# System-wide model of redox-regulated chromatin dynamics

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## 1 Introduction

### 1.1 Background

A deeper understanding of the cellular dynamics and the transcription regulation are one of the central goals of modern biology. In eukaryotic cells, chromatin structure has been shown to be a highly dynamic property, which regulates essential cellular functions, such as gene transcription, DNA replication and repair. Chromatin is an array of nucleosomes formed by 147 bp of DNA which are wound around a histone octamer containing two copies of each core histone proteins H2A, H2B, H3 and H4, or, alternatively, from histone variants that specialize chromatin at particular regions. This packaging has a structural role, by allowing compaction of DNA in the nucleus, but has a repressive effect on transcription, since it hinders the binding of transcription factors and transcriptional machinery to gene promoters and coding regions.

Nucleosomes exhibit at least three dynamic properties *in vivo*: repositioning (that is, altering the position of the nucleosome on the DNA), compositional alteration (such as the replacement in *S. cerevisiae* of H2A histone with its variant, Htz1) and covalent modification (modifications of individual residues of histones). These dynamic properties are mediated by nucleosome modifying and nucleosome remodeling complexes, which work in concert to regulate the local and global properties of chromatin[1, 2]. Histone covalent modifications in particular have been the focus of many recent studies, which have revealed that the acetylation, methylation, phosphorylation, ubiquitination and sumoylation of the core histones have a crucial role in dynamically regulating gene activity[3, 4, 5, 6].

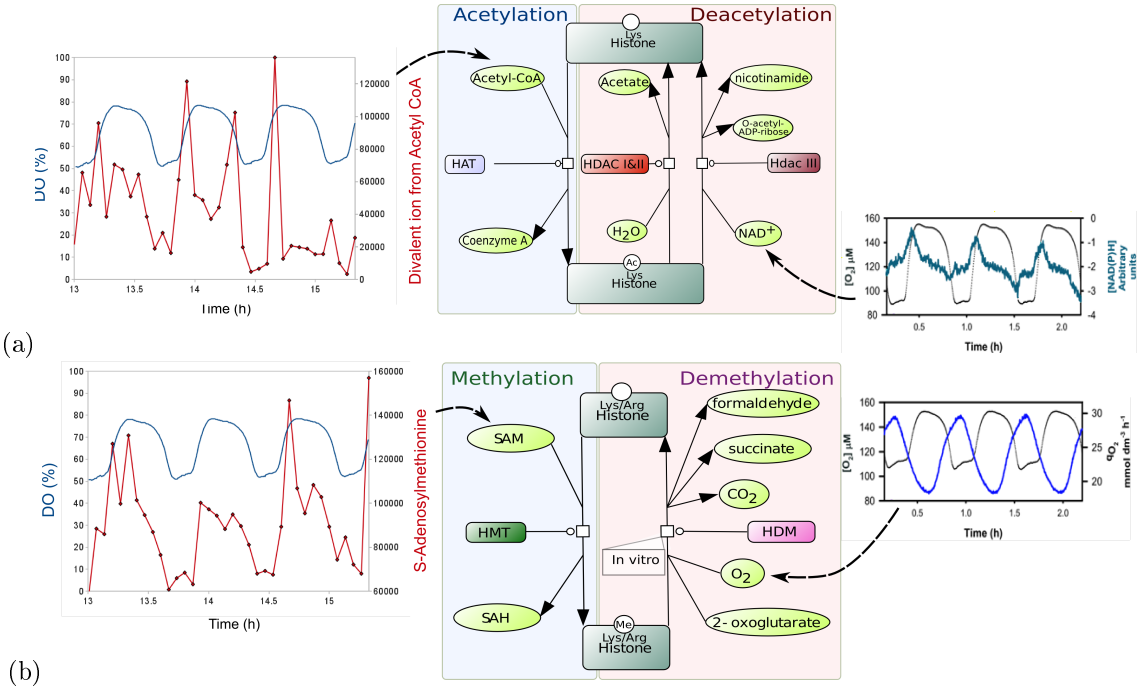



Figure 1: Metabolic regulation of histone modifications.The known covalent histone modifications are conditioned by the availability of certain currency metabolites, which have been monitored online (NAD(P)H, $O_2$) or measured by CE-MS (Acetyl-CoA, SAM), revealing strong oscillatory patterns; (a) Acetylation, (b) Methylation enzymatic reactions.

It has been postulated that the histone modification events are dependent on the availability of the "currency metabolites"[7] *in vivo*, such as acetaldehyde (precursor of acetyl-CoA required for acetylation - fig. 1(a)), and

$H_2S$ (precursor of S-adenosyl methionine required for methylation - fig. 1(b)), and that chromatin dynamics are therefore interwoven with cellular metabolism[8, 9, 10].

For the study of the dynamic correlation between the metabolic state, chromatin state and transcription regulation, *S. cerevisiae* grown in continuous culture represent an ideal organism, since they can be grown in precisely controlled conditions, can be easily manipulated and, under many conditions, the individuals auto-synchronize to produce a temperature-compensated oscillation, that involves respiratory switching between high respiratory activity (oxidative phase) and low respiratory activity (reductive phase). These metabolic oscillations can be conveniently tracked by on-line measurements, such as dissolved oxygen, NAD(P)H and $H_2S$. Moreover, it was shown that many core metabolites, as well as mRNA concentrations (fig. 2), oscillate with phase relationships to this respiratory cycle[11, 12].

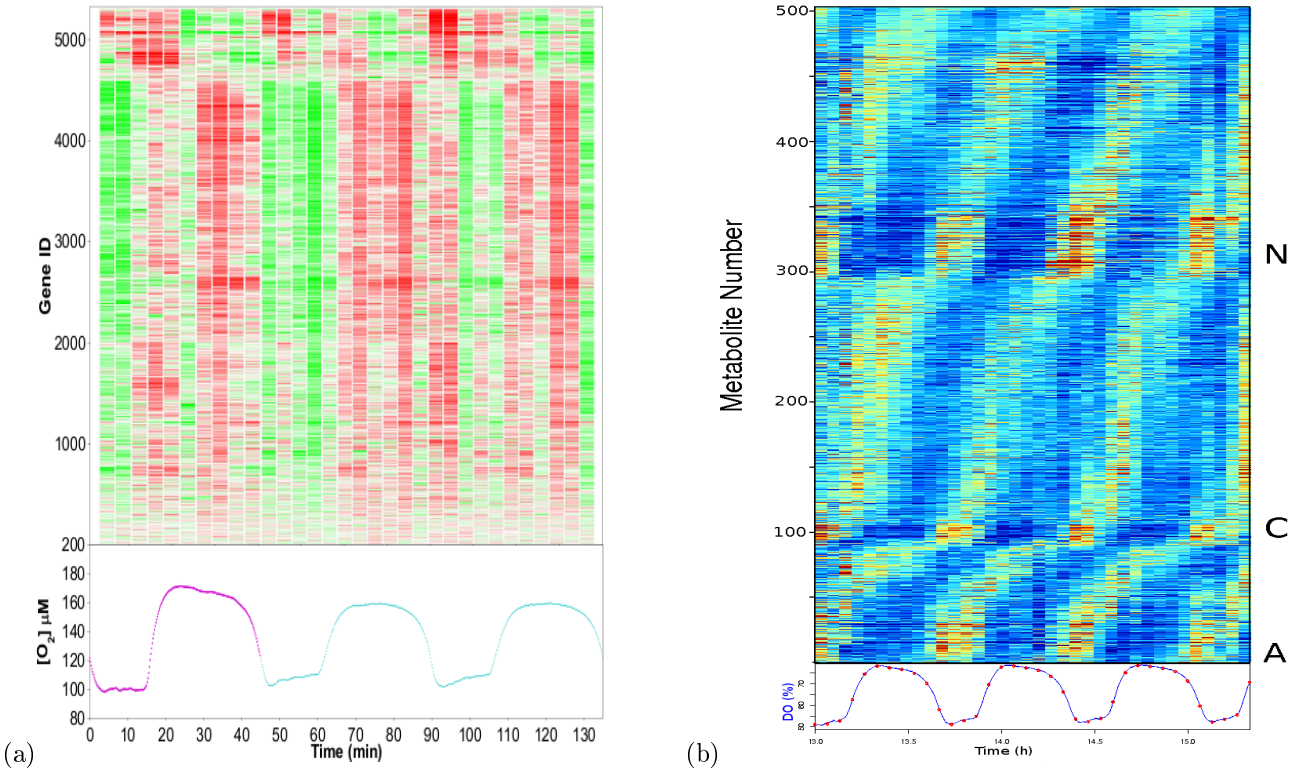



Figure 2: Waves of transcription and metabolite production. (a) A heatmap of scaled GenechipTM expression data[13] from 32 microarrays show >90% of transcripts show oscillatory dynamics and their maximum production has distinct phase relationships with respiratory activity. Green represents scaled expression $< 0.33$ and red represents scaled expression $> 3$. The lower graph shows dissolved oxygen concentration for the experiment where the magenta line depicts the concentrations of the first 10 microarrays and the cyan line the last 22. The samples from these were taken 3 months apart.
(b) A contour plot of scaled CE-MS metabolite data show that ~500 peaks show oscillatory dynamics and have a similar production structure to transcription. Green represents scaled expression $< 0.33$ and red represents scaled expression $> 3$. The lower graph shows dissolved oxygen concentration (black; uM) and loess smoothed fluorimeter signal NAD(P)H (dark cyan; V). C represents cation, A anion and N nucleotide.

Essential cellular substrates with redox activity, such as the above mentioned S-adenosyl methionine and acetyl-CoA have been implicated in the synchronization of the respiratory oscillation[14, 15]. It is also likely that energetics (e.g., ATP:ADP) play a key role in the global regulation of transcript level, since nucleosome remodeling and several histone modification events are ATP-dependent. Generally, the role of these metabolites is often overlooked in global regulation, perhaps due to measurement difficulties.

## 1.2 Objectives

The main theme of my research is to investigate the dynamic relationship between the metabolic state, chromatin state and transcription, both computationally and experimentally. Although much work has been done to investigate the core metabolism dynamics experimentally, few studies have approached this computationally. Therefore, I plan to construct a computational model of the core metabolism and of the histone modifications by gathering data from the literature, and study the way the core metabolism, histone modifications and transcription feed back on each other.

Similarly, even though many studies have been conducted to study particular histone modifications, the global dynamics of these events are still yet to be elucidated. Thus, I intend to produce system-wide time-series experimental data of global protein modification levels (e.g. acetylation, methylation), co-enzyme concentrations (e.g. acetyl-CoA, S-adenosyl methionine) and metabolite concentrations (e.g. acetate and $H_2S$ ) with respect to redox oscillations.

These results together will give new insights on the global correlation between the metabolome, proteome and transcriptome and can have significant medical benefits, such as elucidating the well-established links between cancer and aging and the general or NAD-dependent Sir2-mediated histone deacetylation[16].

### 1.3 Progress

We have conducted a series of Chromatin Immunoprecipitation experiments on time-series samples, aimed at better understanding the dynamics of the transcription regulation with regard to histone positioning and modifications. Although the results are still preliminary and further work is needed, they suggest that: (a) the waves of mRNA quantities shown in previous studies are indeed regulated at transcriptional level; (b) histone acetylation shows differences over the respiratory cycle and correlates with differentially expressed genes and (c) nucleosome positioning upstream of transcription start site (TSS) may be a dynamic, global property over the respiratory cycle.

## 2 Materials and Methods

### 2.1 Culture growth and sample preparation

The *S. cerevisiae* strain used was IFO0233 and was grown in continuous culture as described in [14]. A total of 40 samples were taken 4 minutes apart over approximatelly 3 respiratory cycles. Each sample of 1.5 ml yeast culture was crosslinked with 42$\mu$l of 37% formaldehyde and mixed 12 minutes. The reaction was stopped with 175$\mu$l glycine and mixed for another 12 minutes. Finally, the samples were spun down briefly using a desktop centrifuge, decanted and stored at -80C.

For ChIP experiments, the frozen pellets were thawed on ice, washed in FA-Lysis buffer, resuspended in 700$\mu$l of the same buffer and disrupted by bead-beating, using 0.5 mm diameter glass beads and beat for 5x30 seconds with 1 minute cooling breaks on ice. After removing the beads, 150 $\mu$l of the cell lysate was diluted in FA-Lysis buffer to a volume of 1.5 ml and sonicated for 15 min (cycles of 30 sec. on and 30 sec. off). Finally the sonicated samples were centrifuged twice and the final supernatant - the chromatin extract - was stored at -80C.

### 2.2 Chromatin Immunoprecipitation and qPCR

For each ChIP, we transfered 75$\mu$l of chromatin extract and 225$\mu$l FA-Lysis buffer to a non-stick microcentrifuge tube and incubated with 2$\mu$l of antibody for 3h to overnight at 4C on a rotary wheel. The IP reaction was centrifuged for 10 min at 4C and the supernatant was transfered to a Spin-X column containing 30$\mu$l of protein A agarose slurry (pre-incubated with 1 mg/ml BSA; Pierce). The samples were incubated for 2h at 4C on a rotary wheel and centrifuged at 3200 rpm for 2 minutes. The eluate was discarded and the column was washed sequentially (5 minutes each) with 700$\mu$l of FA-Lysis buffer, FA500, LiCl wash solution and TES. Finally, the column was transfered to new microcentrifuge tube and incubated at 37C for 30 minutes with agitation after adding 100$\mu$l of elution buffer. The sample was eluted by centrifugation at 9000 rpm for 2 min and treated with Proteinase K (20$\mu$g in 100$\mu$l of water; Roche) at 65C overnight. Finally, the sample was purified using the QIAquick PCR purification kit (Qiagen) following the manufacturer instructions. The resulting DNA samples were stored at -20C. For total chromatin control samples (inputs), 0.75$\mu$l of chromatin extract was mixed with 100$\mu$l of elution buffer, treated with Proteinase K and purified as described above. The antibodies used were: anti-PolII (Abcam, ab5408), anti-H3 (Abcam, ab1791) and anti-H3K9acetyl (Millipore 07-352).

For PCR reactions, 2$\mu$l of each DNA sample was amplified by real-time PCR using Absolute QPCR SYBR green reagents (Abgene). The primer sequences are given in table 1:

## 3 Results

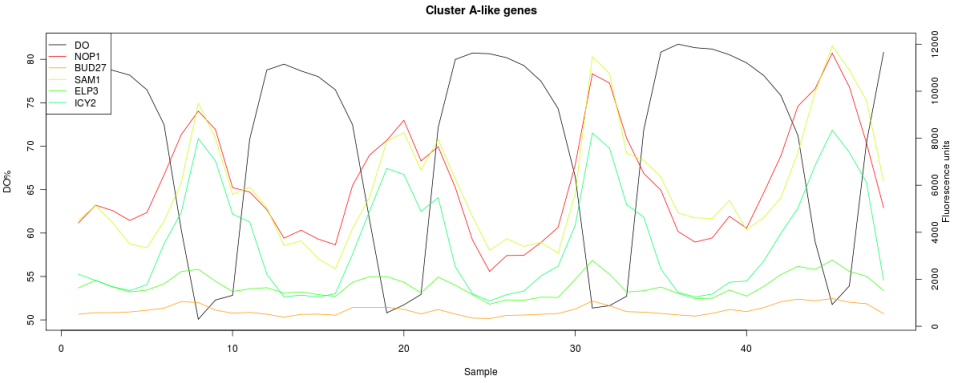
### 3.1 Polymerase II binding correlates with mRNA temporal profile

As a first experiment, we wanted to confirm that the oscillatory patterns in mRNA profiles from previous microarray time-series experiments ([13]) were regulated at a transcriptional level and not exclusively related to mRNA stability, degradation, etc. We therfore performed ChIP experiments against PolII on 14 successive

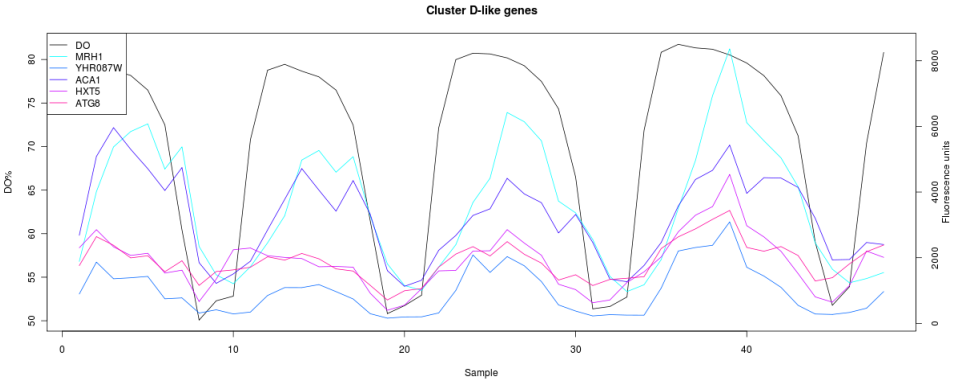
| Gene | Name | 5' Primer | 3' Primer |
|---|---|---|---|
| ACA1 | ACA1 | GCTGCAATGACCCCGTATG | TCGCCAGATGGGATAGAAGG |
| | ACA1P | CAATGCTCGCTTCTGCTGG | TGATACCACAAAAAGCTGCGC |
| | ACA1P2 | CGACTAAAAGCGCAGAAACTGTATT | TCGTTACCTGGTGTTATTCCAGTACT |
| MET2 | MET2P | CTTGTTCACGGATATTTCTTGCTTT | GAGAAACTTTAGACGGACCCTGTG |
| MRH1 | MRH1 | ACGTTTTGCAGCCAGACTCTG | CCGAAGTGGTTAGCAATTGGA |
| | MRH1P | TGTCACCATCTCGTCTCCCTT | CCGCACTAAGAACTAGCCGG |
| | MRH1P2 | AATAACTTTTTCTTTCTCTACATCCAATTTT | GGAAGATGATTAGATATTAAGTGAAATTAGGTATTA |
| NOP1 | NOP1 | TCAGATCTAAGTTGGCTGCCG | AGAAGTACCGGAAGCAGCACC |
| | NOP1P | GCCAAGAAGTTTTCCTTACATCG | GCAATGGGCAACTTAGATTTATCC |
| PUT1 | PUT1P2 | AAATCGCATGAACTAAGCCCA | GAGAAGAGCTGCTACGGACTTGT |
| SAM1 | SAM1 | CTGAATCCGTTGGTGAAGGTC | CGCAACTTTGGAGTGAGGGT |
| | SAM1P | GAAATGGCAATATCTCCCCCA | GACGCGCCCTCTAGAACAGA |
| | SAM1P2 | TTGGGACGTATATATCGACTGGTG | TTTTTAGGGTAAATTCCTGGTTTTTTACT |
| — | TEL | TAACAAGCGGCTGGACTACTTTC | GATAACTCTGAACTGTGCATCCACTC |

Table 1: Primers used in this study

timepoints (one respiratory cycle) and used the resulting DNA for qPCR with four primer sets corresponding to the coding regions of genes shown to oscillate in the microarray dataset. Two of these genes (NOP1 and SAM1) were shown to peak in the oxidative phase, while the other two (MRH1 and ACA1) were shown to peak at the end of the reductive phase (see fig. 3). For normalizing the results, we used a primer set corresponding to a telomeric region on chromosome VI that is considered not to be transcribed. The results (fig. 6) show a strong correlation to the microarray data: PolII is found bound on NOP1 and SAM1 at high levels during the oxidative phase, when the number of transcripts of these genes are at their highest. PolII binding to MRH1 and ACA1 occurs mostly at mid-reductive phase and shows broader peaks, relating very well with the mRNA data which shows the highest mRNA concentration increase for these genes in mid-reductive phase. Statistical analysis of these results show that the correlations between the genes in oxidative phase and reductive phase respectively are significant (fig. 5).




(a)




(b)

Figure 3: Microarray time-series experiment[13]: (a) shows the expression profile of several genes peaking in the oxidative phase, while (b) shows the expression of genes in reductive phase relative to dissolved oxygen traces.

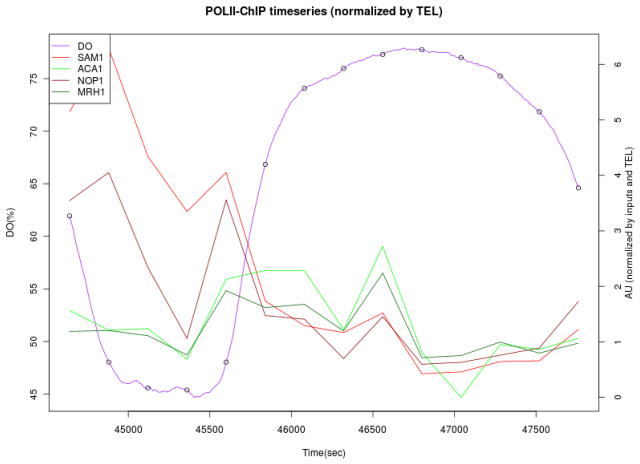



Figure 4: qPCR experiments on anti-PolII ChIP time-series. The values were normalized by total chromatin control and telomeric region qPCRs. Circles on the DO trace show sampling times.

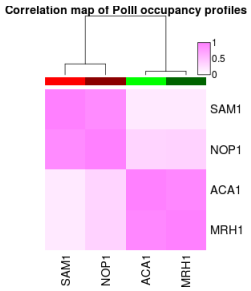



Figure 5: Anti-PolII ChIP correlation map. The values represent correlation coefficients; ACA1-MRH1 and SAM1-NOP1 correlations are statistically significant (p-values: 6.787e-07 and 7.558e-06 respectively)

## 3.2 Nucleosome remodeling and histone modification upstream of TSS.

We continued this study by looking for differences in histone modifications between the two groups of genes that were shown to be differentially transcribed. We therefore performed ChIP against an acetylated site on histone H3 (H3K9) and against histone H3. Since nucleosome-mediated transcriptional control is considered most proeminent on the promoter region, the qPCR experiments were done using primers around the -100 bp region relative to the transcriptional start site (TSS).

As a first result, histone H3 (and presumambly the whole nucleosome) closest to the transcription start site seems to be displaced during the oxidative phase at all promoters and then repositioned towards late reductive phase (fig. 6). There is no statistical difference between the two groups of genes, suggesting that nucleosome positioning upstream of TSS is not related to the differential expression of these groups of genes (fig. 7).

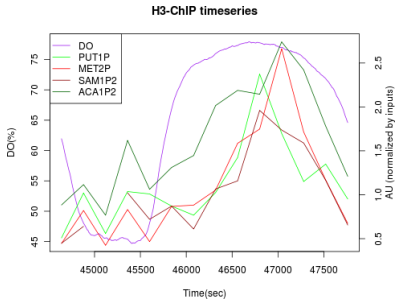



Figure 6: qPCR experiments on anti-H3 ChIP time-series. The values were normalized by total chromatin control. Circles on the DO trace show sampling times.

The anti-H3K9acetyl ChIP experiments also showed a strong correlation between different promoters. and, more interestingly, reveal acetylation "spikes" during the respiratory cycle (fig. 8). However, since we had no control with which to normalize the data, these results may simply be due to technical errors and require further confirmation. On the other hand, unlike anti-H3 ChIP results, this dataset shows a clear correlation with the

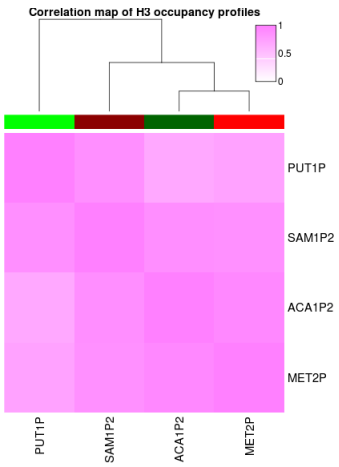



Figure 7: Anti-H3 ChIP correlation map

gene expression groups: the genes that are coexpressed also show higher similarity in H3K9 acetylation profiles, suggesting that acetylation is linked to diferentially expressed groups of genes (fig. 9).

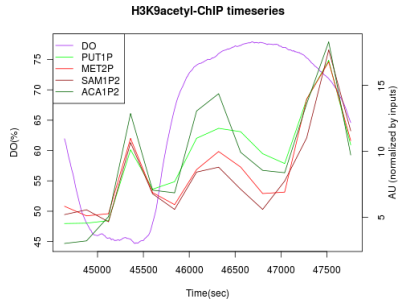



Figure 8: qPCR experiments on anti-H3K9acetyl ChIP time-series. The values were normalized by total chromatin control. Circles on the DO trace show sampling times.

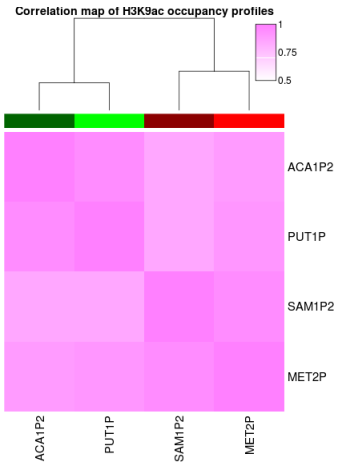



Figure 9: Anti-H3K9acetyl ChIP correlation map

# 4 Conclusion and future work

These results strongly suggest that the oscillation of mRNA levels and the differential mRNA patterns of different groups of genes previously observed are regulated at a transcriptional level. Moreover, nucleosome remodeling in the promoter region (as suggested by anti-H3 ChIP experiments) seems to be a global dynamic property that correlates with the respiratory cycle, possibly due to the ATP dependece of this process. Finally, histone acetylation correlates with gene transcription profiles and shows interesting peaks over the respiratory cycle which could be linked to the spikes in Acetyl-CoA levels.

As further work, we intend to

- repeat the PolII ChIP experiments over three respiratory cycles for confirmation;
- investigate the nucleosome remodeling at promoters by ChIP and Western Blot experiments against several histone antibodies;
- investigate the temporal profile of several histone modifications by ChIP and LC-MS/MS experiments.

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