35th Annual International Conference of the IEEE EMBS
Osaka, Japan, 3 - 7 July, 2013

The Dynamics of Cellular Energetics during Continuous yeast Culture.

Cornelia Amariei, Rainer Machné, Kalesh Sasidharan, Willi Gottstein, Masaru Tomita, Tomoyoshi Soga, David Lloyd and Douglas B. Murray

***Abstract*—A plethora of data is accumulating from high throughput methods on metabolites, coenzymes, proteins, and nucleic acids and their interactions as well as the signalling and regulatory functions and pathways of the cellular network. The frozen moment viewed in a single discrete time sample requires frequent repetition and updating before any appreciation of the dynamics of component interaction becomes possible. Even then in a sample derived from a cell population, time-averaging of processes and events that occur in out-of-phase individuals blur the detailed complexity of single cell organization. Continuously-grown cultures of yeast can become spontaneously self-synchronized, thereby enabling resolution of far more detailed temporal structure. Continuous on-line monitoring by rapidly responding sensors (O_2 electrode and membrane-inlet mass spectrometry for O_2, CO_2 and H_2S; direct fluorimetry for NAD(P)H and flavins) gives dynamic information from time-scales of minutes to hours. Supplemented with capillary electophoresis and gas chromatography mass spectrometry and transcriptomics the predominantly oscillatory behaviour of network components becomes evident, with a 40 min cycle between a phase of increased respiration (oxidative phase) and decreased respiration (reductive phase). Highly pervasive, this ultradian clock provides a coordinating function that links mitochondrial energetics and redox balance to transcriptional regulation, mitochondrial structure and organelle remodelling, DNA duplication and cell division events. Ultimately, this leads to a global partitioning of anabolism and catabolism and the enzymes involved, mediated by a relatively simple ATP feedback loop on chromatin architecture.**

I. Online Measurements

It has been known for almost 60 years that continuously-grown high-cell density budding yeast cultures tend to auto-synchronise their behaviour resulting in an oscillation in respiratory activity(Finn & Wilson, 1954; Satroutdinov, Kuriyama, & Kobayashi, 1992; von Meyenburg, 1968), which is most easily accessible by measuring the residual dissolved oxygen of the culture and can be maintained for months (Fig. 1). This dynamic state has periods ranging from 40 minutes to several hours and occurs despite temperature, pressure, pH, media flow rate and reaction volume remaining constant during the culture(Murray, Beckmann, & Kitano, 2007). This challenges the paradigm that continuous cultures will reach a chemically static (chemostat) state when grown under continuous constant conditions, where one substrate (for example carbon, nitrogen or phosphate) becomes limiting(Monod, 1950). Each cycle comprises phases of high (oxidative) and low (reductive) respiratory activity. The oxidative and reductive phases can be empirically defined by oxygen consumption rates(Fig. 1a). Moreover, relative NAD(P)H concentration (Fig. 1b) and flavin oxidation state (Fig. 1c) indicate a more reducing environment during the reductive phase. Carbon dioxide production rates (Fig. 1d), hydrogen sulphide production rates (Fig. 1e) and heat transfer rates (Fig. 1f) are all used to precisely define the physiological state of the culture at any given phase of the oscillation.

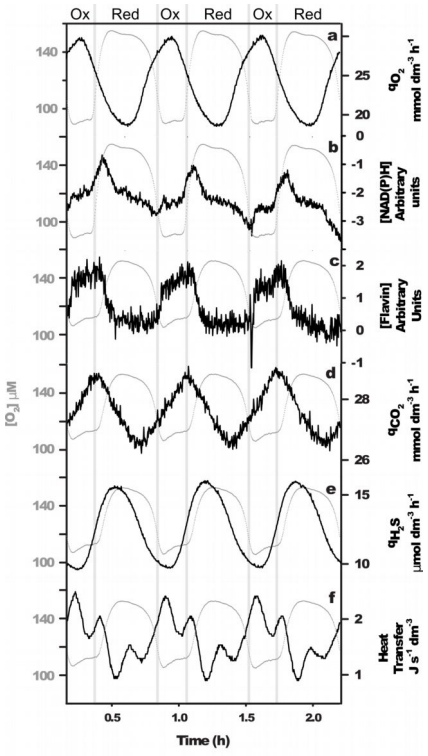

Figure 1. Continuous online measurements during the respiratory oscillation of *Saccharomyces cerevisiae*. The dry biomass was 8.1 g/L and the cell doubling time was 8.13 h. The thin dotted represents the residual dissolved oxygen concentration (measured by an electrode). Oxygen uptake rates were calculated from an off-gas sensor array (qO_2; a)(Murray et al., 2007). NAD(P)H (b) and flavin oxidation state was measured by *in-situ* flurorimetry(Murray, Engelen, Lloyd, & Kuriyama, 1999). Carbon dioxide excretion rates (qCO_2; d), Hydrogen sulphide production rates (qH2S; e) and heat transfer (f) were all calculated as previously described(Murray et al., 2007). The vertical gray lines represnt the transition between redox states (Ox- oxidative, Red – Reductive). Adapted from Murray, Haynes, & Tomita, 2011.

II. Omic Approaches

Beyond continuous measurements, respiratory oscillations have also been frequently sampled for the analysis of biological function, transcript abundance(Klevecz, Bolen, Forrest, & Murray, 2004; Li & Klevecz, 2006; Nikolai Slavov & Botstein, 2011; Tu, Kudlicki, Rowicka, & McKnight, 2005) and metabolite concentrations(Hans, Heinzle, & Wittmann, 2003; Murray et al., 2007; Sasidharan, Soga, Tomita, & Murray, 2012; Nikolai Slavov & Botstein,

*This work is part funded by Yamagata Prefecture, Tsuruoka City, Japan Society for the promotion of Science, Japan Science and Technology Agency and Vienna Science and Technology Fund

Cornelia Amariei, Kalesh Sasidharan, Masaru Tomita, Tomoyoshi Soga and Douglas B. Murray are with the Institute for Advanced Biosciences, Keio University, Tsuruoka, Japan. (dougie@ttck.keio.ac.jp)

Rainer Machné, is with Theoretical Biochemistry Institute, Vienna University, Austria.

David Lloyd is with the Biosciences Department, Cardiff University, Cardiff, UK.

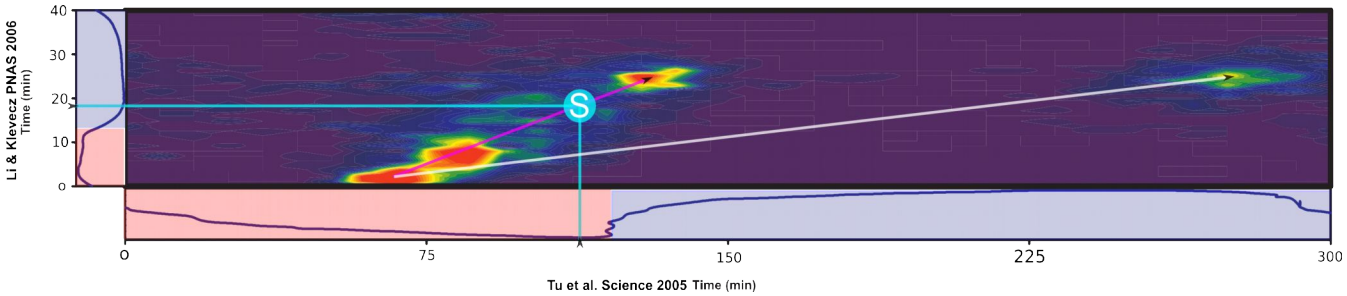

Figure 2. A phase-phase plot of two oscillation periods. The left and lower insets indicate the residual dissolved oxygen concentration in the culture for the 40 min (Li & Klevecz, 2006) and 300 min (Tu et al., 2005) oscillation, respectively. The residual dissolved oxygen for the 40 min cycle was from direct measurements and the 300 min oscillation cycle values were derived from digitized from the original paper(Tu et al., 2005). The blue area represents the reductive phase and the red area represents the oxidative phase, calculated from the minimum and maximum of the first derivative of the residual dissolved oxygen. S represents when S-phase is at a maximum during both periods. The heatmap behind shows the when transcript abundance for the 5200 yeast genes peak. This simple analysis already shows common clusters in the dataset (for a more comprehensive cluster analysis see (Machné & Murray, 2012)). The magenta line represents a biosynthetic or catabolic program common to both periods in time and the cyan line represents phase coherent clusters, i.e., have similar phase angles in both datasets.

2011; Tu et al., 2007). These studies lead to the startling conclusion that the vast majority of cellular physiology oscillates with specific phase relationships to respiratory state. Further computational analyses comparing oscillation periods of 40 and 300 min have highlighted two super-groups of transcripts that translate into anabolic (oxidative phase) and catabolic (reductive phase) processes(Machné & Murray, 2012). The metabolic outputs from these superclusters generally appeared antiphase to the transcript abundances.

Moreover, in both oscillation periods DNA replication was gated at a specific phase, leading to the hypothesis that the major function of the oscillator was to separate DNA synthesis from respiratory activity (measured by oxygen uptake rates), i.e., the reactive oxygen species (ROS) – DNA synthesis temporal partitioning hypothesis(Klevecz et al., 2004; Tu et al., 2005). This was the case for the 40 min oscillation (Fig. 2) as S-phase occurs when oxygen uptake rates are lower. However, when the maximum DNA replication phase was plotted for the 300 min oscillation it occurred during the peak of oxygen consumption. The confusion on what defines the reductive and oxidative phases may have lent support to the ROS-DNA synthesis hypothesis(Murray, 2006). It is therefore critical to define these phases according to measured oxygen uptake rates(N. Slavov, Macinskas, Caudy, & Botstein, 2011). It is interesting to note in Fig. 2 that the slope during the 300 minute oscillation of the "Biosynthetic or anabolic Program" (Murray et al., 2007) is 0.46, this may arise from the 2% glucose used in the 40 minute case and the 1% glucose in the 300 minute case. Additionally, S-phase appears to occur at the same point during this "program". Further evidence that supports a different scenario was provided by the observation that no measurable S-phase occurred during the respiratory oscillation at the end of batch growth (N. Slavov et al., 2011). It is apparent that DNA synthesis occurs during the same phase of the "biosynthetic program" (Fig. 2). Overall, these results suggest the cell division cycle is only coupled weakly with the respiratory oscillation and that the energy input (from glucose) can determine the phase relationships between the cell division cycle and the "biosynthetic program".

III. Cellular Energetics

Therefore, we tested ATP availability during the oscillation (Fig. 3), by calculating the ATP:ADP ratios. We observed an oscillation between 1-1.2 (reductive) to 4-5.5 (oxidative) each cycle (Fig. 3). The maximum ATP availability was observed during the oxidative phase, i.e., coinciding with maximum respiratory chain activity. In Fig. 2 the data already clusters into distinct groups, we further investigated this using a multidimensional model based clustering approach adapted from flow cytometric analysis(Lo, Hahne, Brinkman, & Gottardo, 2009). Initially, we could identify two superclusters (oxidative and reductive),

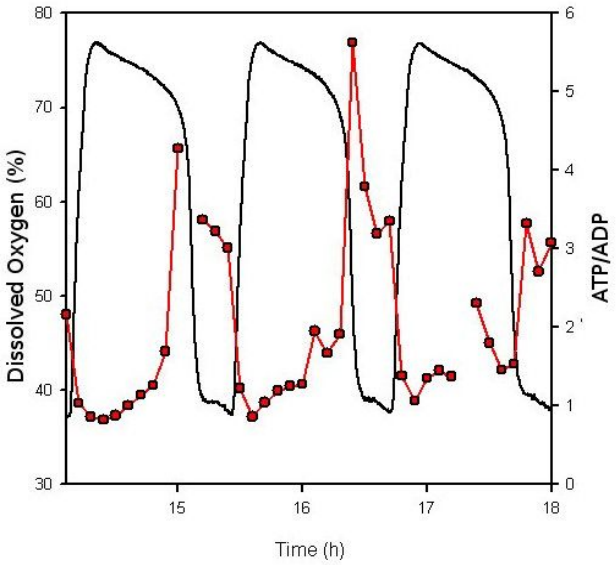

Figure 3. ATP/ADP ratios (■) during the respiratory oscillation. ATP and ADP were measured by anionic capillary electrophoresis mass spectrometry(Sasidharan et al., 2012; Soga et al., 2009). The continuous black line represents residual dissolved oxygen concentration.

that could further be clustered into five fine-grain clusters (A, AB and B in the oxidative phase, and C, and D in the reductive phase) that encompass ~34 % of the yeast genome (Machné & Murray, 2012). These clusters were then analysed for ontology enrichment to reveal a series of events from ribosomal assembly in cluster A and AB, anabolic metabolism in cluster AB and B, mitochondrial ribosomal assembly in cluster C and then catabolic metabolism and stress response in cluster D. We statistically compared the clusters to a compendia of high-throughput datasets that exist for yeast. In Figure 4, for example, we see that from a collection of 1327 microarray hybridisations(McCord, Berger, Philippakis, & Bulyk, 2007) performed in many independent experiments that encompass knockout, time series perturbations we can reveal that these experiments generally showed oxidative or reductive phase responses. The ATP data and the cross-correlation between these expression

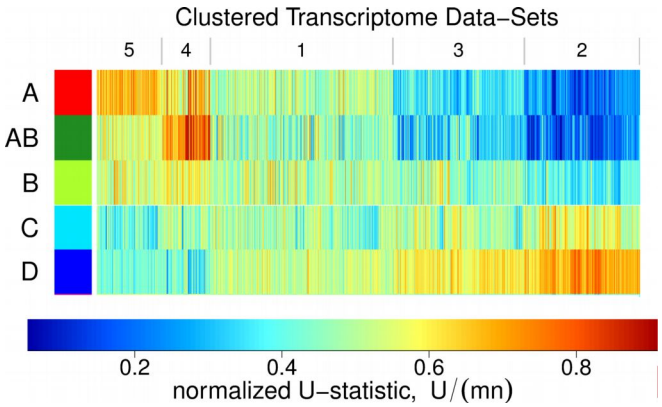

Figure 4. A comparison of the expression clusters that form during the respiratory oscillation with a compendia of 1327 microarray hybridisations performed in yeast(McCord et al., 2007) The hue represents the normalised rank sum where yellow to red indicates an upregulation and cyan to blue represents a downregulation of the any target hybridisation compared to the cluster. The column numbers indicate clusters of hybridisations derived using the SOTA algorithm and plotted in decreasing order (from left to right) using the oxidative phase cluster A as a reference (Machné & Murray, 2012)

clusters, when taken together, imply a general regulatory mechanism which underlies such global and pervasive expression dynamics. Correlation between ATP/ADP ratios and mitochondrial state 3 respiratory rates (Chance & Williams, 1955) has been experimentally demonstrated *in vivo* in synchronous cultures(Lloyd, Poole, & Edwards,

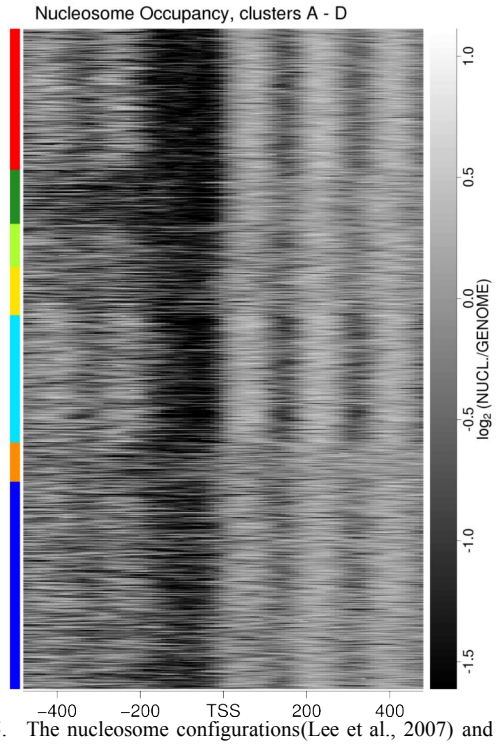

Figure 5. The nucleosome configurations(Lee et al., 2007) and the gene expression clusters that form during the respiratory oscillation. White and black shows where nucleosomes are present or absent respectively. The vertical coloured bar corresponds to the cluster identifier (Fig. 4). The y axis represents distance from the transcriptional start site (TSS) .Yellow and orange represent differential clusters between the 40 minute and 300 min cycles(Machné & Murray, 2012).

1982), and this phenomenon of intracellular respiratory control correlated with the cycles of respiratory oscillation confirmed in yeast(Lloyd, Salgado, Turner, Suller, & Murray, 2002).

We hypothesised that this mechanism must involve a fundamental signature in promoter architecture of the target genes. Therefore, we cross-correlated nucleosome configurations with the expression clusters genes(Lee et al., 2007; Machné & Murray, 2012) (Fig. 5). Nucleosome architecture showed distinctive patterns for each of the cluster genes. Cluster A and C genes show coherent nucleosomes with a large nucleosome free region. Cluster AB genes were depleted up stream and these genes, encoding for ribosomal proteins, were the most highly expressed genes in yeast. Cluster D genes did not have clearly defined nucleosomes upstream of the transcriptional start site. This configuration is termed as fuzzy(Lee et al., 2007).

These data point to a sequential activation of gene expression during the respiratory oscillation that is coupled to cellular energy state. Chromatin remodelling in yeast is mediated by at least two complexes, RSC which is involved in maintaining nucleosome-free promoters for efficient transcription(Fischer, Saha, & Cairns, 2007) and the ISWI complex Isw2 which shifts nucleosomes over promoter regions to inhibit transcription(Whitehouse, Rando, Delrow, & Tsukiyama, 2007) and appears to influence genes in cluster D mainly(Machné & Murray, 2012) . The activity of both these complexes are ATP dependent therefore we propose a dual negative feedback loop that shapes gene expression to the energetic landscape (Fig. 6). This involves ATP activation of the RSC complex resulting in the expression of genes with well defined or depleted nucleosome free regions (cluster A, AB and C) while simultaneously ATP represses the expression of cluster D genes. Decreased intracellular ATP results in reversal of all these processes.

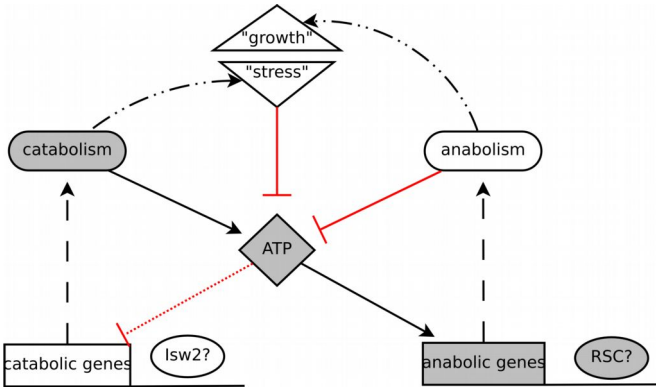

Figure 6. A simple dual negative feedback model the direct influence of ATP on gene expression.

Recently, the respiratory oscillation has been correlated with the growth and the general stress responses of yeast (Machné & Murray, 2012; Nikolai Slavov & Botstein, 2011). Our current simple model has the potential to describe both of these phenomena under the umbrella of global ATP availability and chromatin remodelling, and would require only minimal regulation framework to switch cellular state, i.e., cells would express growth or stress expression profiles based on cellular energetics and its feedback on the chromatin remodelling complexes.

References

Chance, B., & Williams, G. R. (1955). Respiratory enzymes in oxidative phosphorylation. III. The steady state. *The Journal of biological chemistry*, *217*(1), 409–27.

Finn, R. K., & Wilson, R. E. (1954). Fermentation Process Control, Population Dynamics of a Continuous Propagator for Microorganisms. *J Agri Food Chem*, *2*(2), 66–69.

Fischer, C. J., Saha, A., & Cairns, B. R. (2007). Kinetic model for the ATP-dependent translocation of *Saccharomyces cerevisiae* RSC along double-stranded DNA. *Biochemistry*, *46*(43), 12416–26.

Hans, M. A., Heinzle, E., & Wittmann, C. (2003). Free intracellular amino acid pools during autonomous oscillations in Saccharomyces cerevisiae. *Biotechnology and bioengineering*, *82*(2), 143–51.

Klevecz, R. R., Bolen, J., Forrest, G., & Murray, D. B. (2004). A genomewide oscillation in transcription gates DNA replication and cell cycle. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(5), 1200–5.

Lee, W., Tillo, D., Bray, N., Morse, R. H., Davis, R. W., Hughes, T. R., & Nislow, C. (2007). A high-resolution atlas of nucleosome occupancy in yeast. *Nature genetics*, *39*(10), 1235–44.

Li, C. M., & Klevecz, R. R. (2006). A rapid genome-scale response of the transcriptional oscillator to perturbation reveals a period-doubling path to phenotypic change. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(44), 16254–9.

Lloyd, D., Poole, R. K., & Edwards, S. W. (1982). *The cell division cycle: temporal organization control of cellular growth and reproduction*. London: Academic Press.

Lloyd, D., Salgado, L. E. J., Turner, M. P., Suller, M. T. E., & Murray, D. B. (2002). Cycles of mitochondrial energization driven by the ultradian clock in a continuous culture of *Saccharomyces cerevisiae*. *Microbiology (Reading, England)*, *148*(Pt 11), 3715–24.

Lo, K., Hahne, F., Brinkman, R. R., & Gottardo, R. (2009). flowClust: a Bioconductor package for automated gating of flow cytometry data. *BMC bioinformatics*, *10*, 145.

Machné, R., & Murray, D. B. (2012). The Yin and Yang of Yeast Transcription: Elements of a Global Feedback System between Metabolism and Chromatin. (V. Saks, Ed.)*PLoS ONE*, *7*(6), e37906.

McCord, R. P., Berger, M. F., Philippakis, A. A., & Bulyk, M. L. (2007). Inferring condition-specific transcription factor function from DNA binding and gene expression data. *Molecular systems biology*, *3*,

Monod, J. (1950). The technique of continuous culture. *Ann. Inst. Pasteur*, *79*, 390–410.

Murray, D. B. (2006). The respiratory oscillation in yeast phase definitions and periodicity. *Nature reviews. Molecular cell biology*, *7*(12).

Murray, D. B., Beckmann, M., & Kitano, H. (2007). Regulation of yeast oscillatory dynamics. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(7), 2241–6.

Murray, D. B., Engelen, F. A., Lloyd, D., & Kuriyama, H. (1999). Involvement of glutathione in the regulation of respiratory oscillation during a continuous culture of *Saccharomyces cerevisiae*. *Microbiology*, *145*, 2739–2745.

Murray, D. B., Haynes, K., & Tomita, M. (2011). Redox regulation in respiring *Saccharomyces cerevisiae*. *Biochimica et biophysica acta*, *1810*(10), 945–58.

Sasidharan, K., Soga, T., Tomita, M., & Murray, D. B. (2012). A yeast metabolite extraction protocol optimised for time-series analyses. (M. F. Tuite, Ed.)*PloS one*, *7*(8), e44283.

Satroutdinov, A. D., Kuriyama, H., & Kobayashi, H. (1992). Oscillatory metabolism of *Saccharomyces cerevisiae* in continuous culture. *FEMS microbiology letters*, *77*(1-3), 261–7. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1334018

Slavov, N., Macinskas, J., Caudy, A., & Botstein, D. (2011). Metabolic cycling without cell division cycling in respiring yeast. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(47), 19090–19095.

Slavov, Nikolai, & Botstein, D. (2011). Coupling among growth rate response, metabolic cycle, and cell division cycle in yeast. *Molecular biology of the cell*, *22*(12), 1997–2009.

Soga, T., Igarashi, K., Ito, C., Mizobuchi, K., Zimmermann, H.-P., & Tomita, M. (2009). Metabolomic profiling of anionic metabolites by capillary electrophoresis mass spectrometry. *Analytical chemistry*, *81*(15), 6165–74.

Tu, B. P., Kudlicki, A., Rowicka, M., & McKnight, S. L. (2005). Logic of the yeast metabolic cycle: temporal compartmentalization of cellular processes. *Science (New York, N.Y.)*, *310*(5751), 1152–8.

Tu, B. P., Mohler, R. E., Liu, J. C., Dombek, K. M., Young, E. T., Synovec, R. E., & McKnight, S. L. (2007). Cyclic changes in metabolic state during the life of a yeast cell. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(43), 16886–91.

Von Meyenburg, H. K. (1968). The budding cycle of *Saccharomyces cerevisiae*. *Pathol. Microbiol.*, *31*(2), 117–127.

Whitehouse, I., Rando, O. J., Delrow, J., & Tsukiyama, T. (2007). Chromatin remodelling at promoters suppresses antisense transcription. *Nature*, *450*(7172), 1031–5.