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Effects of *cis* and *trans* regulatory variations on the expression divergence of heat shock response genes between yeast strains

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ABSTRACT

Phenotypic variation among individuals in a population can be due to DNA sequence variation in protein coding regions or in regulatory elements. Recently, many studies have indicated that mutations in regulatory elements may be the major cause of phenotypic evolution. However, the mechanisms for evolutionary changes in gene expression are still not well understood. Here, we studied the relative roles of *cis* and *trans* regulatory changes in *Saccharomyces cerevisiae* cells to cope with heat stress. It has been found that the expression level of ~300 genes was induced at least two fold and that of ~500 genes was repressed at least two fold in response to heat shock. From the former set of genes, we randomly selected 65 genes that showed polymorphism(s) between the BY and RM strains for pyrosequencing analysis to explore the relative contributions of *cis* and *trans* regulatory variations to the expression divergence between BY and RM. Our data indicated that the expression divergence between BY and RM was mainly due to *trans* regulatory variations under either the normal condition or the heat stress condition. However, the relative contribution of *trans* regulatory variation was decreased from 76.9% to 61.5% after the heat shock stress. These results indicated that the *cis* regulatory variation may play an important role in the adaption to heat stress. In our data, 43.1% (28 genes) of the 65 genes showed the same trend of *cis* or *trans* variation effect after the heat shock stress, 35.4% (23 genes) showed an increased *cis* variation effect and 21.5% (14 genes) showed an increased *trans* variation effect after the heat shock stress. Thus, our data give insights into the relative roles of *cis* and *trans* variations in response to heat shock in yeast.

1. Introduction

Expressional changes in a gene can arise from *cis* changes, or *trans* changes, or both. *Cis* changes are allele-specific, affecting only the expression of the allele linked to the changes but not the expression of the other allele. *Cis* changes are mainly referred to changes in the *cis*-regulatory elements of the promoter region, though a change in the transcribed region that influences the mRNA stability could also affect the expression level of the allele. In contrast, *trans* changes are not allele-specific and thus can affect the expression of the gene regulated. *Trans* effect can be due to changes that affect the timing, level, stability, or activity of the TFs or other regulators that control the expression of TF genes or the target genes. Much effort has been made to understand the relative contributions of *cis* and *trans* regulatory changes to the expression divergence within and between species. In *Drosophila*, several studies have indicated that *cis* variation plays a more important role than *trans* variation in the expression divergence both within and between species of *Drosophila melanogaster* and *Drosophila simulans* (Osada et al., 2006; Rifkin et al., 2003; Wittkopp et al., 2004, 2008). However, the data of McManus et al. (2010) indicated that *trans* regulatory variation plays a more important role in the expression divergence between *D. melanogaster* and *Drosophila sechellia*. McManus et al. (2010) suggested that the inconsistent observations between *D. melanogaster*/*D. simulans* and *D. melanogaster*/*D. sechellia* may be due to the differences in the sensitivity of the methods used in different studies, or may lie in the special evolutionary characteristics of *D. sechellia*, which displays less intraspecific genetic variation and has maintained a small population size. In *Arabidopsis*, the expression divergence between two *Arabidopsis thaliana* strains, Columbia (Col) and Vancouver (Van), was suggested to be mainly due to *trans* regulatory changes (Zhang and Borevitz, 2009).

In yeast, several studies have shown that *trans* variation plays a more important role in the expression divergence between BY and RM, two strains of *Saccharomyces cerevisiae* (Brem et al., 2002; Emerson et al., 2010; Sung et al., 2009; Yvert et al., 2003). On the other hand, Tirosh et al. (2009) reported that the expression divergence between two yeast species, *S. cerevisiae* and *Saccharomyces paradoxus*, is mainly due to *cis* regulatory changes. They argued that *trans* effects might be attributed primarily to differential interpretation

Abbreviations: TF, transcription factor; BY, BY4743; RM, RM11-1a/α; SNP, single nucleotide polymorphism.

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of sensory signals but not to mutations directly in transcriptional regulators. After analyzing the whole genome expression profiles carried out by deep sequencing, Emerson et al. (2010) proposed that *trans* variation is more sensitive to selective constraints, so that the expression divergence between *S. cerevisiae* and *S. paradoxus* is mainly due to *cis* variation effects, although the intraspecific expression divergence is mostly due to *trans* variation effects.

To date, the expression divergence studies of flies and yeasts were mostly conducted under the standard lab condition. However, stress conditions are frequently encountered in the wild. Therefore, we studied the heat-shock effect, which is one of the most studied stress responses and is an important factor for the survival of microorganisms, to examine the relative contributions of *cis* and *trans* variations to the expression divergence of heat shock response genes between BY and RM.

2. Materials and methods

2.1. Yeast strains and growth conditions

The yeast strains used in this study were BY4743 (or simply BY) (*MATa/α his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 LYS2/lys2Δ0 met15Δ0/MET15 ura3Δ0/ura3Δ0*), RM11-1a/α (or simply RM) (*MATa/α leu2Δ0/LEU2 LYS2/lys2Δ0 ura3Δ0/ura3Δ0 ho::KAN*) and the hybrid strain WL201 (BY4741 × RM11-1α, *MATa/α his3Δ1/HIS3 leu2Δ0/LEU2 LYS2/lys2Δ0 met15Δ0/MET15 ura3Δ0/ura3Δ0*). Co-culture was established using a mixture of approximately equal numbers of BY and RM cells. The mixture of the two strains was then grown in the same culture to rule out the possible environmental effect. All yeast cultures were conducted in YPAD media (1% yeast extract, 2% peptone, 0.02 g/L adenine and 2% dextrose) at 30 °C under 250 rpm shaking.

2.2. Induced heat-shock response gene expression

Freshly prepared overnight yeast cultures were used to prepare the 100 ml starting cultures, at $OD_{600} = 0.1$, and the yeast cells were grown in YPAD media at 30 °C with 250 rpm shaking. The mid-log phase vegetative yeast cells were used for the heat-shock treatment. Basically, the yeast cells were grown to $OD_{600} = 1$, and the cultures were transferred to 37 °C water bath for 15 min of heat-shock treatment. Then, the yeast cells were harvested by centrifugation, 6000 × *g* for 5 min at 4 °C, and the cell pellets were stored at −80 °C for further experiments.

2.3. Quantification of allele expression using pyrosequencing

Total RNA was extracted from both untreated and heat-shock treated yeast cells by the hot acid phenol method (Sung et al., 2009). An aliquot of 5 μg total RNA from each sample was used for cDNA synthesis. The reverse transcription was carried out with oligo-dT primers and the Super-script II kit (Invitrogen) following the manufacturer's instructions. After identification of strain-specific single nucleotide polymorphism (SNP) in the coding region of a gene, a 150–200 base-pair (bp) fragment of the coding region, containing the strain-specific SNP, in the hybrid samples, WL201, and in the co-culture samples of BY and RM, was amplified and sequenced by pyrosequencing (supplemental Table 3). A sequencing primer located 1–5 bp upstream of the strain-specific SNP was used in the pyrosequencing reaction for measuring the relative abundances of the two alleles in genomic DNA and in cDNA samples from both co-culture and hybrid pools as described in Sung et al. (2009). The pyrosequencing instrument software (PSQ 96MA 2.1.1) reports a peak height directly proportional to the number of molecules incorporated into the growing DNA chain. The ratio of allele-specific frequencies ($RM_{co\text{-}cult}/BY_{co\text{-}cult}$, RM_{hybrid}/BY_{hybrid}), which corresponds to the relative transcript abundances of the BY and RM alleles in the sample, was reported by the pyrosequencing software, PSQ 96MA 2.1.1. The cDNA ratios were then normalized with the genomic DNA measurements for both co-culture and hybrid samples as described in Wittkopp et al. (2004). Because both alleles were extracted and measured in the same sample, this method is insensitive to differences in extraction efficiency, eliminating the need for controlling the quantification of total RNA recovery. The relative expression ratio of BY and RM alleles of each gene was estimated from at least 3 replicates.

2.4. Rules for inferring cis and trans variation effects

Let $R_1 = BY_{hybrid}/RM_{hybrid}$ be the ratio of the expression levels of the BY and RM alleles in the hybrid diploid and $R_2 = BY_{co\text{-}cult}/RM_{co\text{-}cult}$ be the ratio when the two strains are grown in the same culture (co-culture). If R_2 is different from 1 (i.e., $R_2 \neq 1$), the difference in the expression levels of the two alleles can be due to the *cis* effect or the *trans* effect or both. Note that in a hybrid, the *trans* effect for both BY and RM alleles is the same, so that any difference in the expression level between the two alleles in a "hybrid" (i.e., if $R_1 \neq 1$) is completely due to the *cis* effect, whereas if $R_1 = 1$, then the expression divergence observed in the co-culture of BY and RM is completely due to the *trans* effect. On the other hand, if $R_2 \neq 1$ and $R_2 = R_1$, then the expression difference between the two alleles in the "co-culture" is completely due to the *cis* effect, because it shows that homogenization (hybridization) of the genetic background does not reduce the expression differences between the two alleles. Thus, the *cis*- and *trans*-effects on the expression differences between $BY_{co\text{-}cult}$ and $RM_{co\text{-}cult}$ can be judged by the allele specific expression in the hybrid according to the following guidelines:

1. If $R_2 \neq 1$ and $R_2 = R_1$, the expression difference is due to the "*cis* effect alone"
2. If $R_2 > 1$ and $R_1 = 1$, the expression difference is due to the "*trans* effect alone"
3. If $R_2 > 1$ and $R_1 > 1$:
   (a) if $(R_1-1)/(R_2-1) \leq 0.25$, the expression difference is mainly due to the *trans* effect — we say it is a "major *trans* effect"
   (b) if $0.25 < (R_1-1)/(R_2-1) < 0.75$, we say the expression difference is due to "both *cis* and *trans* effect"
   (c) if $(R_1-1)/(R_2-1) > 0.75$ (but $\neq 1$), the expression difference is mainly due to the *cis* effect — we say it is a "major *cis* effect"
4. If $R_2 > 1$ but $R_1 < 1$, then to be on the same scale as R_2, we consider $1/R_1$.
   (a) If $R_2 > 1/R_1$, it is a "major *trans* effect", because it can be shown that the *cis* effect $(1/R_1-1)$ is smaller than half of the *trans* effect.
   (b) If $R_2 \leq 1/R_1$, it is due to "both *cis* and *trans* effect" because the *cis* effect $(1/R_1-1)$ is greater than half of the *trans* effect.
5. If $R_2 \approx 1$ but $R_1 \neq 1$, the expression difference is due to "both *cis* and *trans* effect"; that is, the *cis* and *trans* effects in the co-culture are equal, so that $R_2 = 1$, but the *cis* effect $(R_1 \neq 1)$ is seen in the hybrid.

All the above equalities and inequalities are to be statistically tested. We used the student *t*-test to examine if $R_1 = 1$ or if $R_2 = 1$. We calculated the standard error (S.E.) from at least 3 replicates and examined the null hypothesis of $R_1 = 1$ or $R_2 = 1$. We used the two-tailed *t*-test to examine if $R_1 = R_2$.

In the above, we considered the case of $RM_{co\text{-}cult}/BY_{co\text{-}cult} \geqq 1$. If $RM_{co\text{-}cult}/BY_{co\text{-}cult} < 1$, then $R_2 = BY_{co\text{-}cult}/RM_{co\text{-}cult}$ instead of $R_2 = RM_{co\text{-}cult}/BY_{co\text{-}cult}$ and $R_1 = BY_{hybrid}/RM_{hybrid}$ instead of $R_1 = RM_{hybrid}/BY_{hybrid}$ should be used in conditions (2), (3), and (4).

2.5. Selection of heat-shock response genes

It has been shown that in *S. cerevisiae* the expression of 324 genes was induced at least two-fold and the expression of 484 genes was repressed at least two-fold in response to a heat shock (Causton et al., 2001; Gasch et al., 2000). In this study, we focused on the former set

of genes because these genes might be important for yeast to adapt to heat stress. Using Gene Ontology (GO) to analyze the molecular function of these heat stress induced genes, we found that 137 genes have a catalytic activity, 37 genes have an oxidoreductase activity, 5 genes have an aldo-keto reductase activity, 13 genes are involved in unfolded protein binding and 132 genes have no known functions. We randomly selected 65 genes from the 324 heat induced genes for this study; see the list of the selected genes in Supplemental Table 1.

The sequence database of BY strain was from the *Saccharomyces* Genome Database (http://www.yeastgenome.org/) and the RM strain sequences were from Broad Institute (http://www.broadinstitute.org/annotation/genome/saccharomyces_cerevisiae/).

3. Results and discussion

3.1. Increase of cis-variation effect on intraspecific expression divergence by heat shock

We randomly selected 65 heat induced genes for pyrosequencing analysis to examine the relative contributions of *cis* and *trans* regulatory variations to the expression divergence between BY and RM under normal condition or under heat-shock condition (see Materials and methods). Under the normal condition, 16.9% (11/65) of these differentially expressed heat-shock induced genes were classified as due to "*trans* effect alone", 60.0% (39/65) as due to "major *trans* effect", 16.9% (11/65) as due to "both *cis* and *trans* effect", 4.6% (3/65) as due to "major *cis* effect", and 1.5% (1/65) as due to "*cis* effect alone" (Fig. 1). After the heat-shock treatment, these proportions became 20.0% (13/65), 41.5% (27/65), 23.1% (15/65), 4.6% (3/65), and 10.8% (7/65), respectively (Fig. 1). These observations indicated that the expression divergence between BY and RM was mainly due to *trans* regulatory variations both under normal growth condition and under heat-shock condition, because 76.9% and 61.5% of the genes, respectively, were affected mainly by *trans* regulatory variations. However, the *cis* variation effect was increased after the cells were subjected to a heat shock. In Fig. 1, the "*cis* effect alone" class increased from 1.5% to 10.8% and the "both *cis* and *trans* effect" class increased from 16.9% to 23.1%.

3.2. Many genes showed the same trend of regulatory variation effect

In the above analysis, the *cis* variation effect was increased after heat shock because 35.4% (23 genes) of the 65 genes showed an increase in *cis* variation effect — classified as "*cis* effect increase", while only 21.5% (14 genes) showed an increase in *trans* variation effect — classified as "*trans* effect increase" (Fig. 2). Interestingly, 43.1% (28 genes) of the 65 genes showed the same trend of *cis* or *trans* variation effect after heat shock — classified as "same variation effect". That is, close to half of the genes showed the same trend of regulatory variation effect under normal condition and under heat shock condition.

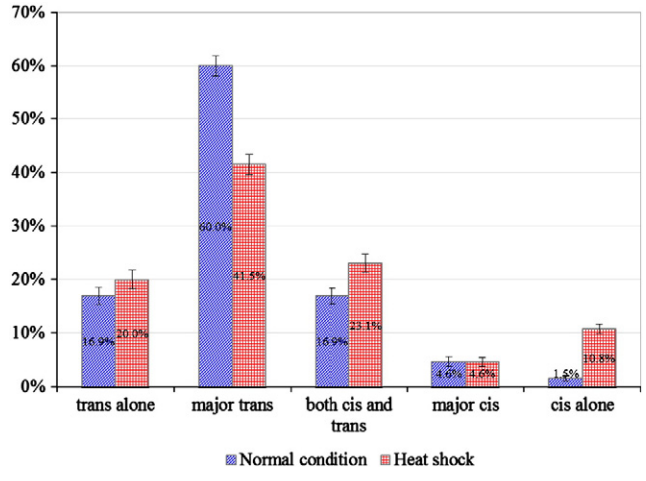

Fig. 1. Relative contributions of *trans* and *cis* effects to the expression divergence between BY and RM under normal condition and under heat shock stress condition. The expression divergence was mainly due to *trans* regulatory variations in either condition: for the two conditions, ~76.9% and ~61.5% of the genes under study were mainly affected by *trans* regulatory variations. Sampling variances were estimated by bootstrapping 500 replicates. ($p<0.05$, *chi*-square test).

3.3. TATA box-containing genes

Only about one-fifth of yeast genes (19%) contain a TATA box. It has been found that TATA box-containing genes tend to respond to environmental signals (Basehoar et al., 2004). Moreover, a comparative study of genome-wide expression profiles from four related yeast species, *S. cerevisiae*, *S. paradoxus*, *S. mikatae* and *S. kudriavzevii*, showed that TATA box-containing genes tend to have a higher expression divergence than TATA box-less genes (Tirosh et al., 2006). However, it was also reported that TATA box-containing promoters tend to be more conserved in sequence than TATA-less promoters (Cliften et al., 2003). Therefore, what causes the stronger tendency of expression divergence for TATA box-containing genes is not clear.

We examined whether the effects of *cis* and *trans* variations on the expression divergence between BY and RM for TATA box-containing genes were different from those for TATA box-less genes. Among the 65 heat-shock induced genes examined, 43 were TATA box-containing genes and 22 were TATA box-less genes. Under the normal condition, 14.0% (6 genes) of these 43 differentially expressed TATA box-containing genes were classified as due to "*trans* effect alone", 60.5% (26 genes) as due to "major *trans* effect", 18.6% (8 genes) as due to "both *cis* and *trans* effect", 4.7% (2 genes) as due to "major *cis* effect", and 2.3% (1 gene) as due to "*cis* effect alone". Also under the same condition, 22.7% (5 genes) of the 22 differentially expressed TATA box-less genes were classified as due to "*trans* effect alone", 59.1% (13 genes) as due to "major *trans* effect", 13.6% (3 genes) as due to "both *cis* and *trans* effect", 4.5% (1 genes) as due to "major *cis* effect" and 0% as due to "*cis* effect alone" (Fig. 3). Our data indicated that under normal growth condition, the effects of *cis* and *trans* regulatory variations to the expression divergence of heat-shock induced genes were significantly different between TATA box-containing genes and TATA box-less genes ($p<0.05$, *chi*-square test). The relative contributions of *cis* and *trans* regulatory variations to the expression divergence of heat-shock induced genes were also significantly different between TATA box-containing genes and TATA box-less genes under heat-shock condition ($p<0.05$, *chi*-square test). Under the heat-shock condition, 14.0% (6 genes) of the 43 TATA box-containing genes examined were classified as due to "*trans* effect alone", 41.9% (18 genes) as due to "major *trans* effect",

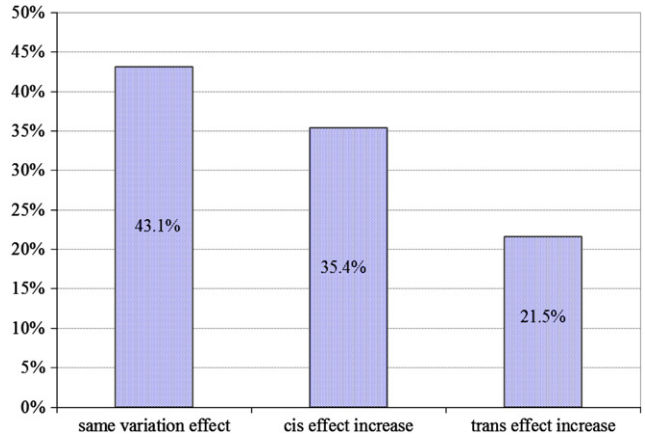

Fig. 2. Comparison of the effects of *trans* and *cis* variations on the expression divergence between normal condition and heat shock condition. The effect of *cis* regulatory variation increased after the heat shock stress.

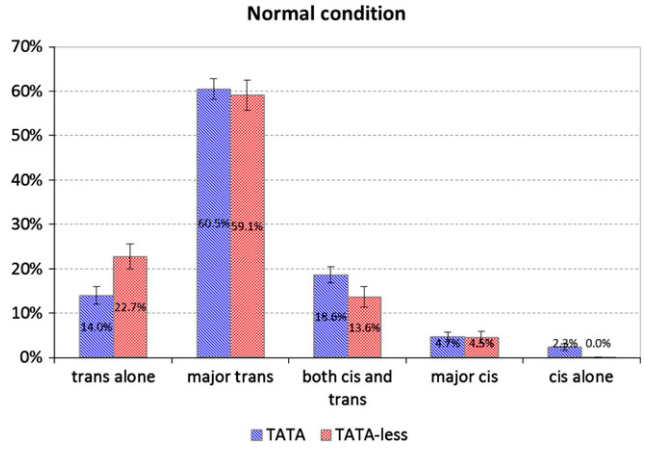

Fig. 3. The relative contributions of *cis* and *trans* variation to the expression divergence between BY and RM under normal condition. The genes studied are classified into TATA box-containing genes and TATA box-less genes. Sampling variances were estimated by bootstrapping 500 replicates. ($p<0.05$, *chi*-square test).

25.6% (11 genes) as due to "both *cis* and *trans* effect", 7.0% (3 genes) as due to "major *cis* effect", and 11.6% (5 genes) as due to "*cis* effect alone". Under the same condition, 31.8% (7 genes) of the 22 TATA box-less genes examined were classified as due to "*trans* effect alone", 40.9% (9 genes) as due to "major *trans* effect", 18.2% (4 genes) as due to "both *cis* and *trans* effect", 0.0% as due to "major *cis* effect" and 9.1% (2 genes) as due to "*cis* effect alone" (Fig. 4).

3.4. Stronger regulatory variation effects on TATA box-containing genes under heat shock condition

We further examined the dynamic changes of *cis* and *trans* variation effect of these TATA box-containing genes and TATA box-less genes under normal condition and under heat shock condition. Our data indicated that 22.7% of the TATA box-less genes showed an increase in *cis* variation effect, 18.2% showed an increase in *trans* variation effect, while 59.1% of genes showed the same trend of *cis* or *trans* variation effect after heat shock (Fig. 5). That is, more than half of the TATA box-less genes showed the same trend of regulatory variation effect under normal condition and under heat shock. On the other hand, 41.9% of the TATA box-containing genes showed an increase in *cis* variation effect, 23.3% showed an increase in *trans* variation effect, while only 34.9% of genes showed the same trend of *cis* or *trans* variation effect after heat shock (Fig. 5). The TATA box-containing

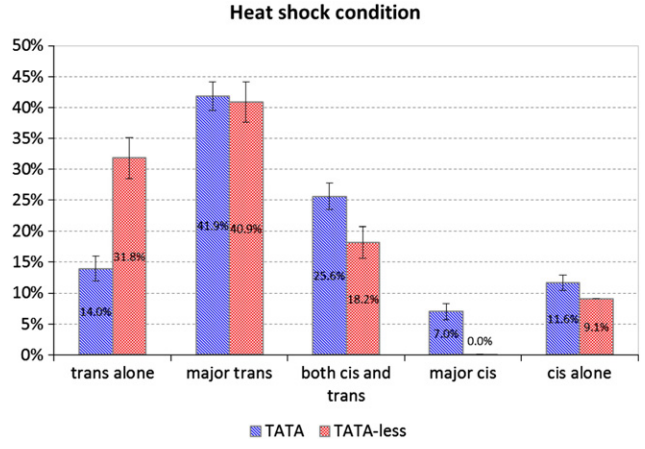

Fig. 4. The relative contributions of *cis* and *trans* variation to expression divergence between BY and RM under heat-shock condition. The genes studied are classified into TATA box-containing genes and TATA box-less genes. Sampling variances were estimated by bootstrapping 500 replicates. ($p<0.05$, *chi*-square test).

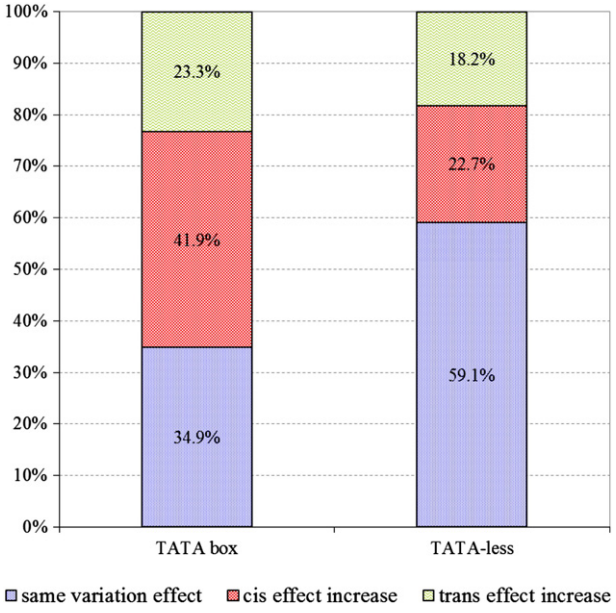

Fig. 5. Dynamic changes of *trans* and *cis* variation effect on the expression divergence of TATA box-containing genes and TATA box-less genes in normal condition and under heat shock condition. ($p<0.05$, *chi*-square test).

genes showed stronger regulatory variation effect, either increase in *cis* or *trans* variation effect, under heat shock condition, compared to TATA box-less genes ($p<0.05$, *chi*-square test).

4. Conclusions

We were interested in knowing the relative contributions of *cis* and *trans* regulatory variations to the expression divergence between two yeast strains, BY and RM, under heat-shock stress, and this information may provide basic genetic variation information on the expression divergence of yeast populations. Our data suggested that the expression divergence of the heat shock response genes of BY and RM was mainly due to *trans* regulatory variation effect in both normal growth condition and under heat-shock condition, because 76.9% and 61.5% of the genes, respectively, were affected mainly by *trans* regulatory variations (affected either by *trans* effect alone or major *trans* effect). However, the *cis* variation effect was increased after the cells were subjected to a heat shock. Our results suggested that the *trans* variations might under stronger selection constrain compared to the *cis* variations as suggested in Emerson et al. (2010).

We have tried to identify the possible reasons for the different regulatory variation effect of these heat shock induced genes. We examined the molecular function, selection constrain (ka/ks), numbers of upstream transcription factor binding sites, expression level, nucleosome occupancy of the genes, and the polymorphism(s) of the intergenic regions, but found no direct evidence to explain the different regulatory variation effect under heat shock stress. However, our analysis showed that the distribution of relative contribution of *cis* and *trans* variation effect were significantly different between TATA box-containing genes and TATA box-less genes in both normal condition and under heat shock condition. Our data indicated that most of the TATA box-less genes showed the same trend of regulatory variation effect under normal condition and under heat shock condition, and most of the TATA box-containing genes showed regulatory variation effect, either increase in *cis* or *trans* variation effect, under heat shock condition. Our data suggested that the heat shock induced TATA box-less genes might have stronger selection constrain on expression divergence compared to TATA box-containing genes and this is in agreement with the previous observation of Tirosh et al. (2006).

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.gene.2012.06.034.

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