Full Paper

Spatially coordinated replication and minimization of expression noise constrain three-dimensional organization of yeast genome

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Abstract

Despite recent advances, the underlying functional constraints that shape the three-dimensional organization of eukaryotic genome are not entirely clear. Through comprehensive multivariate analyses of genome-wide datasets, we show that cis and trans interactions in yeast genome have significantly distinct functional associations. In particular, (i) the trans interactions are constrained by coordinated replication and co-varying mutation rates of early replicating domains through interactions among early origins, while cis interactions are constrained by coordination of late replication through interactions among late origins; (ii) cis and trans interactions exhibit differential preference for nucleosome occupancy; (iii) cis interactions are also constrained by the essentiality and co-fitness of interacting genes. Essential gene clusters associate with high average interaction frequency, relatively short-range interactions of low variance, and exhibit less fluctuations in chromatin conformation, marking a physically restrained state of engaged loci that, we suggest, is important to mitigate the epigenetic errors by restricting the spatial mobility of loci. Indeed, the genes with lower expression noise associate with relatively short-range interactions of lower variance and exhibit relatively higher average interaction frequency, a property that is conserved across Escherichia coli, yeast, and mESCs. Altogether, our observations highlight the coordination of replication and the minimization of expression noise, not necessarily co-expression of genes, as potent evolutionary constraints shaping the spatial organization of yeast genome.

Key words: 3D genome organization, long-range chromatin interactions, replication, expression noise, evolutionary constraints, essential genes

1. Introduction

Eukaryotic genes and their regulatory elements communicate with each other through a complex wiring of long-range interactions. It is now well established that distal enhancers can physically juxtapose to their cognate promoters for transcriptional regulation. Interestingly, distant genes can also co-localize in nuclear space. The prevailing view is that the genes spatially cluster at concentrated foci of RNA polymerase II, also known as transcription factories. It is suggested that the spatial convergence of genes at transcription factories provide a topological basis of co-expression of engaged genes; however, such proposals have not been subjected to proper scrutiny. Recent advent of high throughput derivatives of Chromosome Conformation Capture (3C) has availed genome-wide quantitative data of long-range chromatin interactions across diverse spectra of model systems. Briefly, in 3C-derived techniques, the chromatin is cross-linked with formaldehyde, restriction
digested, and the open ends of cross-linked products are ligated in diluted conditions to prefer intra-molecular ligation over inter-molecular. In HiC, the ligated junctions are then pulled down and sequenced using deep sequencing to unravel all-to-all chromatin interactions.\textsuperscript{21} HiC has revealed large topologically associated domains (TADs) that exhibit high density of intra-connectivity of chromatin and are largely conserved across cell lineages.\textsuperscript{27,28} TADs are tightly associated with the chromatin type and replication timing, and are marked by CTCF on boundaries.\textsuperscript{27,28,30} Wide-spread enhancer-to-promoter interactions, that are mostly cell-type specific, have been uncovered across several systems.\textsuperscript{26,31-33} Zhang et al.\textsuperscript{34} have suggested differential usage of enhancers during embryonic stem cell differentiation. Some studies have also revealed promoter-to-terminator interactions commonly found for housekeeping genes,\textsuperscript{34,35} possibly ascribing a circular template for recurrent transcription. Most interesting of all is the widespread promoter-to-promoter interactions among genes impinging from neighbouring regions to form discrete multi-gene complexes.\textsuperscript{34,35} However, what functional and evolutionary constraints might have shaped the large-scale organization of promoter–promoter interactions is not entirely clear. Although the genes within multi-gene complexes are shown to be co-expressed,\textsuperscript{34,36} whether or not co-expression of engaged genes is dependent on their spatial, but not the linear, proximity remains to be seen. Moreover, it is hypothesized that interacting promoters can influence transcriptional states of each other and that the promoter of one gene can function as an enhancer of other gene.\textsuperscript{34} Nevertheless, these proposals are yet not established as fact. Importantly, most of these studies have primarily focussed on intra-chromosomal (referred as ‘cis’ in this study) interactions and whether or not distant genes converging from different chromosomes (referred as trans interactions) have functional association is yet not clear. Comprehensive statistical analyses of accumulated HiC like datasets can answer several questions pertaining to non-random genome organization. Here, we ask whether we can delineate evolutionary constraints of three-dimensional organization of genome.

Multivariate analyses provide a statistical platform to assess the association of several different functional variables in an unbiased manner. Availability of various genome-wide datasets and high-resolution data of cis as well as trans chromatin interactions makes budding yeast an ideal candidate for multivariate analysis to identify the potential functional constraints shaping the non-random spatial organization of genome. The article by Duan et al.\textsuperscript{23} suggested following key features of three-dimensional organization of budding yeast genome: (i) interactions among the centro-meres, (ii) interactions among the sites of early origin and not the late origins, and (iii) interactions among t-RNA genes. A few follow-up studies suggested a link between chromatin interactions and co-expression of involved genes.\textsuperscript{37,38} Another report, on the contrary, dismissed the claims of proximity of co-expressed genes in yeast.\textsuperscript{29} Moreover, the possibility that cis and trans chromatin interactions might have been shaped under different evolutionary constraints has not been explored. In this study, using comprehensive statistical analysis, we show that functional and evolutionary constraints of cis and trans interactions are significantly distinct and are not necessarily associated with co-expression of genes. We show that the trans interactions are primarily constrained by coordinated replication through converged early origins, while cis interactions are shaped by coordination through late origins and by the minimization of expression noise of engaged genes in an evolutionarily conserved manner.

2. Materials and methods

2.1. Data sources

We obtained the publicly available genome-scale datasets from different sources; details of which are given in Supplementary Table S1.

2.2. Methods

Detailed methodology to process the datasets is given in the Supplementary Material.

2.2.1. Binning of data

The interaction frequency data (frq) were clustered into bins of equal size of 1 unit, and average value of each functional attribute was calculated for each bin. The master tables for the binned and the original data are given in the Supplementary Information.

2.2.2. Correlogram analyses

Correlograms were plotted for the binned data using ‘corrgram’ R-package (http://cran.r-project.org/web/packages/corrgram/index.html). Pearson’s correlation coefficients were calculated using cor.test() function in R and P-values for multiple comparisons (no. 55) were corrected using Bonferroni’s method. Significant P-values after correction are marked with triple asterisk (***) in correlograms.

2.2.3. Partial least squared regression

Partial least squared regression (PLSR) models the relation between input matrix X (\(n \times p\) matrix with \(n\) dimensions of \(p\) input variables, genomic/functional attributes in this case) and response matrix Y (\(n \times 1\) matrix with \(n\) dimensions of 1 response variable, interaction frequency in this case) by decomposing them as following:

\[
X = TP^T + E
\]

\[
Y = UQ^T + F
\]

Where T and U are \(n \times r\) matrices with \(r\) extracted latent vectors (or scores), P and Q are \(p \times r\) and \(1 \times r\) matrices of X and Y loadings, respectively. E and F are \(n \times p\) and \(n \times 1\) matrices of residuals. In kernel PLS regression, following inner relation between T and U is assumed;

\[
U = TB^T + H
\]

where B is the \(r \times r\) diagonal matrix of regression coefficients and H is matrix of residuals. Accordingly, equation (2) can be rewritten as

\[
Y = TBQ^T + (HQ^T + F)\]

which defines the final PLS regression model,

\[
Y = TC^T + F^*
\]

where \(C^T = BQ^T\) and \(F^* = HQ^T + F\)

PLS’ R-package was used for this (http://cran.r-project.org/web/packages/pls/index.html). Prior to multivariate regression analyses, the columns of X were Z-normalized.

2.2.4. Random re-sampling to compile null distributions

To assess the non-random connectivity among replication origins, we used re-sampling approach given by Witten and Noble.\textsuperscript{39} Briefly, the positions of the origins in the genome were randomized 10\(^4\) times keeping the chromosomal distribution same as in the original set.

2.2.5. Partial correlation matrices

Partial correlation matrices were calculated using the function cor() in R, and compared against the null distribution generated by random re-sampling of the origins.

2.2.6. Partial least squares regression

Partial least squares regression (PLSR) models the relation between input matrix X (\(n \times p\) matrix with \(n\) dimensions of \(p\) input variables, genomic/functional attributes in this case) and response matrix Y (\(n \times 1\) matrix with \(n\) dimensions of 1 response variable, interaction frequency in this case) by decomposing them as following:

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which defines the final PLS regression model,

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P-value was calculated using following equation:

\[ p = \frac{1}{B} \left( 1 + \sum_{k=1}^{B} \left( \frac{1}{k_b} \right)^{b} \right) \]

Where, \( B \) = number of re-samplings (no. 1,000); \( k_b \) = Observed number of interactions among randomized coordinates; \( k \) = Expected number of interactions among original coordinates; \( k' \) = Expected number of interactions among original coordinates.

We mapped the feature coordinates onto restriction fragments and considered a restriction fragment only once regardless of how many feature coordinates fall inside. This takes care of local clustering of feature coordinates.

3. Results

3.1. Distinct functional attributes of cis and trans chromatin interactions in yeast

Chromatin in the interphase nucleus is generally present in the form of chromosomal territories. The chromatin loci embedded inside the territory would, therefore, be expected to have greater number of cis interactions, while the ones near territorial edge would have abundant trans interactions. This is particularly true for the metazoan genomes; the distinction into such intra- and inter-territorial organization of genes is not well studied in yeast however. If yeast genome conforms into distinct territories, certain regions of chromosomes would exhibit greater number of either cis or trans interactions marking intra-territorial and inter-territorial locations of the loci. Therefore, we first tested whether the number of cis interactions of each locus correlated with the trans interactions of corresponding locus in the yeast genome. We smoothed the number of cis and trans interactions using an arbitrary window of 20 kb along the chromosomes and plotted number of cis vs. trans interactions. We observed that the number of cis and trans interactions of each locus poorly correlated with each other (\( p = 0.05 \)) and that there were regions, which were enriched either with cis or trans interactions (highlighted in ellipses in Fig. 1a). The observed distinction of cis and trans interactions was also confirmed by plotting the \( Z \)-normalized number of cis and trans interactions side-by-side as a function of chromosomal coordinates. As shown in the Fig. 1b, there were domains in the genome having greater number of either cis or trans chromatin interactions. We further calculated average frequency of all the cis and all the trans interactions of each genomic locus independently. By plotting average cis and trans interaction frequencies of corresponding genomic loci against each other, we once again observed regions having higher cis interaction frequency and relatively lower trans frequency and vice versa supporting that there were regions in the genome having high frequency of either cis or trans interactions (Supplementary Fig. S1). We further showed an example of chromosome 4 in the rendered 3D model of yeast genome (as provided by Duan et al.) in Fig. 1c, highlighting a region enriched with local clusters of abundant cis interactions (dashed box). Rest of the chromosome 4 could be seen as an extended arm extensively intermingling with other chromosomes. Quantitative data for Fig. 1c were shown in the Fig. 1d. The plot clearly showed greater number of cis interactions in the boxed area, which corresponds to the same boxed area as in 3D model. Our observations through Fig. 1 highlighted that certain genomic domains can have preferred folding in cis while others intermingles extensively with other chromosomes in trans, supporting the notion of ‘chromosome territories’. This also raised a possibility of cis and trans interactomes to have evolved

Figure 1. Preferred domains of cis and trans chromatin interactions. (a) Scatter plot of number of cis and trans interactions of each locus in yeast genome. X-Y coordinates were binned into 100 bins and represented as shade intensity. (b) Linear view of yeast genome (chr1–16 are concatenated in that order) depicting \( Z \)-normalized number of cis and trans chromatin interactions of each locus. Thicker lines represent moving average of 10 values. (c) 3D model of yeast genome, as provided by Duan et al., rendered using VMD software. As an example, a region rich in cis interactions on chromosome 4 is highlighted in dashed box. (d) \( Z \)-normalized number of cis and trans interactions of each locus on chromosome 4 as a function of chromosomal coordinates. Boxed area highlights the same cis-rich region as in (c). Data plotted in a, b, and d are given in the Supplementary Table S2. This figure is available in black and white in print and in colour at DNA Research online.
under distinct evolutionary constraints. We, therefore, attempted to delineate the functional attributes that explained best the observed distinction of cis and trans chromatin interactions by analysing the datasets of cis and trans chromatin interactions independently.

Multi-dimensional genomic datasets, i.e. data where measurements were done for all the yeast genes across several different time-points or conditions, were used to calculate similarity (mostly Pearson’s correlation coefficient; Fig. 2; Table 1; Materials and Methods) between interacting genes. Analysis of correlations among several functional attributes suggested that the frequency of trans interactions was best correlated with the similarity in % replicated DNA or co-replication (‘rep’, Table 1) of interacting loci ($r = 0.47$, $P$-value < $10^{-4}$, Fig. 3a). Interestingly, co-expression of genes (i) through cell cycle (cct), (ii) following transcription factor perturbation (tfp), and

Figure 2. Flow chart of overall analysis. Pairs of interacting genes were obtained from chromatin interaction data and split into cis and trans pairs. For each interacting pair of genes, epigenetic/functional similarity was calculated, mostly by calculating Pearson’s correlation between attribute profiles across conditions or time points. Vectors of cis and trans interaction frequencies were compiled and binned into equal size of 1 unit change in interaction frequency. The functional similarity scores were accordingly averaged for each bin. The resulting interaction frequency and functional similarity matrices (Y and X) were than subjected to correlation and PLSR analysis. The functional attributes that exhibited best association in the multivariate analyses were subjected to comprehensive follow-up analyses. This figure is available in black and white in print and in colour at DNA Research online.
Correlation analysis does not always imply if a variable independently accounts for the observed response (interaction frequency in this case) or it is due to co-linearity among variables. To address this, we performed PLSR of the variables to identify the ones that explained the observed variance in the frequency of chromatin interactions. The analyses suggested that 32.3 and 36.9% of total variance in trans and cis interaction data, respectively, were explained by one major component, primarily comprised of co-replication (rep) followed by similar susceptibility to chromatin factor perturbations (cfp) of interacting genes in case of trans interactions and co-fitness (cof) followed by functional similarity (fsm) of interacting genes in case of cis interactions (Fig. 3d and e). The second best component explained only 5.6 and 3.9% of variance in trans and cis interaction frequency, respectively. Details of relative contributions of functional attributes to each component were given in the Supplementary Fig. S5. Importantly, second component was also associated with co-replication to a large extent (second best contributor) in case of trans interactions and co-fitness (cof) followed by functional similarity (fsm) of interacting genes in case of cis interactions [Supplementary Fig. S5]. Interestingly, the cell-cycle-related co-expression (cct) of genes, in general, did not show significant contributions to the first two components in PLSR analysis of either trans or cis interactions. Overall, the PLSR and the correlation analyses concomitantly suggested that the trans interactions in yeast were primarily shaped by similarity in replication states of interacting loci, while cis interactions were mainly constrained by co-fitness of interacting genes highlighting an overall distinction of constraints shaping cis and trans chromatin interactomes.

### 3.2. trans and cis interactions associate with the coordination of early and late replication, respectively

It has been shown that the early firing, but not the late firing, origins from different chromosomes non-randomly collide with each other in the nuclear space. We first confirmed this observation ($P$-value = 0.07, 0.09, and 0.13, respectively; Fig. 3a, Supplementary Fig. S2). On the contrary, cis interactions among loci, which were at least 20 kb apart, did not exhibit strong correlation with co-replication ($P$ = 0.14; $P$-value < 10^{-3}; FDR > 0.01) and were, instead, strongly correlated with the co-fitness of interacting genes as measured through chemical genomic screens ($P$ = 0.25; $P$-value < 10^{-4}; FDR < 0.01; Fig. 3b). Again, the general cell-cycle-related co-expression of genes showed weak correlation, though slightly higher compared with the trans interactions ($P$ = 0.11 vs. 0.07). To further scrutinize the significance of observed correlations, we rewired the chromatin interactions using the strategy given in the ‘Materials and methods’ section to obtain null distribution and calculate P-values (Supplementary Fig. S3). Examples of correlograms generated from rewired cis and trans interactions were shown in Supplementary Fig. S3a and b, and their comparisons with observed values of correlations were drawn in Supplementary Fig. S3c and d. In particular, we confirmed that (i) the correlation of trans interaction frequency with the coordinated replication and that of cis interaction frequency with the co-fitness of interacting genes were (i) significant compared with rewired control ($P$-value = 1.0e-09 and 3.3e-09, respectively, FDR < 0.001 for both comparisons, Supplementary Fig. S3a-e); and (ii) significantly different for cis and trans interactions, i.e. correlation with ‘rep’ was significantly higher for trans ($P$-value = 3.1e-12) and lower for cis, while correlation with co-fitness was significantly higher for cis ($P$-value = 8.5e-08) and lower for trans interactions. We also confirmed these observations for the chromatin interactions that were concomitantly captured in the HiC-library generated using EcoRI restriction enzyme, highlighting the robustness of the analyses (Supplementary Fig. S4a and b). We further cross-validated the differential association of cis and trans interaction frequencies with the coordinated replication (rep) and co-fitness (cof) of engaged genes using recently published HiC data-set of exponentially growing budding yeast cells (Fig. 3c).

### Table 1. Abbreviations used for different kind of functional similarities between genes

| Abbreviations | Attribute | Similarity | No. in Supplementary Table S1 |
|---------------|-----------|------------|-------------------------------|
| frq           | Interaction frequency | cis and trans chromatin interaction frequencies between genes were taken from Duan et al. and normalized using HiCNorm package | 1 |
| cct           | Cell cycle time course | Pearson correlation between time course expression values of interacting genes | 2 |
| cfp           | Chromatin factor perturbation | Pearson correlation between expression values of interacting genes across chromatin factor mutant strains | 3 |
| tpf           | Transcription factor perturbation | Pearson correlation between expression values of interacting genes across transcription factor mutant strains | 4 |
| env           | Environmental response | Pearson’s correlation between expression values of interacting genes across different environmental conditions | 5 |
| ace           | Acetylation | Pearson’s correlation between ChIP enrichment values of interacting gene promoters across different histone acetylations | 7 |
| met           | Methylation | Pearson’s correlation between ChIP enrichment values of interacting gene promoters across different histone methylations | 8 |
| rep           | Co-replication | Pearson’s correlation between % replication DNA of 500 bp genomic bins, as provided by the authors, mapping to interacting pairs of genes | 9 |
| cof           | Co-fitness | Co-fitness of interacting genes were directly taken from this study | 11 |
| ppi           | Protein-protein interaction | Socio-Affinity (SA) index, which measures the log-odds of observed number of times two proteins interact relative to the expected value deduced from their frequency in the dataset, were obtained for each protein-protein interaction | 14 |
| fsm           | Functional similarity | Functional similarity (fsm) was calculated using ‘GOSemSim’ R-package. | 16 |
| erc           | Evolutionary rate co-variation | Evolutionary rate co-variation (erc) was taken as it is from the source | 17 |
Interestingly, we observed that the late, but not the early, firing origins non-randomly interacted in cis (P-value = 1.9e-02; Fig. 4a), suggesting that the close physical proximity might be a common property of origins of replication and that early origins were trans-interacting and late origins were cis-interacting. Early and late origins showed significantly lesser number of cis and trans-interactions with each other compared with null distribution, i.e. early-to-late interactions were under-represented compared with null distribution, strongly suggesting that they do not interact with each other in cis or trans (P-value$_{cis}$ = 0.003, P-value$_{trans}$ = 0.012; Supplementary Fig. S6). It is noteworthy that the significant P-values in the Fig. 4a did not approach extreme partly due to small sample sizes ($n = 78$ and 122 for early and late origins) and stringent null models.

These observations suggested that the correlation between trans interaction frequency and correlated replication seen in Fig. 2a might be associated with the non-random trans interactions among early origins. On similar lines, relatively weaker correlation ($\rho = 0.14$) of cis interaction frequency with the coordinated replication might be
associated with the non-random cis interactions among late origins (Fig. 3b). To further explore whether trans and cis interactions associate with the coordination of early and late replication, respectively, we split the yeast genome into early and late replicating domains as per data from McCune et al.41 and accordingly compiled early-to-early and late-to-late interaction categories. Early-to-early (EE) interaction category exhibited significant correlation between coordinated replication and the trans, but not the cis, interaction frequency ($\rho_{\text{trans}} = 0.37$, $\rho_{\text{cis}} = 0.0$; Fig. 4b). On the contrary, in the late-to-late category, cis and trans interactions showed marginally differing correlations with coordinated replication ($\rho_{\text{cis}} = 0.26$, $\rho_{\text{trans}} = 0.29$; Fig. 4b). However, importantly, the cis interaction frequency in the late-to-late category showed greater significance and power of correlation with the coordinated replication compared with the trans interaction frequency ($P\text{-value}_{\text{cis}} = 8.7 \times 10^{-4}$, $P\text{-value}_{\text{trans}} = 2.7 \times 10^{-4}$; power$_{\text{cis}} = 0.99$ and power$_{\text{trans}} = 0.83$; Fig. 4b). Since the Pearson’s correlation coefficient decreases and the power of the observed effect increases exponentially as the sample size increases, we reason that the differing sample sizes of cis and trans interactions of late replicating regions ($n = 711$ and 146 binned values, respectively) explain the observed difference in significance of apparently similar correlation values. It is also notable that the cis interactions, as a whole, were only weakly associated with the coordinated replication as demonstrated in Fig. 3b, the significance of correlation of late replication with the cis interactions was, therefore, non-trivial. This is also reinforced by our earlier observation that late origins interacted in cis, but not in trans. These results supported that the trans and cis interactions were organized vis-a-vis to correlated early and late replication states, respectively, possibly marking spatial segregation of early and late replication factories. Furthermore, replication is the major cause of mutations in the genome, and correlation between replication timing and mutation rates has been observed in the past.42 We, therefore, asked whether spatially proximal loci exhibited similar mutation rates. We obtained synonymous single-nucleotide substitution rates, which were adjusted for neutrality, for this purpose (Supplementary Table S1). Again, we observed stronger correlation between similarity in mutation rates and the trans interaction frequency and relatively weaker correlation with the cis interaction frequency ($\rho_{\text{cis}} = 0.14$, $\rho_{\text{trans}} = 0.36$; $P\text{-value}_{\text{cis}} = 0.63$, $P\text{-value}_{\text{trans}} = 2.2e^{-05}$; Supplementary Fig. S7). Early-to-early interactions in trans and late-to-late interactions in cis exhibited greater significance of correlations with the similarity in mutation rates of engaged genes ($P\text{-value}_{\text{EE,trans}} = 0.15$, $P\text{-value}_{\text{EE,cis}} = 1.4e^{-03}$, $P\text{-value}_{\text{LL,trans}} = 5.4e^{-02}$, $P\text{-value}_{\text{LL,cis}} = 0.46$; Fig. 4b), highlighting a possible role of 3D genome organization in mutagenic mechanisms that might have shaped the co-evolution of spatially proximal genes in yeast. We hypothesize that the genes sharing the same sub-nuclear compartment might experience similar microenvironment that causes single-nucleotide mutations during replication or similar deficiency/efficiency of DNA repair factors.

### 3.3. trans and cis interactions exhibit differential preference for nucleosome occupancy

Since the process of replication is intricately linked with the chromatin structure, we tested whether the observed distinction between trans

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**Figure 4.** Association of cis and trans chromatin interactions with the coordinated replication and co-varying mutation rate. (a) Spatial connectivity (observed/expected number of interactions) among CLB5-independent early firing origins and CLB5-dependent late firing origins, with respect to null distribution generated through re-sampling-based method. Cartoon on the left-hand side represents the atypical cell-cycle curve representing number of cells (y-axis) as a function of replicated DNA content (x-axis). The first peak represents cells in G1 phase with 2N DNA followed by S-phase, and the second peak representing cells in G2 phase with 4N total DNA. The vertical bars in the cartoon represent early and late S-phase respectively. (b) Significance of correlation between interaction frequency and coordinated replication (upper panel), and similarity of mutation rate (lower panel) for cis and trans interactions. Interactions are split into EE (early-to-early) and LL (late-to-late) categories. Horizontal dotted lines represent P-value of 0.05. Over each bar, corresponding Pearson’s correlations and 95% confidence values are given. Datasets of b are given in the Supplementary Tables S5 and S6. This figure is available in black and white in print and in colour at DNA Research online.
and cis interactomes was associated with nucleosome occupancy and explains their association with early and late replication, respectively. Z-normalized nucleosome occupancy in 3 kb bins across yeast genome was plotted against the number of cis, trans interactions, and the ratio of the two. The plots clearly showed significantly negative and positive correlations with cis and trans interactions, respectively ($\rho_{cis} = -0.12, P_{trans} = 0.13; P_{cis} < 2.2e^{-16}, P_{trans} < 2.2e^{-16};$ Fig. 5a). Accordingly, the ratio of number of cis and trans interactions inversely scaled with the nucleosome occupancy suggesting that cis and trans interactions, in general, associate with low and high nucleosome occupancy, respectively ($\rho_{cis/trans} = -0.10; P_{cis/trans} = 7.2e^{-08};$ Fig. 5a). The Pearson’s correlation coefficients observed in the Fig. 5a, though statistically significant, were relatively small in magnitude, which can be attributed to large sample size ($n = 4,031$). It is also noteworthy that the present study does not aim to claim the scaling of cis and trans interactions with the nucleosome occupancy, but the distinct level of nucleosome occupancy of regions significantly enriched with cis or trans interactions. Therefore, we identified the cis- and trans-rich regions using sliding window (20 kb) approach and applying cut-off for cis/trans peaks as 50% of maximal window value in the genome (Fig. 5b). Demarcating the regions that were enriched with cis- or trans interactions clearly highlighted greater nucleosome occupancy in the regions enriched with trans interactions and vice versa ($P_{cis/trans} < 2.2e^{-16};$ Fig. 5b, boxplot). These observations suggested that the nucleosome-depleted ‘open chromatin’ regions in the yeast genome were more likely to fold in cis, possibly highlighting a mechanism of spatially insulating the highly transcribing open chromatin from the nucleosome-rich repressive or regulated domains. We indeed observed that cis-enriched, nucleosome-depleted domains had relatively higher transcriptional activity compared with trans-enriched regions ($P = 5.0e^{-03};$ Fig. 5b, boxplot). We also confirmed that these correlations were not the artefacts of abundant trans interactions among centromeres, which are generally heterochromatized and might show higher nucleosomal occupancy. We removed the regions that were proximal (<50 kb) to centromeres and recalculated the correlations. We consistently found significant negative and positive correlations of nucleosome occupancy with the cis and trans interaction frequencies, respectively ($\rho_{cis} = -0.12, \rho_{trans} = 0.19; P_{cis} < 2.2e^{-16}, P_{trans} < 2.2e^{-16}.$)

To reconcile the observation in the context of replication, we assessed the nucleosome occupancy around the early origins and compared with that of late origins. To nullify the differential levels of

Figure 5. Association of cis and trans chromatin interactions with the nucleosome occupancy. (a) Left: Regression plot between the ratio of cis to trans interactions and nucleosomal occupancy. Right: regressions showing relationships between nucleosome occupancy and cis/trans interaction frequency. (b) Linear view of nucleosome occupancy and gene expression (SAGE counts) aligned along the number of cis/trans chromatin interactions. cis-Enriched and trans-enriched regions are highlighted. The peaks were identified as windows having sliding mean (averaged over 10 consecutive windows) greater than the 50% of the maximum window average in the genome. Boxplots on the right-hand side show distributions of nucleosome occupancy and SAGE counts in the cis-rich and trans-rich regions. (c) Left panel: average nucleosome occupancy (G1 phase) around ±10 kb of early and late origins. Right panel: average nucleosome occupancy profile around early and late origins. Shades represent the standard errors across all the early and late origins respectively. The datasets of b and c are given in Supplementary Tables S7 and S8, respectively. This figure is available in black and white in print and in colour at DNA Research online.
replicated DNA at early and late origins during S phase, we considered nucleosome occupancy in G1 phase of cell cycle only. Since we showed that the \textit{trans} interactions were associated with early origins as well as with higher overall nucleosome occupancy, by inference, we expected the early origins to be associated with the higher nucleosome occupancy and vice versa for late origins. Indeed, we confirmed greater overall nucleosome occupancy around early origins compared with late origins (±10 kb, \textit{P}-value = 5.0e-02, Fig. 5c). Interestingly, by analysing the nucleosome occupancy at single nucleosome resolution, we observed that the early origins were located in a narrow (~350 bp) nucleosome-depleted region (NDR) despite having an overall higher nucleosome occupancy (Fig. 5c). Such nucleosomal organization had been shown to facilitate the replication initiation. Late origins, on the contrary, were located in a relatively wider NDR (~750 bp), significance of which is not entirely clear yet (Fig. 5c). However, we speculated that the late origins might need accessibility to additional sequence features for the regulatory factors involved in late replication. Indeed, such sequence features have been proposed earlier by others too.\textsuperscript{44,45} Role of \textit{cis} regulatory elements in late firing of origins is further discussed in the Discussion section.

3.4. Association of \textit{cis} interactions with the essential gene clusters

Strong correlation between \textit{cis} interaction frequency and the co-fitness of interacting genes suggested that the genes with similar fitness defects across different environments tend to co-localize more frequently. This would also mean that genes with the same extreme growth defects, like lethality, would tend to interact with greater interaction frequency. We, therefore, tested whether the essential genes were interacting with each other in \textit{cis} with a significantly greater frequency compared with random null model. We extracted the \textit{cis} and \textit{trans} interactions that had essential genes on both restriction fragments. Indeed, the resampling approach uncovered significantly greater frequency of \textit{cis} (\textit{P}-value = 5e-02), but not the \textit{trans} (\textit{P}-value = 0.16), interactions among essential genes (Fig. 6a). Essential genes are generally located in the regions having an overall low nucleosome occupancy and consequently exhibit lower expression noise.\textsuperscript{46} We, therefore, propose a hypothesis that the essential genes should also be relatively stable in the three-dimensional nuclear space to keep the expression noise low. Greater mobility might introduce noise in the expression, which can be deleterious in case of essential genes. We presumed that a stable locus would be expected to show: (i) high average value of its interaction frequencies to all its \textit{cis}-interacting partners, that we referred as ‘average interaction frequency’; (ii) relatively short-range interactions and lower variance in the genomic distances between interacting loci; and (iii) greater consistency of chromatin interactions across biological replicates in HiC experiments, measured as overlap of interactions of a locus commonly captured in \textit{Eco}RI and \textit{Hind}III HiC libraries to all its interactions captured in \textit{Hind}III library. Although it is not possible to distinguish biological variations from technical variations in the present scenario, some studies have shown that variation between biological replicates are largely biological than technical and can be attributed to genetic, epigenetic, or

Figure 6. Association of \textit{cis} interactions with the gene essentiality. (a) Average frequency of \textit{trans} and \textit{cis} interactions among essential genes overlaid on the null distribution of average values across 1,000 random samples. Cartoon on the top depicts all \textit{cis} interactions of a locus (grey-coloured node), from which the average interaction frequency of that locus was calculated. (b) Average frequency of \textit{cis} interactions of genomic loci having and not having essential gene clusters. (c) Mean and variance of genomic distances between interaction sites at genomic loci having and not having essential gene clusters. Cartoon on the top represents the genomic distance between \textit{cis}-interacting sites of grey-coloured node. (d) Average \textit{cis} interaction frequency as a function of relative distance from the essential gene cluster (defined as >2 essential genes in 5 kb window). (e) Average \textit{cis} interaction frequency (moving average of three consecutive restriction fragments) and essential gene density along Chromosome 3. Data plotted in b–e are given in the Supplementary Table S9. This figure is available in black and white in print and in colour at \textit{DNA Research} online.
stochastic mosaicism of cells. 47–52 The chromatin loci exhibiting greater consistency of chromatin interactions in biological replicates can, therefore, be considered relatively restrained in the nuclear space compared with the ones showing greater variations. By applying the aforementioned measures, we observed that the loci having essential gene clusters (>2 gene in 5 kb bin) consistently showed: (i) higher average interaction frequency (P-value = 3.8e–06; Fig. 6b), (ii) relatively short-range interactions as depicted by mean distance between interacting sites (P-value = 2.9e–02; Fig. 6c); (iii) lower variance in genomic distances between interacting sites (P-value = 3.5e–02; Fig. 6c); and (iv) greater overlap between biological replicates of HiC data, compared with rest of genome (Supplementary Fig. S8). Further, the average interaction frequency decreases as a function of genomic distance from the essential gene clusters, marking the selective enrichment of high frequency interaction on and around essential gene clusters (Fig. 6d and e, Supplementary Fig. S9). Thus, the higher average interaction frequency, relatively short-range interactions of lower variance, and consistency across HiC replicates might suggest spatially clustered and stable nature of genomic regions having essential gene clusters. We also confirmed the link between gene essentiality and the average interaction frequency analysing average fitness defect, as measure from chemical genomics data, at sites of high average interaction frequency (P-value = 9.0e–06; Supplementary Fig. S10).

3.5. **cis** interactions are constrained by minimization of expression noise

To directly test whether or not spatially restrained loci were associated with the lower noise in the expression, we extracted high noise and low noise genes (upper and lower quartile of genome-wide abundance-corrected expression noise data) and assessed their average interaction frequencies, mean and variance of genomic distances between interacting sites, and the overlap ratio of their interactions captured in the HiC replicates. The genes with low expression noise exhibited greater average interaction frequency (P-value = 2e–02; Fig. 7a), relatively lower mean and variance of genomic distances between interacting loci (P-value (mean) = 9e–03, P-value (variance) = 4e–02; Fig. 7b), and greater overlap ratio of interactions in the HiC biological replicates (P-value = 3.3e05; Fig. 7), supporting the hypothesis that spatially restrained loci would tend to have lower expression noise. Indeed, the expression noise of essential genes inversely scaled with the average **cis**, but not the trans interaction frequency of the loci (Supplementary Fig. S11). Further, it is known that the genes that are toxic when over-expressed also exhibit low expression noise. 46 We, therefore, tested whether these genes are also spatially constrained by high frequency of **cis** interactions. Surprisingly, genes exhibiting over-expression toxicity did not show significantly greater average interaction frequency as in the case of essential gene clusters (Supplementary Fig. S12). We propose that spatially restrained or ‘tethered’ property is specifically associated with the mechanism that minimizes stochastic loss of expression but not the abrupt gain of expression.

To further scrutinize our observations, we performed following additional analyses. (i) We identified the regions of significantly greater average frequency of **cis** interactions from the interactions commonly present in the two HiC replicates. The distribution plot of average **cis** interaction frequency across yeast genome revealed a distinct population of regions having significantly greater interaction frequency

![Figure 7](https://academic.oup.com/dnares/article-abstract/23/2/155/1745431)

**Figure 7.** Association of **cis** interactions with expression noise and chromatin fluctuations. (a) Distribution of average **cis** interaction frequencies for low (≤1st quartile) and high noise (≥3rd quartile) genes. Cartoon on the top depicts all **cis** interactions of a locus (grey-coloured node), from which the average interaction frequency of grey-coloured locus was calculated. (b) Distribution of mean and variance of genomic distances between interacting sites for low and high noise genes. Cartoon on the top represents the genomic distance between **cis**-interacting sites of grey-coloured node. (c) Distribution of overlap ratio of chromatin interactions between HindIII and EcoRI HiC replicates for low and high noise genes. Horizontal dotted line represents the genome-wide median value. (d–f) Scatter plot between and experimentally determined chromatin fluctuations and (d) average **cis** interaction frequency, (e) expression noise, and (f) variance of genomic distances between interacting sites on yeast Chromosome 12. (g) Expression noise, average **cis** interaction frequency, variance of genomic distances, and chromatin fluctuations aligned along Chromosome 12. Data plotted in a–c and d–g are given in Supplementary Tables S9 and S10, respectively. This figure is available in black and white in print and in colour at *DNA Research* online.
than the rest (Supplementary Fig. S13a). This is analogous to identifying ‘peaks’ in ChIP-Seq genomic tracks. The ‘peak’ nature of certain regions was also confirmed by plotting QQ plot of the average interaction frequency against a random normal distribution (Supplementary Fig. 13b and c). We extracted the genes located in these regions and compared the expression noise with the null distribution. Again, we observed significantly lower (P-value = 1e–03) noise for the genes located in the regions of high average interaction frequency. (ii) Lower diversity of interactions might also represent relatively stable state of a locus. We measured the variation in chromatin environment using coefficient of variation (σ/μ) and observed that the genes having greater variation in their chromatin interactions exhibited higher expression noise (P-value = 3e–02; Supplementary Fig. S14). (iii) More importantly, we compared the experimentally determined chromatin mobility of several loci with their average cis interaction frequencies, variances of distances between interacting sites, and the expression noise (Fig. 7d–g, Supplementary Fig. S15). The analyses showed striking correlation of chromatin fluctuations with the expression noise (ρ = 0.78, Fig. 7d), moderate correlation with the variance of genomic distances between interacting loci (ρ = 0.39, Fig. 7e), and strong anticorrelation with the cis interaction frequency (ρ = −0.65, Fig. 7f), strongly supporting our proposal that the chromatin mobility associates with the expression noise of the underlying genes.

Although we largely restrict all our claims to the budding yeast genome, we now present a few lines of evidence which suggest that minimization of expression noise through physically restrained environment might be a general evolutionary constraint shaping the genome organization in other radically different systems like bacteria and the mammalian cells. The expression noise data had been available for Escherichia coli too and very recently genome organization data of E. coli have also been generated using Genome Conformation Capture technique. It would, therefore, be interesting to test whether the proposed relationship between interaction frequency and expression noise also exists in Bacteria. We performed similar analyses in E. coli by taking normalized interaction frequency data for 10 kb bins as provided by Xie et al., number of essential genes and the maximal expression noise value in each bin. We found strikingly similar association among gene essentiality, expression noise, and average interaction frequency in E. coli, as in yeast (Fig. 8a–c). This is an important observation highlighting an evolutionarily conserved association between expression noise and genome organization despite having radically distinct mechanisms of gene expression. Further, genome-wide single-cell gene expression data are now available for several model systems. We obtained one such dataset for mouse embryonic stem cells (mESCs), for which genome-wide chromatin interaction data was also available. We calculated the noise in gene expression for mESCs and corrected for the transcript abundance using residuals of lowess fit between transcript abundance and noise. Again, the average interaction frequency of low noise genes was significantly greater than that of high noise genes (P-value = 3.0e–11; Figure 8. Association of chromatin interactions with the expression noise in other systems (a) Average frequency of interactions among genomic bins having essential genes overlaid on the null distribution of average values across 1,000 random re-samplings in Escherichia coli. (b) Violin plots of average interaction frequencies of loci having distinct densities of essential genes per 5 kb in E. coli genome. The categories >0, >1, and >2 are inclusive and not mutually exclusive. (c) Distribution of interaction frequencies for low (≤1st quartile) and high noise (≥3rd quartile) genes in E. coli. (d) Distribution of interaction frequencies for low (≤1st quartile) and high noise (≥3rd quartile) genes in mESCs. (e) Distribution of number of RNAPII-associated chromatin interactions of low and high noise genes in mESCs. Mapped E. coli and mESC datasets are given in Supplementary Table S11 and S12. This figure is available in black and white in print and in colour at DNA Research online.
Fig. 8d). Due to lack of whole-genome single-cell gene expression data along with HiC data on other cell types of mammalian genome, our observation on mESCs is presently not generalizable to different cell types in higher eukaryotes.

The above analyses clearly strengthened our proposal that essential genes exhibit lesser variation in its chromatin environment and that such a property contributes in reducing the expression noise of engaged loci.

4. Discussion

It is known that spatial positioning of genes in the nucleus is highly non-random. Genes positioned interior to the nucleus experience transcriptionally permissive environment, while the ones located near the nuclear periphery are generally repressed or lowly expressed. Whether or not spatial co-localization of genes plays an active role in regulating essential genomic functions is presently subjected to intense scrutiny. It has been presumed that genes co-localize to synchronize their transcriptional states. Besides sporadic reports of co-expression of individual loci, genome-wide proposal has been made in case of multi-gene complexes (relatively short-range intra-chromosomal interactions) in mammals and for both intra- and inter-chromosomal interactions in lower eukaryotes like yeast. However, these studies are not subjected to comprehensive multivariate analyses and in the absence of other genomic variables, these reports do not present an unbiased view of possible functional constraints shaping the three-dimensional genome organization of eukaryotic genome. Here we analysed several functional attributes of yeast genome to identify the potential constraints of genome-wide chromatin interactions. Our analyses suggested that inter- and intra-chromosomal interactions were under distinct evolutionary constraints. While inter-chromosomal interactions were primarily associated with the coordination of Clb5-independent replication, intra-chromosomal interactions were constrained by the coordination of Clb5-dependent replication and minimization of expression noise of engaged loci. Correlation of interaction frequency with the co-expression of engaged genes was very weak. The correlation coefficient of similar magnitude was also reported earlier by others. However, the authors compared the correlation coefficient with the average correlation of all possible gene pairs in the genome and found the correlation of 0.09 to be significantly higher. We argue that genome-wide average might not serve as an appropriate control, because the sample size of gene pairs will be disproportionately greater for the whole genome compared with the ones that are spatially proximal. Rewired contact networks and re-sampling-based approach can serve as better control. Re-assessment of reports claiming co-localization of co-regulated genes have been questioned elsewhere. More importantly, in the absence of other functional variables, it is not entirely justified to claim that co-expression if the major constraint of genome organization.

The specific association of trans and cis interaction frequency with early and the late replication, respectively, supports the notion of spatially segregated early and late replication factories, which had been proposed earlier by several authors. Interestingly, a recent report has clearly shown that androgen-induced proximity between TMPRSS2 and ERG genes is due to AR-controlled replication, but not the transcription. Further, the association of preferred domains of cis and trans interactions with the low and high nucleosome occupancy, respectively, supports the spatial segregation of open and closed chromatin. The observation that the early origins were flanked by well-positioned nucleosome around a narrow trough of nucleosome-depleted region was in line with earlier reports suggesting the role ORC mediated nucleosome positioning in establishment of pre-initiation complex around early origins. Relatively wider NDRs around late origins can be explained through following arguments: (i) Late firing of origins might need additional cis regulatory elements in the proximity. It is known that after recycling from early origins, replication factors are present in a very limited amount for late origins. Late origins, therefore, cannot afford the delay caused by the remodelling of nucleosomes to open up the binding sites of replication factors. Constitutively open chromatin and spatial co-localization of late origins therefore might help in efficient and rapid usage of replication factors and ensure the complete replication of the genome. (ii) Late origins are known to be present predominantly in the intergenic regions (IGRs) between convergent genes. The abundant occurrence of transcription termination around convergent IGRs can interfere with the nucleosome stability at late origins. The nucleosome depletion around late origins can thus be an artefact of genomic neighbour- hood. (iii) It is known that the radial positioning early origins in the nuclear space is mostly random, while late origins often localize towards nuclear periphery. The accessibility to sequences flaking late origins can help targeting the late origins to nuclear periphery.

The association of chromatin interactions with the mutation rate variation suggests a potential role of spatial genome organization in mutagenic mechanisms. Coordinated replication through spatial convergence might also ascribe similar susceptibility to genetic errors at engaged loci. Such properties had earlier been proposed in the context of cancer genomes.

Intra-chromosomal interactions, on the other hand, were also constrained by co-fitness of genes. It has been proposed that the gene pairs with higher co-fitness represent their close functional similarity. It can, therefore, partly be explained by the co-functionality of cis-interacting genes as shown in the Fig. 2e. We further attempted to explain the link between fitness defect and genome organization by taking essential genes as example. We hypothesized that the high frequency intra-chromosomal interactions restrict the mobility of engaged loci, while low frequency interactions of a locus might represent a relatively mobile state of chromatin. The presumption that the high interaction frequency and the lower coefficient of variation represent spatially restrained loci in genome is also supported by comparison with the experimentally determined mobility of certain loci (Fig. 8d-g, Supplementary Fig. S12). Restricted mobility of interacting loci might be important to reduce the transcriptional noise of interacting genes. We reconciled this hypothesis by taking the example of essential genes. Essential gene clusters are known to exhibit lower expression noise and consistently remain in transcriptionally permissive open chromatin state. Their non-randomly greater frequency of interactions aligns to our hypothesis. It was noteworthy that we did not consider the chromatin interactions between loci which were ≤20 kb apart, and therefore, the linear clustering of essential genes would not have impacted this observation. Further, the association of low noise genes with greater mean interaction frequency of loci and vice versa across bacteria, yeast, and mouse genomes clearly suggests that spatially stable nature of genes assist in minimizing the stochastic transcriptional errors. Indeed, it has earlier been proposed that the long-range chromatin interactions might occur at the cost of increased expression noise. To which sub-nuclear compartment these loci might tether to? We propose that for active genes, the most likely tethering foci would be transcription factories. Though this would need thorough scrutiny, we explored the RNApolII-association chromatin interaction data generated through ChIA-PET technique for this purpose. Multi-gene complexes in that data have been proposed to be equivalent to transcription factories.
transcriptional noise associate with these complexes. We observed that the number of RNAPII-tethered promoter-to-promoter interactions, marking multi-gene complexes, was significantly higher for low noise genes compared with high noise genes (P < 3e-11), suggesting that genes engaged in multi-gene complexes or transcription factories tend to have lower transcriptional noise (Fig. 8e). Other nuclear compartments like nucleoli have been shown experimentally to constrain the chromatin movement, and their disruption leads to increased chromatin mobility.72

Altogether, our unbiased approach of analysing distinct functional constraints provides a different perspective of evolution of three-dimensional genome organizations, which appears to be conserved in bacteria, yeast, and mouse. Escherichia coli genome is known to have an organization tightly linked with the replication,57 and here we showed that it is also associated with gene essentiality and expression noise, very similar to what we observed in yeast. Finally, based on our observations, we propose a model for functional constraints shaping the cis and trans organization of chromatin. This is illustrated in the Fig. 9.

5. Conclusion

The study suggests that there are different set of functional constraints that shape intra- and inter-chromosomal interactomes in eukaryotes. Distinct spatial organization of early and late origins and the underlying coordination of replication strongly support the presence of discrete early and late replication factories. A tethered intra-chromosomal microenvironment might ascribe physical stability to a locus which can be important to reduce the transcriptional noise of engaged loci, particularly, the ones that are functionally indispensable. Therefore, coordinated replication and gene essentiality, not necessarily the co-expression of genes, seem major functional and evolutionary constraints shaping the three-dimensional genome organization of eukaryotes as well as of prokaryotes.

Authors’ contribution

A.S. performed most of the analyses. M.B. helped with the data analysis of mouse embryonic stem cells and performed some of the control analyses. K.S.S. conceived and supervised the project. K.S.S. has performed the statistical tests and drawn the figures.

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Conflict of interest statement

None declared.

Supplementary data

Supplementary data are available at www.dnaresearch.oxfordjournals.org.

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