BIOINFORMATICS ORIGINAL PAPER

Vol. 27 no. 2 2011, pages 147–152
doi:10.1093/bioinformatics/btq637

Genome analysis

Advance Access publication November 11, 2010

Differential effects of chromatin regulators and transcription factors on gene regulation: a nucleosomal perspective

Dong Dong[1], Xiaojian Shao[1,2] and Zhaolei Zhang[1,3,4,*]

[1]Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, 160 College Street, Toronto, ON, M5S 3E1, Canada, [2]College of Science, China Agricultural University, Beijing 100083, China, [3]Department of Molecular Genetics, University of Toronto, 1 King's College Circle, Toronto, ON, M5S 1A8, Canada and [4]Banting and Best Department of Medical Research, University of Toronto, 112 College Street, Toronto, ON, M5G 1L6, Canada

Associate Editor: John Quackenbush

ABSTRACT

Motivation: Chromatin regulators (CR) and transcription factors (TF) are important *trans*-acting factors regulating transcription process, and many efforts have been devoted to understand their underlying mechanisms in gene regulation. However, the influences of CR and TF regulation effects on nucleosomes during transcription are still minimally understood, and it remains to be determined the extent to which CR and TF regulatory effect shape the organization of nucleosomes in the genome. In this article we attempted to address this problem and examine the patterns of CR and TF regulation effects from the nucleosome perspective.

Results: Our results show that the CR and TF regulatory effects exhibit different paradigms of transcriptional control in *Saccharomyces cerevisiae*. We grouped yeast genes into two categories, 'CR-sensitive' genes and 'TF-sensitive' genes, based on how their expression profiles change upon deletion of CRs or TFs. We found that genes in these two groups have very different patterns of nucleosome organization. The promoters of CR-sensitive genes tend to have higher nucleosome occupancy, whereas the promoters of TF-sensitive genes are depleted of nucleosomes. Furthermore, the nucleosome profiles of CR-sensitive genes tend to show more dynamic characteristics than TF-sensitive genes. These results reveal that the nucleosome organizations of yeast genes have a strong impact on their mode of regulation, and there are differential regulation effects on nucleosomes between CRs and TFs.

Availability: http://www.utoronto.ca/zhanglab/papers/bioinfo_2010/

Contact: zhaolei.zhang@utoronto.ca

Supplementary information: Supplementary data are available at *Bioinformatics* online.

Received on August 14, 2010; revised on November 3, 2010; accepted on November 5, 2010

1 INTRODUCTION

Transcriptional regulation in eukaryotic cells is a very important and complicated process. In the model organism *Saccharomyces cerevisiae*, this process involves a precise orchestration of complex molecular events, and is mediated by a series of regulators to create transcription factories, such as transcription factors (TFs), chromatin regulators (CRs) and the general transcription machinery with related regulators (such as RNA polymerase molecules) (Berger, 2000; Li *et al*., 2007; Orphanides and Reinberg, 2002; Pugh, 2000). Many studies have attempted to investigate the mechanisms of TFs and CRs and how they work together to regulate the gene transcription process (Buck and Lieb, 2006; Field *et al.*, 2008; Hartley and Madhani, 2009; Kim and O'Shea, 2008; Yarragudi *et al*., 2004). In yeast, the activation of TFs is usually affected by environmental stimuli through signaling pathways, which further determines the level of mRNA abundance (Harbison *et al*., 2004). One common strategy is to use regulatory feedback loops, in which the TFs are embedded in the feedback loops and tune gene expression with the corresponding needs (Amit *et al*., 2007; Segal *et al*., 2003). CRs and TFs are often coupled together and jointly regulate the transcriptional state of specific genes (Li *et al*., 2007). Some of the CRs can utilize the energy of ATP hydrolysis to affect the interaction of histone and DNA as a result of nucleosome delocalization, such as histone sliding, eviction or replacement (Li *et al*., 2007; Rando and Chang, 2009). This in turn affects the accessibility of TFs to their binding targets, leading to transcriptional activation or repression.

Gene transcription in the cell need to be tightly regulated, not only to optimize the expression level at different physiological conditions, but also to provide the cells the capacity to quickly adjust genes' expression level in response to external signals and perturbations. It has been recognized that, in addition to regulation by TFs, CRs also play important roles in regulating gene expression. The arrangements of nucleosomes along the DNA sequences are pivotal mechanism influencing gene regulation (Choi and Kim, 2009; Field *et al.*, 2009; Lam *et al*., 2008; Lee *et al*., 2006). A number of recent genome-wide *in vivo* and *in vitro* experiments have revealed some general principles that govern nucleosome organization (Kaplan *et al*., 2009; Lee *et al*., 2007). Although it was considered that DNA sequence itself largely determines the mode of binding by nucleosomes, this concept is still under debate (Stein *et al.*, 2009; Zhang *et al*., 2009). For example, it has been reported that the nucleosome positioning can be the consequence of the regulatory activities of *trans*-acting factors (Badis *et al*., 2008; Buck and Lieb, 2006; Hartley and Madhani, 2009; Kim and O'Shea, 2008; Lam *et al*., 2008; Li *et al*., 2007). Among these *trans*- acting factors, TFs can compete with nucleosomes for accessing to the DNA sequences (Komili and Silver, 2008), and CRs can help to conduct chromatin assembly and organization, leading to nucleosome arrays of uniform spacing (such as ISWI family) (Ito *et al.*, 1997; Langst *et al*.,

*To whom correspondence should be addressed.

1999). For example, Whitehouse and Tsukiyama (2006) showed that ATPase Isw2 acts to position nucleosomes over less favorable sequence elements at the POT1 promoter. About 35% of Isw2 regulated genes (∼400 genes) were subject to detectable chromatin remodeling compared with wild-type cells, and the transcription of these genes can be initiated after Isw2 is deleted (Whitehouse *et al*., 2007).

In this study, we analyzed the different effects of CR and TF regulations on nucleosomes, e.g. nucleosome positioning, packing, dynamics and histone activities. We also described how nucleosomal effects can have an impact on gene expression. Our results indicated that TFs and CRs might have distinct strategies on the gene regulation by influencing nucleosome organization at the promoter region.

2 MATERIALS AND METHODS

2.1 Gene expression data

In this study, transcription plasticity, mRNA abundance and transcription frequency were regarded as effects of transcriptional regulation. Transcription plasticity data were obtained from Tirosh and Barkai (2008), which was calculated as expression variation among ∼1500 transcription profiles. mRNA abundance and transcription rate were retrieved from Holstege *et al.* (1998).

To study the CR and TF regulatory effects on transcriptional regulation, we compiled two previously assembled expression compendiums in *S.cerevisiae*, in which various CR and TF genes were either deleted or mutated (Hu *et al.*, 2007; Steinfeld *et al.*, 2007). (i) The CR effect (CRE): we removed the expression profiles under histone perturbations, such as the deletion of histone tails (H3 and H4), histone methyltransferase, acetyltransferase, deacetyltransferase, etc., and only the deletions of CR proteins were chosen. The final refined CR dataset contains only104 expression profiles for CR gene perturbation. (ii) The TF regulation effect (TRE): expression compendium with individual deletions of 269 TFs was compiled from Hu *et al.* (2007). We further checked the percentage of missing expression values for each gene, and genes with >10% missing expression values were excluded in the final refined data. Following previously described procedures (Choi and Kim, 2008), we normalized the refined expression data under each perturbation condition, and then calculated CR and TF regulation effects as the average of absolute values of logarithm of the expression changes across these *trans*-acting factors perturbations, respectively.

To detect the influence of CR and TF regulation effects on nucleosomes, we determined two cohorts of genes that are sensitive to the perturbations of CRs and TFs (the top 20% genes by ranking CRE and TRE, respectively) based on the assumption that significantly differentially expressed genes tend to be the targets of CRs and TFs. To more specifically reveal the distinct regulatory mechanism between CR and TF, genes categorized into both cohorts were not included in any group. These remaining genes are referred to as 'CR-sensitive genes' and 'TF-sensitive genes', respectively.

2.2 Nucleosome data

2.2.1 Nucleosome occupancy High-throughput nucleosome occupancy data measured *in vivo* and *in vitro* were retrieved from Kaplan *et al.* (2009); average nucleosome occupancy at the promoter region [500 bp upstream to 100 bp downstream of the transcription start site (TSS)] was calculated. To compare the differences in nucleosome occupancy between genes, we redefined the nucleosome occupancy over the promoter region as the lowest average nucleosome occupancy across any 100 bp window in the 200 bp region upstream of the TSS. This metric can best reflect the 'open' and 'closed' state of the nucleosome positioning in the promoter regions (Supplementary Table S4).

2.2.2 Nucleosome dynamics We compared several published nucleosome occupancy datasets to reveal the nucleosome dynamic characteristics (Supplementary Table S1). Here, we mainly focused on the '−1 nucleosome' (the first one upstream the TSS) and '+1 nucleosome' (the first one downstream the TSS) of each gene. (i) Nucleosome occupancy in the normal and heat-shock conditions were obtained from Shivawamy *et al.* (2008). They measured the nucleosome occupancy data of yeast S288c strain using deep-sequencing method. To compare the nucleosome occupancy patterns in the normal and heat-shock conditions, we calculated the percentage of nucleosomes in the promoter region that had dramatic changes (>45 bp) before and after heat shock; (ii) With more and more nucleosome occupancy data becoming available, it offers new opportunity to study nucleosome dynamics. Two recently published deep-sequencing-based nucleosome occupancy data were taken from Mavrich *et al.* (2008) and Jiang and Pugh (2009), respectively. We chose these data because they were performed on the same yeast strains (BY4741) and under normal conditions in the same laboratory, and mapped the observed discrepancies of nucleosome occupancy between two datasets. Then, we calculated the fraction of inconsistent nucleosomes (changes >30 bp); (iii) Kaplan *et al.* (2009) measured *in vivo* nucleosome organization from the cells grown in different growth medium (in YPD, galactose and ethanol). We calculated the differences in nucleosome occupancy signals in the promoter region (500 bp upstream to 100 bp downstream of TSS) using analysis of variance (ANOVA) method, and genes with significant nucleosome packing differences ($P < 0.001$) were selected.

2.2.3 Nucleosome fuzziness (i) Lee *et al.* (2007) determined a genome-wide nucleosome map and defined nucleosome occupancy as three states: unoccupied linker region, fuzzily positioned nucleosomes and well-positioned nucleosomes. We used the defined fuzzily positioned nucleosomes in this study, and calculated the fraction of genes with fuzziness region >50 bp. (ii) Genome-wide nucleosome fuzziness data were taken from Mavrich *et al.* (2008), which measured the nucleosome phasing and quantified nucleosome fuzziness by counting the standard deviation of tag distances from consensus position. To examine the nucleosome fuzziness relative to the TSSs, the data were plotted as a moving average of 500 nucleosomes along the focal genes (500 bp upstream to 1000 bp downstream to TSS).

2.3 Epigenetic marks

H2A.Z data were obtained from Albert *et al.* (2007). We restricted the 10% highest H2A.Z occupancy as H2A.Z containing, and calculated the percentage of genes containing H2A.Z in the promoter region. Histone H3 turnover rates were taken from Dion *et al.* (2007).

2.4 TF binding sites and TATA box containing genes

TF binding sites were obtained from MacIsaac *et al.* (2006), which re-analyzed the previously published genome-wide ChIP-chip data (Harbison *et al*., 2004). We only selected those binding sites that have a *P*-value of 0.001 or better and are conserved in two related yeast species. TATA box containing genes were taken from Basehoar *et al.* (2004).

2.5 Partial correlation

In this work, a non-parametric Spearman's rank correlation with corresponding statistical test was used since it has no prior assumption on data distribution. It was always used to uncover linear association between two ranked variables. To perform the joint variable analysis, non-parametric Spearman's partial correlation and the corresponding significance test were used to detect which measurements are the most influential features. Partial correlation can measure the degree of association between two variables with the effect of controlling variables removed. For example, when examining (Yuan *et al*., 2006) which of the two measurements y or z has stronger correlation with x, we can compare the values of $\rho_{x,y|z}$ and $\rho_{x,y|z}$. The bigger

one means that it has stronger association with x. $\rho_{x,y|z}$ indicates the partial correlation between x and y given a set of n controlling variables z. It is defined as:

$$\rho_{x,y|z} = \frac{\rho_{x,y} - \rho_{x,z}\rho_{y,z}}{\sqrt{(1-\rho_{x,z}^2)(1-\rho_{y,z}^2)}}$$

where $\rho_{x,y}$ is the correlation between x and y.

3 RESULTS

3.1 CR and TF have different regulatory effects on gene transcription

As described in a previous work (Choi and Kim, 2009), CRE and TRE were calculated from two expression compendiums, in which individual CRs and TFs were perturbed and genome-wide expression changes were quantified, respectively. To determine how CRE and TRE influence transcriptional regulation, we collected data for transcriptional plasticity (fluctuation in gene expression under various conditions) (Ihmels *et al*., 2002; Tirosh and Barkai, 2008), mRNA abundance and transcription frequency (Holstege *et al.*, 1998) and regarded them as proxies of different aspects of transcription. Table 1 shows that both CRE and TRE are significantly associated with transcriptional plasticity, whereas CRE has higher correlation with transcriptional plasticity than TRE ($\rho = 0.68$, P-value ≈ 0 for CRE, and $\rho = 0.28$, P-value <1e-20 for TRE, respectively). It is in agreement with previous findings that CRs have greater effects on gene expression variation than TFs (Choi and Kim, 2009), and suggested that chromatin regulation plays a pivotal role in expression fluctuations caused by changes in chromatin organization.

It is possible that, even though this observation is very significant, it is only confined to a few gene categories such as TATA box containing genes (Basehoar *et al*., 2004). To control for potential biases, partial correlation coefficients were calculated to measure the association strength between transcriptional plasticity and CRE/TRE after controlling TATA box containing genes, respectively. The result showed that $\rho_{\text{transcriptional plasticity, CRE | TATA box}} = 0.61$ ($P \approx 0$) and $\rho_{\text{transcriptional plasticity, TRE | TATA box}} = 0.24$ ($P <$ 1e-20). The partial correlation coefficients when TATA box is controlled are similar to the corresponding correlations without controlling TATA box, which indicates that the results are independent of TATA box presences. When comparing the influences of CRE and TRE on mRNA abundance and transcription frequency, we found that TRE is more significantly correlated with mRNA abundance ($\rho = 0.05$, $P = 0.18$ for CRE, and $\rho = 0.27$, $P =$ 1e-14 for TRE, respectively) and transcription frequency ($\rho = 0.06$, $P = 0.13$ for CRE, and $\rho = 0.25$, $P =$ 1e-13 for TRE, respectively) than that of CRE (Table 1), indicating that transcript levels are largely determined by the binding of TFs to DNA target.

Table 1. Comparison of the influences of CR and TF regulation effect on transcription plasticity, mRNA abundance and transcription frequency in *S.cerevisiae*

	Transcription plasticity	mRNA abundance	Transcription rate
CRE			
TRE uncontrolled	0.68 (<1e-20)	0.05 (0.18)	0.06 (0.13)
TRE controlled	0.61 (<1e-20)	−0.01 (0.88)	−0.01 (0.86)
TRE			
CRE uncontrolled	0.28 (<1e-20)	0.27 (1e-14)	0.25 (1e-13)
CRE controlled	0.24 (<1e-20)	0.26 (1e-14)	0.23 (1e-13)

Note: when TRE and CRE were uncontrolled, Spearman's correlation coefficients were shown; when CRE or TRE was controlled, partial correlation coefficients were shown; the numbers in parentheses are P-values measured based on the null hypothesis that there is no significant relationships.

We next asked whether CRE and TRE can collectively explain the transcription regulation program in yeast. We carried out the joint effect analysis of CRE and TRE on transcription regulation, i.e. we calculated the partial correlations by controlling CRE or TRE, respectively. The result clearly showed that CRE plays more important roles in influencing transcription plasticity, whereas TRE is more dominant in controlling mRNA abundance and transcription frequency (Table 1). These results further implicated that TF and CR have distinct roles in transcriptional regulation. CRs are more responsible to expression changes by regulating chromatin organization, and have more dynamic characteristics, whereas TFs are responsible for initiating gene transcription and properly regulating mRNA synthesis.

3.2 CR and TF regulatory effects on nucleosome organization

We next asked whether these two classes of regulators also have different effect on nucleosome organization. The rationale for this study is motivated by the fact that the nucleosome structure can have a profound impact on gene expression (Lee *et al*., 2007; Tirosh and Barkai, 2008). To detect the different influences of CRE and TRE on the nucleosome organization, we determined two cohorts of genes that are sensitive to the perturbations of CRs and TFs, respectively (see Section 2, Supplementary Table S2). We next examined nucleosome organizations in the promoter region for the genes in these two groups, as recent studies have shown that nucleosome positioning plays an important role in gene expression activation (Lee *et al*., 2007; Tirosh and Barkai, 2008). After plotting the average nucleosome occupancy measured by experimental method *in vivo* (Kaplan *et al*., 2009), we observed a clear open state (nucleosome depleted) for TF-sensitive genes and a closed state (nucleosome occupied) for CR-sensitive genes, respectively (Fig. 1A). To quantify such difference in nucleosome occupancy, we devised a metric, the Lowest Average Nucleosome Occupancy (termed as 'LANO'), which was calculated over 100 bp sliding windows in the 200 bp region upstream of the transcriptional start site. As shown in Figure 1A, indeed we found the TF-sensitive genes have stronger nucleosome depletion (lower LANO value) in the promoter region than CR-sensitive genes (Wilcoxon rank sum test, $P =$ 4.1e-3). As genes in both cohorts have relatively higher level of transcription plasticity, our result seems to contradict with previous findings that genes with higher level of nucleosome occupancy tend to have dramatic transcriptional plasticity (Lee *et al.*, 2007; Tirosh and Barkai, 2008). With the notion that genes with open nucleosome organization at the proximal region of TSS are constitutively expressed, we dissected TF-sensitive genes into highly expressed and lowly expressed groups by ranking their mRNA abundances. By comparing the 'LANO' scores between these two groups of genes, we found that there was no significant difference between them (Wilcoxon rank sum test, $P = 0.59$). It indicated that nucleosome depletion in promoters cannot guarantee higher

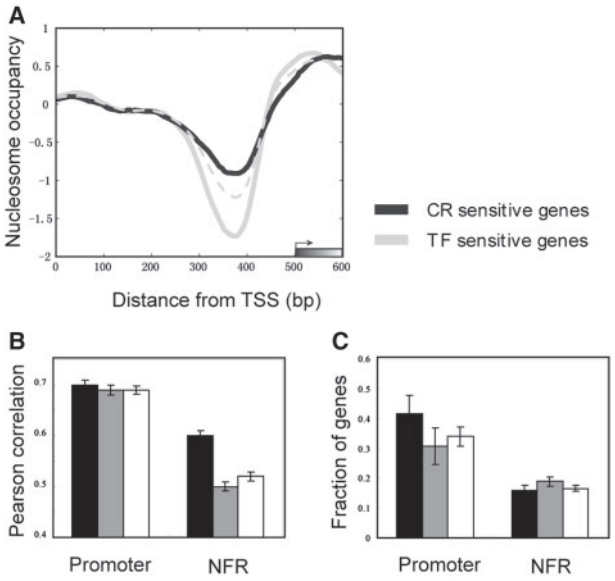

Fig. 1. Different strategies of CR and TF regulation effect on nucleosome organization. (**A**) Nucleosome occupancy of the promoter regions (500 bp upstream, ∼100 bp downstream relative to TSS) in TF- and CR-sensitive genes. The promoter regions of TF-sensitive genes show a nucleosome-free region, whereas the promoter regions of CR-sensitive genes reflect nucleosome occupied organization. The dash curve represents the nucleosome occupancy of all yeast genes. (**B**) Pearson's correlation of promoter regions and nucleosome-free regions (NFR, 200 bp upstream, ∼50 bp upstream relative to TSS) of nucleosome occupancy measured *in vivo* and *in vitro*. (**C**) TF binding sites of CR- and TF-sensitive genes under the promoter regions and nucleosome-free regions. The white bars represent the results for all yeast genes, and the black and gray bars represent the results for CR and TFsensitive genes, respectively.

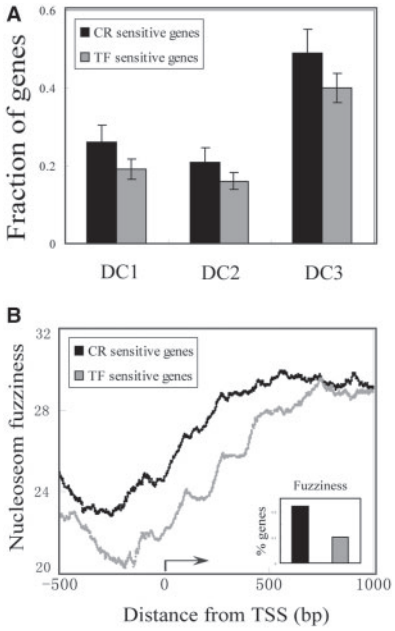

Fig. 2. Dynamic characteristics (DC) of nucleosome organization. (**A**) DC of nucleosome organization in the promoter regions. They were measured by nucleosome positioning before and after heat shock (DC1); nucleosome positioning between different cross-platform datasets (DC2) and nucleosome occupancy among cells grown at different conditions (DC3), respectively. (**B**) Nucleosome fuzziness relative to TSS. Fuzziness is reported as the standard deviation of nucleosome locations for each individual reference nucleosome. The distribution of nucleosome fuzziness is plotted by binning nucleosomes together moving along the genes. The inset figure shows the fraction of genes with linker region longer than 50 bp in the promoter region (500 bp upstream to 1000 bp downstream to TSS). All error bars were calculated by 1000 bootstrapping.

expression level of genes, and transcription plasticity does not mean higher level of nucleosome occupancy.

Until now, the concept of nucleosome positions being encoded by DNA sequences is still under debate. we were interested to examine the role of DNA sequence in determining nucleosome positioning for CR- and TF-sensitive genes. In this work, we compared the relationship between *in vitro* and *in vivo* maps of nucleosome organization at promoters, and found no difference between TF- and CR-sensitive genes ($\rho = 0.70$ and 0.69, respectively). Intriguingly, significant difference was found at the nucleosome depleted regions (Fig. 1B, $\rho = 0.6$ and 0.5, Wilcoxon rank sum test, P-value ≈ 0). TF-sensitive genes have a relative weaker effect on the positioning of nucleosomes than CR-sensitive genes, which indicated that TFs dramatically influence the resulting nucleosome organization *in vivo*. We next tested whether this difference is caused by the competition between TFs and nucleosomes to access DNA as we examined the difference of TF binding sites in promoters between these two cohorts of genes. We found TF binding sites are more highly enriched in the promoters of CR-sensitive genes than TF-sensitive gene promoters. Interestingly, when focusing on the nucleosome-free region, TF-sensitive genes have significantly more TF binding sites than CR-sensitive genes (Fig. 1C). This result is consistent with previous findings that TFs can influence chromatin organization by competing with nucleosomes for accessing to DNA (Komili and Silver, 2008; Segal and Widom, 2009).

3.3 Dynamic characteristics of nucleosome organization between CR- and TF-sensitive genes

In order to fully describe the extents and patterns of CRs and TFs in driving dynamic characteristics of nucleosome positioning, we next show it from different lines of evidences. (i) We mapped and compared the locations of nucleosomes measured under the normal and heat-shock conditions (Shivaswamy *et al.*, 2008). By comparing nucleosome positions within 45 bp of their loci in both two conditions, the results indicate that ∼66% of nucleosomes retain their nucleosome patterns after heat shock. Nucleosomes with dramatic positional changes are more highly enriched in CR-sensitive genes than in TF-sensitive genes (Fig. 2A). (ii) With more and more genome-wide maps of nucleosome position being available, we compiled two genome-wide maps of nucleosome positions with high resolutions in the same strain (BY4714) and compared their nucleosome locations (see Section 2, Supplementary Table S3). After mapping the inconsistent nucleosome positions, the result shows that CR-sensitive genes have a more dynamic nucleosome pattern than TF-sensitive genes (Fig. 2A). (iii) We compared the discrepancies of nucleosome occupancy derived from three different growth conditions, and also found that CRsensitive genes are likely to have dynamic nucleosome characteristics. These results suggested that the influence of CRE was markedly higher

than that of TRE on the dynamic characteristics of nucleosome organization.

Most nucleosomes are 'well-positioned' at the same location in the cells, whereas some of them tend to spread out their positions and show a delocalized status, and they have been defined as 'fuzzily positioned' nucleosomes (Mavrich *et al.*, 2008). To evaluate the extent of nucleosomes having different binding locations between CR- and TF-sensitive genes, we calculated the percentage of DNA sequences occupied by fuzzily positioned nucleosomes (Lee *et al.*, 2007) within the promoter regions. Figure 2B (inset figure) shows that it was significantly higher in CR-sensitive genes than in TF-sensitive genes (Wilcoxon rank sum test, $P = 4.3e\text{-}5$), which suggests that CRs are more responsible for the nucleosome dynamic patterns than TFs. Recently, Mavrich *et al.* (2008) measured 'fuzziness score' using standard deviation of all number of reads of nucleosome location. To further validate our result, we plotted the 'fuzziness score' within the focal genes. The result revealed that nucleosome fuzziness increased from the promoters to the 3′ end of the genes in both CR-and TF-sensitive genes (Fig. 2B), and nucleosomes located in CR-sensitive genes show significantly higher level of fuzziness (Wilcoxon rank sum test, $P = 1.7e\text{-}47$).

3.4 Histone modifications induced by CR and TF regulation

Histone proteins are subject to numerous modifications that function as important transcriptional regulatory mechanisms (Li *et al.*, 2007). To detect the histone activities induced by CR and TF regulation, we compiled the histone tails perturbation data and examined the general relationship between transcriptional regulation by TFs and CRs and histone activities. We analyzed genome-wide expression data of yeast strains after deleting the H3 (H3Δ1-28) and H4 (H4Δ2-26) amino termini (Sabet *et al.*, 2003), and found that genes affected in the absence of the amino termini of histone H3 and H4 were extremely regulated by TFs and CRs. These genes might be highly regulated by histone tails through activating or repressing gene expression. Moreover, genes de-repressed by the deletion of histone tails are dramatically sensitive to CRs (showing extremely the right-side tails, Fig. 3A), which indicated that these genes require histone amino termini for proper regulation, particularly repression. For example, genes regulated by histone modification which is associated with repressed transcription will show upregulated expression after histone depletion (right side tails in Fig. 3), and the expression of these genes should be highly depended on CR to activate gene expression. This result is also in line with previous findings that chromatin remodeler ISW2 interacts with histone amino acid termini and represses the expression of many genes, especially genes involved in meiosis (Georgel *et al.*, 1997; Goldmark *et al.*, 2000). This scenario suggested that histone H3 and H4 are regulated by both TFs and CRs, and genes requiring histone tails depend on specific effects of chromatin.

We also examined the histone H3 turnover rate (Dion *et al.*, 2007) and histone-variant H2A.Z occupancy (Albert *et al.*, 2007) within these two cohorts of genes. As expected, histone-variant H2A.Z is highly enriched in the cohort of TF-sensitive genes (60%, relative to CR-sensitive genes 48%, $P = 0.002$, chi-square test), which is in agreement with the findings that H2A.Z is a general feature of promoter chromatin architecture characterized by nucleosome depleted region (Tirosh and Barkai, 2008).In contrast, higher histone turnover rates were observed in the promoters of CR-sensitive genes (0.29) than that in TF-sensitive genes (0.21) ($P = 0.03$, 1000 permutation test).

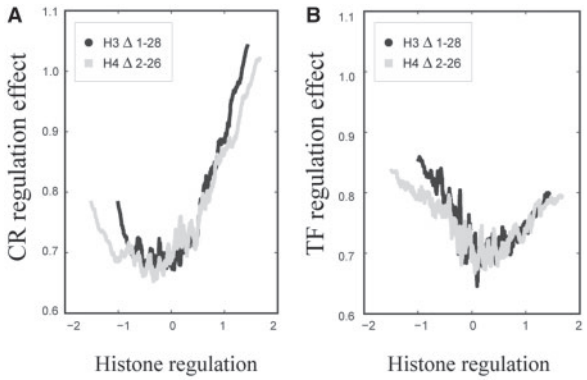

Fig. 3. Impact of CR and TF regulation effect on the activity of histones. (**A**) CR and (**B**) TF regulation effect with the varying sensitivity to histone regulation. Genes were ordered by expression changes resulting from histone H3 (H3Δ1-28) and H4 (H4Δ2-26) amino terminus depletion. Both the average values of CR and TF regulation effect were obtained by a sliding window of 200 ordered genes.

4 DISCUSSION AND CONCLUSION

Recent works elucidating nucleosome positioning in yeast have revealed that the nucleosome organization at the promoter region is characterized by a nucleosome depleted region located at ∼100–200 bp upstream of TSS (Lee *et al.*, 2007; Yuan *et al.*, 2005). This depleted region can be occupied by nucleosomes to adjust expression levels under particular environmental perturbations, whereas promoters of active genes are depleted of nucleosomes to permit TF-binding events required for gene expression activity. Here, we showed that nucleosome organization has distinct patterns in the promoter region under the regulation of CRs and TFs. Notably, our result revealed that depleted nucleosome occupancy does not always cause higher gene expression level in the cohort of TF-sensitive genes. This phenomenon could be largely explained by the competition of TFs with nucleosomes to access specific genomic location. This competition might result in reduced binding efficiency of the cognate TF, and thus tune the gene expression to adapt the varying environmental conditions. It was documented that CRs are capable of remodeling chromatin structures through nucleosome movement and eviction *in vivo* (Li *et al.*, 2007).

Previous studies suggested that an ATP-dependent chromatin remodeling enzyme, the SWI/SNF complex, is involved in nucleosome eviction (Narlikar *et al.*, 2002; Saha *et al.*, 2006), and the Iswi2 enzyme is involved in shifting nucleosomes to a position with an energetically unfavorable DNA sequence (Whitehouse and Tsukiyama, 2006). These works support our finding that dynamic characteristics of nucleosome positioning are more likely to be mediated by CRs than TFs.

Taken together, we performed a comprehensive analysis to detect the differences between CR and TF regulation effects on gene regulation, and found the two different underlying patterns of nucleosome organization at nucleosome-free region. It suggested that CR-sensitive genes tend to have dynamic characteristics of

nucleosomes than TF-sensitive genes. In spite of the different mechanism, however, CR and TF by no means work independently, and they are likely to work jointly during the transcription process. Nevertheless, our results provide a better understanding of how TRE and CRE determine nucleosome positioning and how they affect transcription process.

ACKNOWLEDGEMENTS

We thank Dr Martin Kupiec for providing us the chromatin modifier perturbation compendiums. We would like to thank the Associate Editor and three referees for their constructive comments that have significantly improved the quality of this article.

Funding: Team Grant from the Canadian Institutes of Health Research (CIHR MOP#82940); Discovery Grant from Natural Sciences and Engineering Research Council of Canada (NSERC).

Conflict of Interest: none declared.

REFERENCES

Albert,I. *et al.* (2007) Translational and rotational settings of H2A.Z nucleosomes across the Saccharomyces cerevisiae genome. *Nature*, **446**, 572–576.

Amit,I. *et al.* (2007) A module of negative feedback regulators defines growth factor signaling. *Nat. Genet.*, **39**, 503–512.

Badis,G. *et al.* (2008) A library of yeast transcription factor motifs reveals a widespread function for Rsc3 in targeting nucleosome exclusion at promoters. *Mol. Cell*, **32**, 878–887.

Basehoar,A.D. *et al.* (2004) Identification and distinct regulation of yeast TATA box-containing genes. *Cell*, **116**, 699–709.

Berger,S.L. (2000) Gene regulation. Local or global? *Nature*, **408**, 412–413, 415.

Buck,M.J. and Lieb,J.D. (2006) A chromatin-mediated mechanism for specification of conditional transcription factor targets. *Nat. Genet.*, **38**, 1446–1451.

Choi,J.K. and Kim,Y.J. (2008) Epigenetic regulation and the variability of gene expression. *Nat. Genet.*, **40**, 141–147.

Choi,J.K. and Kim,Y.J. (2009) Intrinsic variability of gene expression encoded in nucleosome positioning sequences. *Nat. Genet.*, **41**, 498–503.

Dion,M.F. *et al.* (2007) Dynamics of replication-independent histone turnover in budding yeast. *Science*, **315**, 1405–1408.

Field,Y. *et al.* (2008) Distinct modes of regulation by chromatin encoded through nucleosome positioning signals. *Plos Comput. Biol.*, **4**.

Field,Y. *et al.* (2009) Gene expression divergence in yeast is coupled to evolution of DNA-encoded nucleosome organization. *Nat. Genet.*, **41**, 438–445.

Georgel,P.T. *et al.* (1997) Role of histone tails in nucleosome remodeling by Drosophila NURF. *EMBO J.*, **16**, 4717–4726.

Goldmark,J.P. *et al.* (2000) The Isw2 chromatin remodeling complex represses early meiotic genes upon recruitment by Ume6p. *Cell*, **103**, 423–433.

Harbison,C.T. *et al.* (2004) Transcriptional regulatory code of a eukaryotic genome. *Nature*, **431**, 99–104.

Hartley,P.D. and Madhani,H.D. (2009) Mechanisms that specify promoter nucleosome location and identity. *Cell*, **137**, 445–458.

Holstege,F.C. *et al.* (1998) Dissecting the regulatory circuitry of a eukaryotic genome. *Cell*, **95**, 717–728.

Hu,Z. *et al.* (2007) Genetic reconstruction of a functional transcriptional regulatory network. *Nat. Genet.*, **39**, 683–687.

Ihmels,J. *et al.* (2002) Revealing modular organization in the yeast transcriptional network. *Nat. Genet.*, **31**, 370–377.

Ito,T. *et al.* (1997) ACF, an ISWI-containing and ATP-utilizing chromatin assembly and remodeling factor. *Cell*, **90**, 145–155.

Jiang,C. and Pugh,B.F. (2009) A compiled and systematic reference map of nucleosome positions across the Saccharomyces cerevisiae genome. *Genome Biol.*, **10**, R109.

Kaplan,N. *et al.* (2009) The DNA-encoded nucleosome organization of a eukaryotic genome. *Nature*, **458**, 362–U129.

Kim,H.D. and O'Shea,E.K. (2008) A quantitative model of transcription factor-activated gene expression. *Nat. Struct. Mol. Biol.*, **15**, 1192–1198.

Komili,S. and Silver,P.A. (2008) Coupling and coordination in gene expression processes: a systems biology view. *Nat. Rev. Genet.*, **9**, 38–48.

Lam,F.H. *et al.* (2008) Chromatin decouples promoter threshold from dynamic range. *Nature*, **453**, 246–U216.

Langst,G. *et al.* (1999) Nucleosome movement by CHRAC and ISWI without disruption or trans-displacement of the histone octamer. *Cell*, **97**, 843–852.

Lee,S.I. *et al.* (2006) Identifying regulatory mechanisms using individual variation reveals key role for chromatin modification. *Proc. Natl Acad. Sci. USA*, **103**, 14062–14067.

Lee,W. *et al.* (2007) A high- resolution atlas of nucleosome occupancy in yeast. *Nat. Genet.*, **39**, 1235–1244.

Li,B. *et al.* (2007) The role of chromatin during transcription. *Cell*, **128**, 707–719.

MacIsaac,K.D. *et al.* (2006) An improved map of conserved regulatory sites for Saccharomyces cerevisiae. *BMC Bioinformatics*, **7**, 113.

Mavrich,T.N. *et al.* (2008) A barrier nucleosome model for statistical positioning of nucleosomes throughout the yeast genome. *Genome Res.*, **18**, 1073–1083.

Narlikar,G.J. *et al.* (2002) Cooperation between complexes that regulate chromatin structure and transcription. *Cell*, **108**, 475–487.

Orphanides,G. and Reinberg,D. (2002) A unified theory of gene expression. *Cell*, **108**, 439–451.

Pugh,B.F. (2000) Control of gene expression through regulation of the TATA-binding protein. *Gene*, **255**, 1–14.

Rando,O.J. and Chang,H.Y. (2009) Genome-wide views of chromatin structure. *Ann. Rev. Biochem.*, **78**, 245–271.

Sabet,N. *et al.* (2003) Global and specific transcriptional repression by the histone H3 amino terminus in yeast. *Proc. Natl Acad. Sci. USA*, **100**, 4084–4089.

Saha,A. *et al.* (2006) Chromatin remodelling: the industrial revolution of DNA around histones. *Nat. Rev.*, **7**, 437–447.

Segal,E. and Widom,J. (2009) What controls nucleosome positions? *Trends Genet.*, **25**, 335–343.

Segal,E. *et al.* (2003) Module networks: identifying regulatory modules and their condition-specific regulators from gene expression data. *Nat. Genet.*, **34**, 166–176.

Shivaswamy,S. *et al.* (2008) Dynamic remodeling of individual nucleosomes across a eukaryotic genome in response to transcriptional perturbation. *PLoS Biol.*, **6**, e65.

Stein,A. *et al.* (2009) Are nucleosome positions in vivo primarily determined by histone-DNA sequence preferences? *Nucleic Acids Res.*, **38**, 709—719.

Steinfeld,I. *et al.* (2007) A genome-wide analysis in Saccharomyces cerevisiae demonstrates the influence of chromatin modifiers on transcription. *Nat. Genet.*, **39**, 303–309.

Tirosh,I. and Barkai,N. (2008) Two strategies for gene regulation by promoter nucleosomes. *Genome Res.*, **18**, 1084–1091.

Whitehouse,I. and Tsukiyama,T. (2006) Antagonistic forces that position nucleosomes in vivo. *Nat. Struct Mol. Biol.*, **13**, 633–640.

Whitehouse,I. *et al.* (2007) Chromatin remodelling at promoters suppresses antisense transcription. *Nature*, **450**, 1031–U1033.

Yarragudi,A. *et al.* (2004) Comparison of ABF1 and RAP1 in chromatin opening and transactivator potentiation in the budding yeast Saccharomyces cerevisiae. *Mol. Cell. Biol.*, **24**, 9152–9164.

Yuan,G.C. *et al.* (2005) Genome-scale identification of nucleosome positions in S.cerevisiae. *Science*, **309**, 626–630.

Yuan,G.C. *et al.* (2006) Statistical assessment of the global regulatory role of histone acetylation in Saccharomyces cerevisiae. *Genome Biol.*, **7**, R70.

Zhang,Y. *et al.* (2009) Intrinsic histone-DNA interactions are not the major determinant of nucleosome positions in vivo. *Nat. Struct. Mol. Biol.*, **16**, 847–852.