standard cultivation conditions, while suppression of acetyl coenzyme A synthetase 1 (AceCS1) has no significant effect. DNA-damage responding transcription factor p53 is a subject for post-translational regulation by acetylation (Chung *et al.*, 2014). The fact, that acetylation pattern of this protein is not altered after exposure to DNA damaging drug doxorubicin (adriamycin) (Potmesil *et al.*, 1984) in cells with disrupted ACL function implicates the existence of mechanism selectively utilizing ACL produced Ac-CoA for histone acetylation purposes. However, exactly opposite effect has been also reported. Over-expression of antiapoptotic protein Bcl-x(L), which takes part in regulation of mitochondrial metabolism, apparently leads to mitochondrial malfunction (Vander Heiden *et al.*, 1999; Gottlieb *et al.*, 2000) and decrement of the Ac-CoA level in Jurkat cells (Yi *et al.*, 2011). The state of decreased level of intracellular Ac-CoA is accompanied by hypoacetylation of non-histone proteins, while acetylation of histones H3 and H4 remains unaffected. The addition of acetate or citrate (AceCS1 and ACL substrates) drives the Ac-CoA to the standard values and restores the acetylation levels of non-histone proteins (Yi *et al.*, 2011). As ACL-mediated production of Ac-CoA strictly depends on mitochondrial function, it can be discussed that over-expression of Bcl-x(L) directly affects the ACL activity. Therefore, it is not clear, whether there is a relevant evidence of some site-specific channeling of ACL produced Ac-CoA. Consistently with Hynes and Murray (Hynes and Murray, 2010), replacement of glucose by acetate causes replenishment of nucleocytosolic Ac-CoA by AceCS1 activity, which leads to dose dependent suppression of effects developed in cells with impaired function of ACL. As HDACs contribute to histone deacetylation, their inhibition by trichostatin A (Yoshida *et al.*, 1990) also leads to suppression of phenotypes specific for cell with suppressed function of ACL (Wellen *et al.*, 2009). Interestingly, cells suffering from activity loss of both ACL and AceCS1 are still able to maintain residual histone acetylation levels (Wellen *et al.*, 2009).

Possible link between ACL function, nutrition and histone acetylation-mediated regulation of transcription has emerged from the studies of adipocyte differentiation using mouse-derived 3T3-L1 cell line. Induction of differentiation leads to histone hyperacetylation and over-expression of *Glut4*, *Hk2*, *Pfk-1* and *Ldh-A*, genes important for glucose exploitation. As already discussed, utilization of glucose is connected to production of Ac-CoA. Therefore, elevation of glucose consumption rate might result in increase of intracellular Ac-CoA, which drives fatty acid synthesis characteristic for

adipocyte differentiation. Process of lipid accumulation as well as transcriptional reprogramming of *Glut4*, *Hk2*, *Pfk-1* and *Ldh-A* is significantly disrupted after ACL silencing. Interestingly, transcription of adipocyte specific marker, adipocyte protein 2 (*Ap2*), is not decreased after suppression of ACL. Hence, expression of *Ap2* seems to be Ac-CoA and histone acetylation independent and activation of glucose utilization machinery and fatty acid accumulation represent events separate from differentiation entry. Histone hyperacetylation has been found in the promoter of *Glut4* and was severely decreased after disruption of ACL. Moreover, histone acetylation and transcription of *Glut4* positively respond to increasing concentration of glucose. As differentiation-induced hyperacetylation of histones is not only specific for *Glut4*, but has been also observed globally, sufficient level of Ac-CoA available for HATs might be essential for induction of specific modes of transcription, which are necessary for metabolic adjustment in response to programmed or environmental changes (Wellen *et al.*, 2009).

4.3. Nuclear pyruvate dehydrogenase complex

The residual histone acetylation level maintained in cancer cells lacking ATP-citrate lyase and acetyl-Coenzyme A synthetase activities suggests the existence of another pathway responsible for production of Ac-CoA available for HAT enzymes (Wellen *et al.*, 2009).

This Ac-CoA source could be represented by pyruvate dehydrogenase complex (PDC), whose nuclear localization has been recently confirmed in mammalian cells (Figure 5. A) (Sutendra *et al.*, 2014). The fact that isolated nuclei respond to the addition of pyruvate by synthesis of Ac-CoA in a dose-dependent manner proves that nuclear pyruvate dehydrogenase complex is fully functional. Production of Ac-CoA mediated by isolated nuclei is significantly corrupted upon siRNA mediated silencing of PDC E1 subunit (Figure 5. B). Disruption of PDC function also results in decreased acetylation of histones H2B, H3 and H4 in both, isolated nuclei treated with pyruvate and whole cells (Figure 5. C), which implies, that nuclear PDC might function as a mediator between carbon metabolism and epigenetics. Interestingly, disruption of PDC function does not induce any changes in acetylation of nuclear proteins p53 and FOXO1, providing another indication of target-specific Ac-CoA channeling towards histones. As PDC also fuels mitochondrial TCA cycle and therefore contributes to citrate production, it directly affects ATP-citrate lyase function. Because of that, hypoacetylation of histones in cells

lacking PDC activity is more severe than in those with disrupted ACL (Figure 5. C). Difference between these two states of inhibition supports the hypothesis of mitochondria-independent function of PDC, which can be important as another direct supplier of nuclear Ac-CoA when rate of citrate, as an ACL substrate, production is decreased (Sutendra *et al.*, 2014).

PDC subunits contain the mitochondrial localization signal (MLS), which restrains the acquirement of native conformation and can only be cleaved in mitochondria. Therefore, to become functional PDC must enter mitochondria before nucleus. It has been shown that active transport of PDC from mitochondria to nucleus occurs after serum mediated induction of G1-S transition (Figure 5. D). The serum induced elevation of nuclear PDC coincides with increase of histone H3 acetylation level, which peaks four hours after induction of S-phase entry (Figure 5. D). This time point is also characterized by elevation of cyclin A level (Figure 5. D). Cyclin A is typically over-expressed upon S-phase entry and is considered to be one of the S-phase markers (Oliver and MacDonald, 2000). Cyclin A transcription is not affected after S-phase induction while PDC expression is disrupted. However, after selective inhibition of mitochondrial PDC fraction, S-phase linked cycline A over-expression is present. Therefore, nuclear fraction of PDC is responsible for induction of cycline A transcription. Similar results have been obtained for P-RB, *E2F* and *CDK2*, which are also characteristic for S-phase entry. Therefore, nuclear PDC-mediated histone acetylation might participate in transcription regulation of S-phase markers and cell cycle progression, linking the carbon metabolism, acetylation and cell-cycle related realization of genetic information (Sutendra *et al.*, 2014).

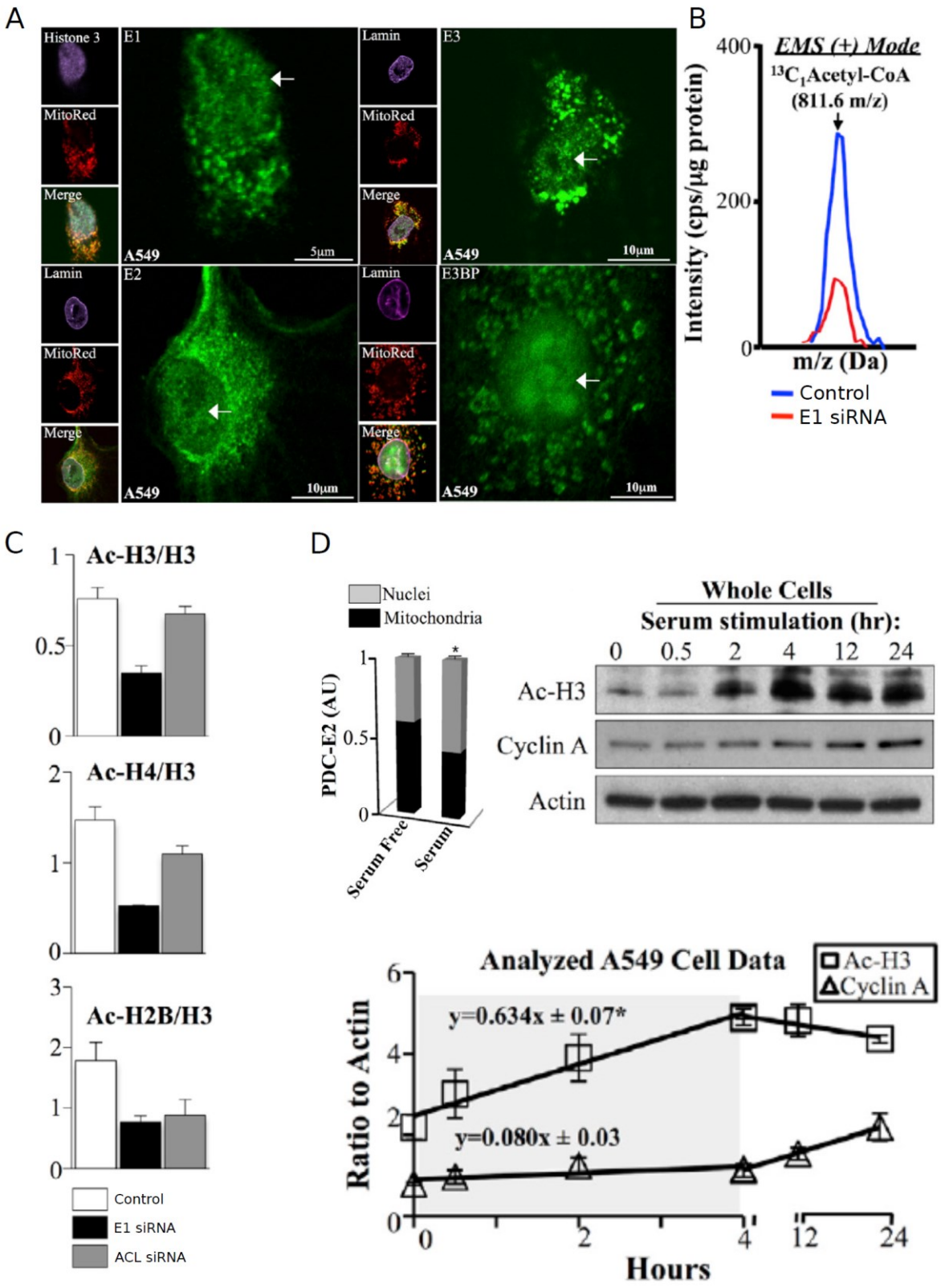

Figure 5. – Pyruvate dehydrogease complex localizes to the nucleus and contributes to nuclear Ac-CoA production and histone acetylation.

(A) Fluorescence microscopy images reveal nuclear localization of all four PDC subunits (E1, E2, E3, E3BP). Immunofluorescence techniques have been used for visualization of PDC subunits and nucleus.

Mitochondria have been stained by fluorescence dye, MitoRed. Arrows indicate nuclear regions where mitochondrial signal is absent but PDC specific fluorescence is present. (B) Mass spectrometry analysis of isolated nuclei treated with $^{13}C_2$-pyruvate. Nuclei isolated from cells exposed to PDC-E1 targeting siRNA show diminished ability to produce Ac-CoA. (C) Quantification of anti-histone western blot from whole cell extract. PDC-E1 and *ACLY* siRNA treated cells show reduced level of H3, H4 and H2B acetylation. PDC knockdown results in more severe effect compared to disruption of *ACLY*. (D) Quantification of anti PDC-E2 immunofluorescence microscopy images shows elevation of nuclear PDC-E2 signal after serum-induced S-phase entry. S-phase induction is accompanied by gradual increase of histone H3 acetylation, which peaks 4 hours after serum treatment. Peaking of ac-H3 signal coincides with elevation of cyclin A protein level. Adapted from (Sutendra *et al.*, 2014).

4.4. Phosphopantothenoylcystein synthetase

The phenotypes, which are detected while fundamental pathways of Ac-CoA synthesis are disrupted are also characteristic for cells lacking the activity of phosphopantothenoylcystein synthetase (Nakamura *et al.*, 2012), enzyme participating in biosynthesis of CoA (Yao and Dotson, 2009). In agreement with previous statements, depletion of Ac-CoA available for HATs caused by inactivation of *Schizosaccharomyces pombe* phosphopantothenoylcystein synthetase (*ppc1*) leads to global loss of histone acetylation. This defect is accompanied by incapability of quiescence phase exit, aberrant nuclear division and high frequency of chromosome loss (Nakamura *et al.*, 2012), providing further clues of importance of Ac-CoA in growth entry and cell-cycle related processes.

4.5. Ac-CoA carboxylase and fatty-acid synthetase

In addition to acetylation processes, nucleocytosolic Ac-CoA functions as a primary substrate for de novo fatty acid synthesis (Waite and Wakil, 1962). Fatty acid synthesis takes place in cytosol; therefore it contributes to consumption of nucleocytosolic Ac-CoA pool. The initial step of fatty acid synthesis is formation of malonyl-CoA by acetyl-CoA carboxylase mediated carboxylation of Ac-CoA. Acetyl-CoA carboxylase is encoded by *ACC1* gene in *Saccharomyces cerevisiae*. Reduced *ACC1* expression results in global histone hyperacetylation and increased mRNA levels of genes known to be regulated by histone acetylation (Galdieri and Vancura, 2012). It is discussed that these effects are caused by relative elevation of HAT substrate concentration, due to impairment of Ac-CoA consumption. Even if there is no evidence of actual increased levels of nucleocytosolic Ac-CoA, the fact that inactivation of Asc2p can be partially rescued by

reducement of Acc1p activity (Galdieri and Vancura, 2012) supports this hypothesis. It has been also reported, that disruption of fatty acid synthesis pathway by inactivation of acetyl-CoA carboxylase (*cut6*) and fatty acid synthetase (*fas1*) as its core components leads to aberrant mitosis in *Schizosaccharomyces pombe* (Saitoh *et al.*, 1996). Same effect can be observed in WT cells treated with fatty acid synthesis inhibitor, cerulenin (Funabashi *et al.*, 1989). Defects induced by fatty acid synthesis impairment seems to be specific for M-phase as G2 arrested cells are not affected by cerulenin treatment, but cell viability rapidly declines after G2-M transition. Similar phenomenon has been also characterized during mammalian oocyte meiosis, during which histone hyperacetylation induced by trichostatin A mediated inhibition of HDAC (Yoshida *et al.*, 1990) interferes with the chromatin condensation and causes abnormal separation of chromatides (Yang *et al.*, 2012). Authors assume that sufficient amount of synthesized fatty acid is required for successful mitosis in *Schizosaccharomyces pombe* (Saitoh *et al.*, 1996). The alternative explanation is that Ac-CoA plays a role in transition trough M-phase and its supra-physiological levels caused by inactivation of its consuming pathway results in mitotic defects, possibly due to histone hyperacetylation-induced alteration of chromatin structure or deregulation of regulatory proteins transcription.

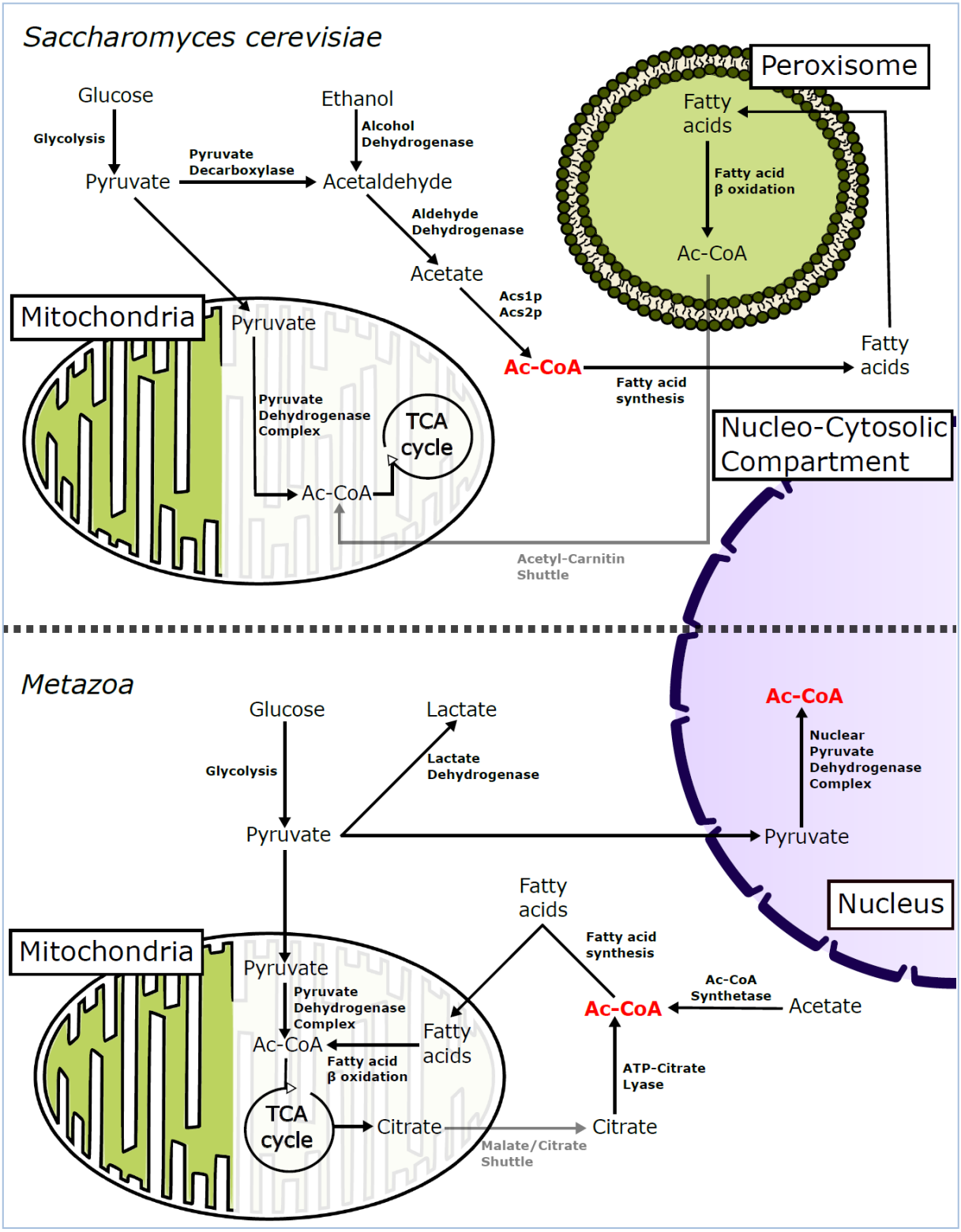

Figure 6. – Schematic overview of pathways producing Ac-CoA available for HATs in *Saccharomyces cerevisiae* and metazoan cells.

Molecules of Ac-CoA marked as red are available for histone acetylation purposes. Image layout inspired by review (Choudhary *et al.*, 2014).

5. Summary

Free living entities encounter extensive changes in availability of nutrients to which they must respond. Among strategies, organisms utilize transcriptional reprogramming, which enable them to optimize subcellular activity and function. Modulation of gene expression takes place on chromatin complex, in which histone proteins undergo various modifications. Several histone N-terminal lysines are subjects to acetylation mediated by histone acetyltransferases (HATs).

Ac-CoA is essential for histone acetylation, the level of which is severely disrupted while utilizable carbon sources are unavailable or fundamental pathways of Ac-CoA production are disrupted. Inactivation of fatty acid synthesis has opposite effect and leads to histone hyperacetylation. Level of histone acetylation also depends on cellular metabolic activity and varies across different lines of mammalian cells. Interestingly, both, hypoacetylation and hyperacetylation of histone proteins cause defects related to cell cycle and cell division. Since Ac-CoA has been shown to be highly fluctuating during yeast metabolic cycles (YMC), in which cell division strictly takes place in distinct interval, it is possible that well controlled temporal fluctuations in intracellular Ac-CoA might be required for proper cell cycle and mitosis.

Under certain conditions, increased level of histone acetylation coincides with activation of transcription, which seems to be not global, but specific for distinct groups of genes. Histone hyperacetylation mediated activation of transcription is important in processes such as differentiation, pluripotency maintenance and yeast metabolic cycles.

Ac-CoA therefore represents a possible transcriptional regulator of basal cellular activities in response to nutrition and takes part in signaling required for cell cycle progression and differentiation.

As other histone modifications, which are connected to different metabolic pathways, also contribute to the chromatin structure and functionality, this work should be considered a fragment of recent knowledge of metabolically driven epigenetic regulation.

6. References

Allfrey, V. G., Faulkner, R., and Mirsky, A. E. (1964). Acetylation and methylation of histones and their possible role in the regulation of rna synthesis. Proc. Natl. Acad. Sci. U. S. A. *51*, 786–794.

Bell, O., Tiwari, V. K., Thomä, N. H., and Schübeler, D. (2011). Determinants and dynamics of genome accessibility. Nat. Rev. Genet. *12*, 554–564. (review)

Van den Berg, M. a, and Steensma, H. Y. (1995). *ACS2*, a *Saccharomyces cerevisiae* gene encoding acetyl-coenzyme A synthetase, essential for growth on glucose. Eur. J. Biochem. *231*, 704–713.

Van den Berg, M. a., De Jong-Gubbels, P., Kortland, C. J., Van Dijken, J. P., Pronk, J. T., and Steensma, H. Y. (1996). The two acetyl-coenzyme A synthetases of *Saccharomyces cerevisiae* differ with respect to kinetic properties and transcriptional regulation. J. Biol. Chem. *271*, 28953–28959.

Buu, L. M., Chen, Y. C., and Lee, F. J. S. (2003). Functional characterization and localization of acetyl-CoA hydrolase, Ach1p, in *Saccharomyces cerevisiae*. J. Biol. Chem. *278*, 17203–17209.

Cai, L., Sutter, B. M., Li, B., and Tu, B. P. (2011). Acetyl-CoA induces cell growth and proliferation by promoting the acetylation of histones at growth genes. Mol. Cell *42*, 426–437.

Dhalluin, C., Carlson, J. E., Zeng, L., He, C., Aggarwal, A. K., and Zhou, M. M. (1999). Structure and ligand of a histone acetyltransferase bromodomain. Nature *399*, 491–496.

Dmitriev, R. I., and Papkovsky, D. B. (2014). *In vitro* ischemia decreases histone H4K16 acetylation in neural cells. FEBS Lett. *589*, 138–144.

Durrin, L. K., Mann, R. K., Kayne, P. S., and Grunstein, M. (1991). Yeast histone H4 N-terminal sequence is required for promoter activation *in vivo*. Cell *65*, 1023–1031.

Friis, R. M. N., Wu, B. P., Reinke, S. N., Hockman, D. J., Sykes, B. D., and Schultz, M. C. (2009). A glycolytic burst drives glucose induction of global histone acetylation by picNuA4 and SAGA. Nucleic Acids Res. *37*, 3969–3980.

Fuge, E. K., Braun, E. L., and Werner-Washburne, M. (1994). Protein synthesis in long-term stationary-phase cultures of *Saccharomyces cerevisiae*. J. Bacteriol. *176*, 5802–5813.

Funabashi, H., Kawaguchi, A., Tomoda, H., Omura, S., Okuda, S., and Iwasaki, S. (1989). Binding site of cerulenin in fatty acid synthetase. J. Biochem. *105*, 751–755.

Galdieri, L., and Vancura, A. (2012). Acetyl-CoA carboxylase regulates global histone acetylation. J. Biol. Chem. *287*, 23865–23876.

Gombert, A. K., Dos Santos, M. M., Christensen, B., and Nielsen, J. (2001). Network identification and flux quantification in the central metabolism of *Saccharomyces cerevisiae* under different conditions of glucose repression. J. Bacteriol. *183*, 1441–1451.

Gottlieb, E., Vander Heiden, M. G., and Thompson, C. B. (2000). Bcl-x(L) prevents the initial decrease in mitochondrial membrane potential and subsequent reactive oxygen species production during tumor necrosis factor alpha-induced apoptosis. Mol. Cell. Biol. *20*, 5680–5689.

Grant, P. a. *et al.* (1997). Yeast Gcn5 functions in two multisubunit complexes to acetylate nucleosomal histones: Characterization of an ada complex and the saga (spt/ada) complex. Genes Dev. *11*, 1640–1650.

Hebbes, T. R., Clayton, a L., Thorne, a W., and Crane-Robinson, C. (1994). Core histone hyperacetylation co-maps with generalized DNase I sensitivity in the chicken beta-globin chromosomal domain. EMBO J. *13*, 1823–1830.

Hebbes, T. R., Thorne, a W., Clayton, a L., and Crane-Robinson, C. (1992). Histone acetylation and globin gene switching. Nucleic Acids Res. *20*, 1017–1022.

Vander Heiden, M. G., Cantley, L. C., and Thompson, C. B. (2009). Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science *324*, 1029–1033. (review)

Vander Heiden, M. G., Chandel, N. S., Schumacker, P. T., and Thompson, C. B. (1999). Bcl-x(L) prevents cell death following growth factor withdrawal by facilitating mitochondrial ATP/ADP exchange. Mol. Cell *3*, 159–167.

Heyland, J., Fu, J., and Blank, L. M. (2009). Correlation between TCA cycle flux and glucose uptake rate during respiro-fermentative growth of *Saccharomyces cerevisiae.* Microbiology *155*, 3827–3837.

Hezroni, H., Tzchori, I., Davidi, A., Mattout, A., Biran, A., Nissim-Rafinia, M., Westphal, H., and Meshorer, E. (2011). H3K9 histone acetylation predicts pluripotency and reprogramming capacity of ES cells. Nucleus *2*, 300–309.

Honma, K., Mochizuki, K., and Goda, T. (2009). Inductions of histone H3 acetylation at lysine 9 on SGLT1 gene and its expression by feeding mice a high carbohydrate/fat ratio diet. Nutrition *25*, 40–44.

Huang, R. C., and Bonner, J. (1962). Histone, a suppressor of chromosomal RNA synthesis. Proc. Natl. Acad. Sci. U. S. A. *48*, 1216–1222.

Hynes, M. J., and Murray, S. L. (2010). ATP-citrate lyase is required for production of cytosolic acetyl coenzyme A and development in *Aspergillus nidulans*. Eukaryot. Cell *9*, 1039–1048.

Choudhary, C., Weinert, B. T., Nishida, Y., Verdin, E., and Mann, M. (2014). The growing landscape of lysine acetylation links metabolism and cell signalling. Nat. Rev. Mol. Cell Biol. *15*, 536–550. (review)

Chung, S. K., Zhu, S., Xu, Y., and Fu, X. (2014). Functional analysis of the acetylation of human p53 in DNA damage responses. Protein Cell *5*, 544–551.

Igual, J. C., Gonazalez-Bosch, C., Franco, L., and Perez-Ortin, J. E. (1992). The *POT1* gene for yeast peroxisomal thiolase is subject to three different mechanisms of regulation. Mol. Microbiol. *6*, 1867–1875.

Inoue, S., Mochizuki, K., and Goda, T. (2011). Jejunal induction of SI and SGLT1 genes in rats by high-starch/low-fat diet is associated with histone acetylation and binding of GCN5 on the genes. J. Nutr. Sci. Vitaminol. (Tokyo). *57*, 162–169.

Jona, G., Choder, M., and Gileadi, O. (2000). Glucose starvation induces a drastic reduction in the rates of both transcription and degradation of mRNA in yeast. Biochim. Biophys. Acta *1491*, 37—48.

Kim, S. C., and Choi, J. K. (2011). Controlling transcriptional programs for cellular adaptation by chromatin regulation. Mol. BioSyst. *7*, 1713–1719.

Kozak, B. U., van Rossum, H. M., Benjamin, K. R., Wu, L., Daran, J. M. G., Pronk, J. T., and Van Maris, A. J. a (2014). Replacement of the *Saccharomyces cerevisiae* acetyl-CoA synthetases by alternative pathways for cytosolic acetyl-CoA synthesis. Metab. Eng. *21*, 46–59.

Lazarow, P. B. (1978). Rat liver peroxisomes catalyze the β oxidation of fatty acids. J. Biol. Chem. *253*, 1522–1528.

Lee, T. I., Causton, H. C., Holstege, F. C., Shen, W. C., Hannett, N., Jennings, E. G., Winston, F., Green, M. R., and Young, R. A. (2000). Redundant roles for the TFIID and SAGA complexes in global transcription. Nature *405*, 701–704.

Liu, X., Little, J. B., and Yuan, Z. (2015). Glycolytic metabolism influences global chromatin structure. Oncotarget *6*.

Moussaieff, A. *et al.* (2015). Glycolysis-Mediated Changes in Acetyl-CoA and Histone Acetylation Control the Early Differentiation of Embryonic Stem Cells. Cell Metab. *21*, 392–402.

Murayama, A. *et al.* (2008). Epigenetic Control of rDNA Loci in Response to Intracellular Energy Status. Cell *133*, 627–639.

Nakamura, T., Pluskal, T., Nakaseko, Y., and Yanagida, M. (2012). Impaired coenzyme A synthesis in fission yeast causes defective mitosis, quiescence-exit failure, histone hypoacetylation and fragile DNA. Open Biol. *2*, 120117.

Oliver, R. J., and MacDonald, D. G. (2000). Comparison of BrdU and cyclin A as markers of the S-phase in oral precancerous lesions. J. Oral Pathol. Med. *29*, 426–431.

Pluskal, T., Hayashi, T., Saitoh, S., Fujisawa, A., and Yanagida, M. (2011). Specific biomarkers for stochastic division patterns and starvation-induced quiescence under limited glucose levels in fission yeast. FEBS J. *278*, 1299–1315.

Potmesil, M., Israel, M., and Silber, R. (1984). Two mechanisms of adriamycin-DNA interaction in L1210 cells. Biochem. Pharmacol. *33*, 3137–3142.

Pronk, J. T., Steensma, H. Y., and Van Dijken, J. P. (1996). Pyruvate metabolism in *Saccharomyces cerevisiae*. Yeast *12*, 1607–1633. (review)

Ramaswamy, V., Williams, J. S., Robinson, K. M., Sopko, R. L., and Schultz, M. C. (2003). Global control of histone modification by the anaphase-promoting complex. Mol. Cell. Biol. *23*, 9136–9149.

Saitoh, S., Takahashi, K., Nabeshima, K., Yamashita, Y., Nakaseko, Y., Hirata, a, and Yanagida, M. (1996). Aberrant mitosis in fission yeast mutants defective in fatty acid synthetase and acetyl CoA carboxylase. J. Cell Biol. *134*, 949–961.

Sato, A., Asano, T., Isono, M., Ito, K., and Asano, T. (2014). Ritonavir acts synergistically with panobinostat to enhance histone acetylation and inhibit renal cancer growth. Mol. Clin. Oncol., 1016–1022.

Schüller, H. J. (1995). Carbon source-dependent regulation of the acetyl-coenzyme A synthetase-encoding gene *ACS1* from *Saccharomyces cerevisiae*. Gene *161*, 75–79.

Slavov, N., Macinskas, J., Caudy, A., and Botstein, D. (2011). Metabolic cycling without cell division cycling in respiring yeast. Proc. Natl. Acad. Sci. U. S. A. *108*, 19090–19095.

Stasevich, T. J. *et al.* (2014). Regulation of RNA polymerase II activation by histone acetylation in single living cells. Nature *516*, 272–275.

Sutendra, G., Kinnaird, A., Dromparis, P., Paulin, R., Stenson, T. H., Haromy, A., Hashimoto, K., Zhang, N., Flaim, E., and Michelakis, E. D. (2014). A nuclear pyruvate dehydrogenase complex is important for the generation of Acetyl-CoA and histone acetylation. Cell *158*, 84–97.

Swiegers, J. H., Dippenaar, N., Pretorius, I. S., and Bauer, F. F. (2001). Carnitine-dependent metabolic activities in *Saccharomyces cerevisiae*: Three carnitine acetyltransferases are essential in a carnitine-dependent strain. Yeast *18*, 585–595.

Takahashi, H., McCaffery, J. M., Irizarry, R. a, and Boeke, J. D. (2006). Nucleocytosolic acetyl-coenzyme a synthetase is required for histone acetylation and global transcription. Mol. Cell *23*, 207–217.

Tse, C., Sera, T., Wolffe, a P., and Hansen, J. C. (1998). Disruption of higher-order folding by core histone acetylation dramatically enhances transcription of nucleosomal arrays by RNA polymerase III. Mol. Cell. Biol. *18*, 4629–4638.

Tu, B. P., Kudlicki, A., Rowicka, M., and McKnight, S. L. (2005). Logic of the yeast metabolic cycle: temporal compartmentalization of cellular processes. Science *310*, 1152–1158.

Tu, B. P., Mohler, R. E., Liu, J. C., Dombek, K. M., Young, E. T., Synovec, R. E., and McKnight, S. L. (2007). Cyclic changes in metabolic state during the life of a yeast cell. Proc. Natl. Acad. Sci. U. S. A. *104*, 16886–16891.

Venkatesh, S., and Workman, J. L. (2015). Histone exchange, chromatin structure and the regulation of transcription. Nat Rev Mol Cell Biol *16*, 178–189. (review)

Waite, M., and Wakil, S. J. (1962). Studies on the mechanism of fatty acid synthesis. XII. Acetyl coenzyme A carboxylase. J. Biol. Chem. *237*, 2750–2757.

Weinert, B. T., Iesmantavicius, V., Moustafa, T., Schölz, C., Wagner, S. A., Magnes, C., Zechner, R., and Choudhary, C. (2014). Acetylation dynamics and stoichiometry in *Saccharomyces cerevisiae*. Mol. Syst. Biol. *10*, 716.

Wellen, K. E., Hatzivassiliou, G., Sachdeva, U. M., Bui, T. V, Cross, J. R., and Thompson, C. B. (2009). ATP-citrate lyase links cellular metabolism to histone acetylation. Science *324*, 1076–1080.

Wenzel, T. J., Luttik, M. a H., Van Den Berg, J. a., and Steensma, H. Y. (1993). Regulation of the PDA1 gene encoding the E1 alpha subunit of the pyruvate dehydrogenase complex from Saccharomyces cerevisiae. Eur. J. Biochem. *218*, 405–411.

Yang, F., Baumann, C., Viveiros, M. M., and De La Fuente, R. (2012). Histone hyperacetylation during meiosis interferes with largescale chromatin remodeling, axial chromatid condensation and sister chromatid separation in the mammalian oocyte. Int. J. Dev. Biol. *56*, 889–899.

Yao, J., and Dotson, G. D. (2009). Kinetic characterization of human phosphopantothenoylcysteine synthetase. Biochim. Biophys. Acta - Proteins Proteomics *1794*, 1743–1750.

Yi, C. H. *et al.* (2011). Metabolic Regulation of Protein N-Alpha-Acetylation by Bcl-xL Promotes Cell Survival. Cell *146*, 607–620.

Yildirim, F. *et al.* (2014). Histone acetylation and CREB binding protein are required for neuronal resistance against ischemic injury. PLoS One *9*.

Yoshida, M., Kijima, M., Akita, M., and Beppu, T. (1990). Potent and specific inhibition of mammalian histone deacetylase both *in vivo* and *in vitro* by trichostatin A. J. Biol. Chem. *265*, 17174–17179.