Nucleus 6:6, 1–5; November/December 2015; © 2015 Taylor and Francis Group, LLC

Topologically Associating Domains: An invariant framework or a dynamic scaffold?

Caelin Cubeñas-Potts and Victor G Corces*
Department of Biology; Emory University; Atlanta, GA USA

Metazoan genomes are organized into regions of topologically associating domains (TADs). TADs are demarcated by border elements, which are enriched for active genes and high occupancy architectural protein binding sites. We recently demonstrated that 3D chromatin architecture is dynamic in response to heat shock, a physiological stress that downregulates transcription and causes a global redistribution of architectural proteins. We utilized a quantitative measure of border strength after heat shock, transcriptional inhibition, and architectural protein knockdown to demonstrate that changes in both transcription and architectural protein occupancy contribute to heat shock-induced TAD dynamics. Notably, architectural proteins appear to play a more important role in altering 3D chromatin architecture. Here, we discuss the implications of our findings on previous studies evaluating the dynamics of TAD structure during cellular differentiation. We propose that the subset of variable TADs observed after differentiation are representative of cell-type specific gene expression and are biologically significant.

Keywords: architectural proteins, chromatin, CTCF, differentiation, epigenetics, heat shock, insulators, TAD, 3D architecture

*Correspondence to: Victor G Corces; Email: vcorces@emory.edu

Submitted: 08/19/2015

Revised: 09/13/2015

Accepted: 09/14/2015

http://dx.doi.org/10.1080/19491034.2015.1096467

Extra View to: Li L, Lyu X, Hou C, Takenaka N, Nguyen HQ, Ong CT, Cubeñas-Potts C, Hu M, Lei EP, Bosco G, Qin ZS, Corces VG. Widespread rearrangement of 3D chromatin organization underlies polycomb-mediated stress-induced silencing. Molecular Cell 2015; 58: 216–31; http://dx.doi.org/10.1016/j.molcel.2015.02.023

Introduction

In recent years, the intricate 3D organization that occurs within a single chromosome has become evident. 3C technologies have demonstrated that chromatin interactions occur in a non-random manner along the chromosome arm, separated into regions of highly interacting chromatin named topologically associating domains (TADs).[1-4] Even more precise interaction mapping has led to the observation that TADs are composed of multiple regions of local chromatin interaction enrichment, which have been named subTADs and contact domains.[1,5,6] In this review, we will highlight our study showing that TAD structure is dynamic in response to heat shock, reevaluate the data analyzing TAD structure during cellular differentiation, and propose that the observed cell-type specific TADs are functionally significant, likely representing novel regulatory element interactions leading to novel gene expression patterns.[3,7]

TAD Structure and Function

Utilizing 2D heatmaps, TADs are readily visualized as regions of enriched chromatin interactions and have been found to span ~880 kb – 1 Mb in mammals and ~60 kb in *Drosophila* (**Fig. 1**).[2-4,8] Because 3C techniques are conducted on large populations of cells, the chromatin contacts observed in TADs likely represent multiple, preferred chromatin conformations in a heterogeneous population of cells.[9] Thus, it is still unclear how many intra-TAD interactions represent chromatin contacts occurring simultaneously within a single cell. TADs are segregated by border regions, which have relatively few chromatin interactions occurring across their loci and are correlated with architectural protein occupancy, high gene density, and active transcription in both flies and mammals.[3,4,10] To date, flies and mammals appear to have one distinction in TAD border identity. In flies, TAD borders are strongly correlated with architectural protein sites that bind > 8 distinct architectural proteins, while mammals have a reverse-forward CTCF motif orientation

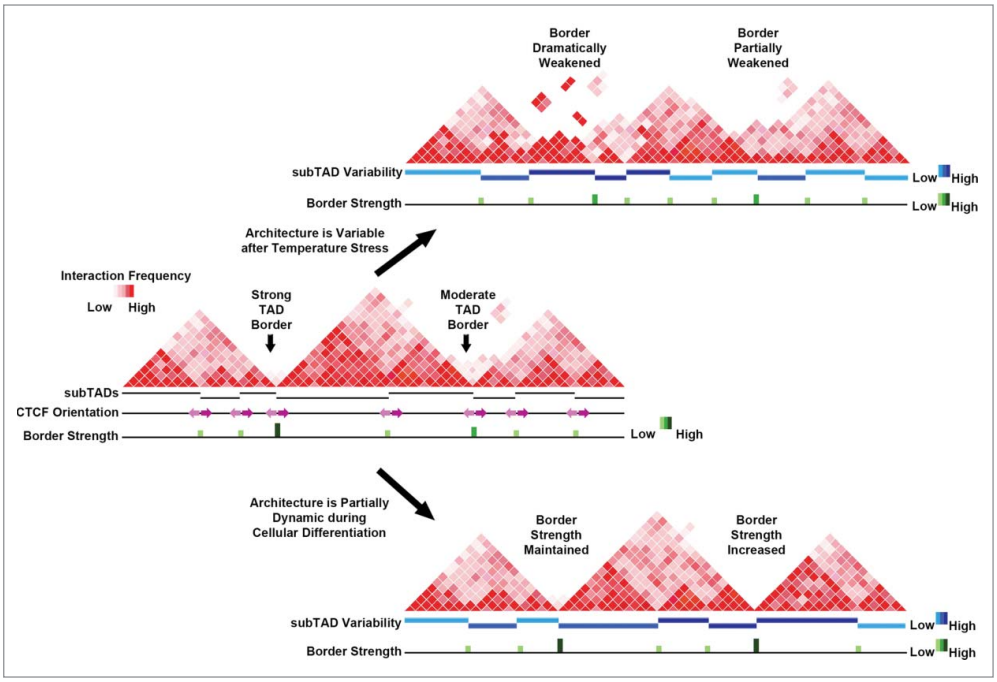

Figure 1. TAD dynamics in response to temperature stress and cellular differentiation. Cartoon schematic illustrating the non-random chromatin interactions occuring along a chromosome, resulting in enrichments that have been named TADs and subTADs. The strength of chromatin interactions is depicted in red. The directionality of chromatin interactions is at least partially regulated by the orientation of CTCF sites in the genome, and has been shown to correlate with both subTAD and TAD borders. A visual depiction of the TAD border strength, a quantitative analysis of the ratio of inter-TAD and intra-TAD interactions surrounding the locus, is shown in green. **Temperature stress**: After heat shock, a dramatic restructuring of the 3D architecture occurs with a striking increase in inter-TAD interactions. Variability is observed in the architecture of both subTADs (shown in blue) and TADs (depicted by changes in border strength). **Differentiation**: During differentiation, cell-type specific enhancer-promoter interactions result in alterations in the subTAD structure (shown in blue). We propose that regions of highly variable subTAD structure also correspond to regions of variable TAD structure (illustrated by the increase in border strength), while regions of low variability in subTADs likely correspond to conserved TADs between cell types.

at TAD borders.[10-12] However, future identification of additional mammalian architectural proteins and a careful analysis of the CTCF motif orientation in *Drosophila* could indicate that the mechanisms of TAD border specification are conserved between mammals and invertebrates. Although the functions of TADs are still poorly understood, TADs have been implicated in replication regulation because TAD borders are highly correlated with the early/late replication domain boundaries and also as important contributors to transcriptional regulation.[13]

There are multiple lines of evidence suggesting that TADs represent compartmentalized regulatory environments. Both fluorescent in situ hybridization (FISH) and 4C analysis have confirmed that intra-TAD interactions occur at a higher frequency than inter-TAD interactions. FISH studies demonstrated that equally spaced genomic loci are in closer spatial proximity if they are intra-TAD compared to inter-TAD, consistent with HiC interactions representing genomic loci interacting in 3D.[1] Furthermore, 4C analyses utilizing cohesin/CTCF binding sites as anchors showed that the anchor interactions occurred predominantly within TADs, confirming the preferential intra-TAD interactions observed by HiC.[14] In addition, there is both correlative and functional evidence to suggest that TAD structure influences which enhancer-promoter interactions occur. First, temporal regulation during development is most highly correlated for genes that are intra-TAD, consistent with genes within TADs having an at least partially shared regulatory environment.[1,15] In addition, a 58 kb deletion in the *Xist* region that removed a TAD border caused ectopic interactions resulting in a partial merging of neighboring TADs and, of particular interest, misregulation of genes within the novel TAD.[1] However, the use of such a large deletion made drawing a direct association between TAD borders and gene regulation challenging. A recent study analyzing inversions and deletions around the WNT6, EPHA4, and PAX3 gene locus, whose precise regulation is required for proper limb development, strongly supports the notion that TAD border elements are critical for the compartmentalization of chromatin interactions. Deletions that included TAD borders or inversions that changed the relative position of genes to the TAD borders both resulted in ectopic interactions extending to the next most proximal TAD border, altered gene expression, and ultimately limb malformations.[16] Furthermore, Lupiañez et al. demonstrated that ectopic interactions only occurred with deletions that included the border element containing the CTCF binding site, providing the strongest evidence to date that TAD structure is functionally relevant in chromatin interaction compartmentalization.[16] However, it is still unclear if the enriched long range chromatin interactions observed within TADs are a cause or simply a consequence of compartmentalized long range chromatin contacts.

Compartmentalization within TADs

TADs are composed of smaller regions of enriched chromatin interactions occurring over shorter genomic distances that have been named subTADs and contact domains (**Fig. 1**).[1,5,6] For example, a genomic locus encompassing 6 TADs in mouse embryonic stem (mES) cells contains 51 subTADs, suggesting that

subTADs are approximately 10-fold smaller in scale than TADs.[5] Consistent with additional compartmentalization within TADs, a single TAD often contains both repressive and active chromatin signatures and genes expressed at similar but distinct levels.[1,2] From a functional perspective, subTADs have been proposed to represent cell-type specific long range enhancer-promoter contacts, because subTADs exhibit a high level of variability during differentiation.[1,5] Thus, understanding the molecular mechanisms governing subTAD interactions could have important implications on the regulation of gene expression. A recent analysis of the protocadherin locus demonstrated that the orientation of CTCF binding sites affects subTAD interactions within this locus. Guo et al. observed that the CTCF motif is arranged in a reverse-forward orientation at the border of subTADs and that this is functionally significant because inversion of the CTCF motif using CRISPR-mediated genome editing changed the directionality of the chromatin interactions.[17] The relevance of CTCF orientation genome-wide was highlighted by analyses showing that interactions defined by CTCF ChIA-PET studies or high resolution HiC are highly correlated with CTCF motifs in the convergent orientation.[6,17] Based on these observations, it is intriguing to speculate that the impact of cell-type specific CTCF occupancy on 3D chromatin architecture will be dictated by the combination of occupancy and orientation of CTCF sites bound in each cell.[18,19] Overall, the discovery of subTADs suggests that the TADs are composed of smaller building blocks of organized chromatin interactions.

The Dynamics of 3D Architecture during Differentiation

A number of studies analyzing TAD dynamics in response to cellular differentiation demonstrated that while the majority of TADs are invariant between cell types, a subset of TADs are variable.[1,3,20] One clear example of conservation of TAD structure between cell types was recently demonstrated for the EPHA4 locus. 4C analysis validated that 3 TADs across the WNT6, EPHA4, and PAX3 locus are conserved between mouse ESCs and limb bud cells and that manipulating the TAD borders resulted in ectopic interactions.[16] However, genome-wide analysis of TAD dynamics during differentiation provided clear evidence that a significant portion of TADs are also variable.[3] Specifically, 678 out of 1967 (34.5%) of TADs detected in human ESCs are not present in lung fibroblasts (IMR90), while 504 out of 1793 (28.1%) of TADs identified in lung fibroblasts are absent in ESCs.[3] Thus, out of the total of 2471 TADs detected in these 2 cell types, only 1289 (52.2%) were found in both ESCs and lung fibroblasts.[3] Thus, the evidence seems to suggest that while a portion of TAD borders are conserved, TAD structure also varies between cell types. We acknowledge that some of the variability in TAD borders during differentiation could be explained by technical variance alone. However, a recent HiC analysis comparing H1 human ES cells, and 4 ES-derived lineages (mesendoderm, mesenchymal stem cells, neural progenitor cells and trophoblast-like cells) found that 85–90% of TADs were reproducible within each cell type, suggesting that the reported variation in TAD borders between ESCs and lung fibroblasts could be biologically significant.[3,20]

Of particular interest to this discussion is the fact that cell type specific chromatin interactions have been detected by HiC methods but have been attributed to alterations in subTAD structure.[1,5] There are many demonstrations of cell type specific subTADs in the literature. For example, HiC analysis of the protocadherin locus in SK-N-SH, H1 hESCs, and nuclear progenitor cells showed that the 2 subTADs encompassing the Pcdhα and Pcdhβ/γ locus were largely conserved while the neighboring 6 subTADs showed a high level of variability.[17] In addition, *in vitro* differentiation of mESCs with retinoic acid caused the loss of a subTAD over the *HoxD* locus.[21] These locus-specific observations along with 5C studies have led to the conclusion that subTADs represent cell type specific enhancer-promoter interactions.[1,5] A genome-wide analysis of high resolution chromatin interactions, which were comparable in size to subTADs, between 6 different human cell types demonstrated that 68.2% and 58.9% of chromatin loops found in B-lympho-blastoid cells were conserved between mesodermal cell lines compared to nonmesodermal cell lines, respectively.[6] Thus, it is plausible that the variable chromatin interactions observed between cell types is biologically significant and gives rise to the variability detected between subTADs during differentiation.

It seems unlikely that subTADs undergo dynamic changes during differentiation while TADs remain static because of 2 particular lines of evidence. First, one recent polymer modeling analysis, which was robustly tested against experimental 5C data, demonstrated that disrupting intra-TAD interactions caused a reciprocal increase in inter-TAD interactions, suggesting that these 2 events are inextricably linked.[9] Consistent with this hypothesis, nearly all of the experimental manipulations of TAD structure including heat shock stress, knockdown of individual architectural proteins, or transcriptional inhibition have affected both intra- and inter-TAD interactions in a reciprocal manner.[7,14,22,23] Thus, if subTAD structure is changing, which is representative of intra-TAD interactions, one would expect an alteration in the TAD structure itself. Second, subTAD and TAD borders appear to be formed by an at least partially conserved molecular mechanism. Both subTAD and TAD borders are correlated with specifically oriented CTCF motifs, suggesting that specific CTCF occupancy could be a critical regulator in determining the directionality of 3D chromatin interactions.[11,17] Because CTCF occupancy has been shown to be regulated by DNA methylation in a cell-type specific manner, one would then hypothesize that both subTAD and TAD border occupancy would be affected.[18,19] However, we cannot discount that there other distinct molecular mechanisms differentiating subTAD and TAD borders. For example, conserved CTCF sites are enriched at TAD borders compared to intra-TAD regions, which could explain why subTAD borders are more affected by cell-type specific CTCF occupancy.[11] Even with this distinction in CTCF conservation between TAD and subTAD borders, we propose that because the

molecular mechanisms generating subTAD and TAD borders are at least partially shared, one would expect both subTAD and TAD borders to be affected to some extent during cell-type specification.

Finally, we propose that the observed changes in TAD structure during differentiation may become even more dramatic if a quantitative measure of border strength, which quantifies the ratio between inter-TAD interactions and intra-TAD interactions on both sides of the border, is utilized rather than the presence or absence of a TAD border.[7,10,14] Many studies that evaluate TAD conservation between cell types show that genome-wide chromatin interactions are highly correlated, which is consistent with a conserved TAD structure.[1,14,20] However, a more quantitative analysis of TAD borders may reveal that although a TAD border does not change physical position between cell types, the strength of the border may vary between cell types, indicative of novel, biologically significant chromatin interactions. For example, a recent analysis of the differentiation of human ES cells into 4 separate lineages demonstrated that 36% of the genome switches between the active A and inactive B genomic compartments, with a subset of the switched regions corresponding to a single TAD.[20] Because two genomic loci within the same compartment interact more frequently than loci in separate compartments, one would predict that the border strength between 2 neighboring TADs in compartment A would strengthen if one TAD switches to compartment B during differentiation.[24] Thus, we support the model that TAD structure is at least partially plastic during cellular differentiation and undergoes remodeling consistent with cell type specific transcriptional profiles.

The Dynamics of 3D Architecture after Stress

To test the hypothesis that alterations in 3D chromatin architecture are functionally representative of differential gene expression profiles, we evaluated the impact of heat shock, a physiological stress that induces a rapid change in transcription, on chromatin interactions.[7] We utilized HiC to map the chromatin interactions after heat shock and observed a striking redistribution of interactions compared to normal temperature: a decrease between neighboring loci within TADs and an increase in long range inter-TAD chromatin interactions.[7] Notably, the novel heat shock-induced chromatin interactions occur among enhancers and promoters over very long distances, and are enriched for Polycomb, suggesting that the altered 3D chromatin interactions may be a mechanism for establishing the transcriptional cessation that occurs after heat shock.[7] To determine whether these altered chromatin interactions affect the global 3D architecture, we measured TAD border strength.[10,14] We observed a reduction in the border strength for the majority of HindIII fragments across the genome after heat shock, consistent with the observed increase in inter-TAD interactions and a weakening of the TAD borders defined under normal temperature.[7] Notably, a subset of fragments exhibited an increase in border strength, possibly indicating the formation of novel TAD borders after heat shock.[7] Altogether, these observations indicate that TAD structure is dynamic in response to stress-induced changes in gene expression.

Next, we investigated the molecular mechanisms regulating heat shock-induced changes to 3D chromatin architecture. Because heat shock is associated with both transcriptional downregulation and the redistribution of architectural protein occupancy, we conducted experiments to distinguish the contribution of both to TAD plasticity. We conducted HiC on cells treated with 2 transcriptional inhibitors, triptolide or flavopiridol, and measured the effects on global 3D chromatin structure. Consistent with heat shock treatment, both triptolide and flavopiridol induced a decrease in intra-TAD interactions, an increase in inter-TAD interactions, and alterations in TAD border strength.[7] However, the changes in chromatin architecture after transcriptional inhibition were far less pronounced than after heat shock, suggesting that other mechanisms also contribute to TAD dynamics. Consistent with a functional role of architectural proteins in driving TAD dynamics, we observed a strong correlation between architectural protein occupancy and changes in border strength after heat shock. The architectural protein occupancy decreased at the TAD borders defined under normal temperature and redistributed to non-border sites, indicative of the formation of novel heat-shock specific TAD borders and a partial loss of the normal temperature TAD borders.[7] To assess the functional contribution of architectural proteins, we conducted knockdown experiments of 2 different architectural proteins: Rad21, a cohesin subunit, and CAPH-2, a condensin II subunit. Similar to transcriptional inhibition, the knockdown of individual architectural proteins caused analogous but weaker changes to 3D architecture when compared to heat shock. Notably, cells depleted of Rad21 and subjected to heat shock showed a marked increase in border strength compared to wild type cells subjected to heat shock, indicating that Rad21 may be critical in mediating the novel chromatin interactions that induce heat shock-mediated changes in 3D chromatin architecture.[7] Altogether, the knockdown analysis and observed architectural protein redistribution after heat shock strongly suggest that architectural protein occupancy and their functional role in mediating long range chromatin interactions is a key contributor to the plasticity of 3D architecture.

Conclusions

In this review we highlight studies demonstrating that, although a large fraction of TAD structure is invariant between cell types, a subset of TADs are variable. We speculate that the observed differences in TAD organization between cell types are a biologically significant representation of cell-type specific gene expression. This is supported by the observation that changes in gene expression during a physiological response to temperature stress is accompanied by changes in 3D chromatin architecture. Of particular interest, the effects on 3D architecture observed after heat shock stress do not strongly alter the appearance of TAD structure when visualized on 2D heatmaps, similar to what has been observed during cellular differentiation. Thus, we suggest that the alterations in 3D chromatin organization during differentiation may be more significant than previously reported if a

quantitative analysis such as TAD border strength is utilized instead of measuring conservation of TAD borders. Because TADs are representative of 3D chromatin interactions, we believe that a partially conserved and variable TAD structure is more consistent with the transcriptional profiles observed between different cell types. The conserved 3D chromatin contacts between cell types likely represent enhancer-promoter interactions of housekeeping genes, while the variable TAD structure is generated by cell type specific long range interactions.

Furthermore, our analyses of 3D chromatin architecture during the heat shock response suggest that while both transcription and architectural protein occupancy contribute to the stress-induced changes in chromatin interactions, architectural proteins may play a more important role. This finding has key implications in understanding the mechanisms regulating the dynamics of cell-type specific TAD structure. We propose that novel cell-type specific enhancer-promoter interactions are mediated by cell-type specific architectural protein occupancy, resulting in altered chromatin interactions and a subset of variation within the TAD structure. This model could be tested in mammalian cells by analyzing the occupancy and orientation of cell-type specific CTCF sites in the genome and comparing sites of differential CTCF occupancy to changes in the global 3D architecture. Overall, our recent findings and the data highlighted in this review support a model suggesting that cells can modulate architectural protein occupancy in order to alter the 3D chromatin architecture of the genome in response to physiological stress and perhaps differentiation cues to establish new patterns of gene expression.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Funding

Work in the authors' lab was supported by US Public Health Service Award R01GM035463 from the National Institutes of Health. The content is solely the responsibility of the authors and does not represent the official views of the National Institutes of Health.

References

1. Nora EP, Lajoie BR, Schulz EG, Giorgetti L, Okamoto I, Servant N, Piolot T, van Berkum NL, Meisig J, Sedat J, et al. Spatial partitioning of the regulatory landscape of the X-inactivation centre. Nature 2012; 485:381-5; PMID:22495304; http://dx.doi.org/10.1038/nature11049
2. Hou C, Li L, Qin ZS, Corces VG. Gene density, transcription, and insulators contribute to the partition of the Drosophila genome into physical domains. Mol Cell 2012; 48:471-84; PMID:23041285; http://dx.doi.org/10.1016/j.molcel.2012.08.031
3. Dixon JR, Selvaraj S, Yue F, Kim A, Li Y, Shen Y, Hu M, Liu JS, Ren B. Topological domains in mammalian genomes identified by analysis of chromatin interactions. Nature 2012; 485:376-80; PMID:22495300; http://dx.doi.org/10.1038/nature11082
4. Sexton T, Yaffe E, Kenigsberg E, Bantignies F, Leblanc B, Hoichman M, Parrinello H, Tanay A, Cavalli G. Three-dimensional folding and functional organization principles of the Drosophila genome. Cell 2012; 148:458-72; PMID:22265598; http://dx.doi.org/10.1016/j.cell.2012.01.010
5. Phillips-Cremins JE, Sauria ME, Sanyal A, Gerasimova TI, Lajoie BR, Bell JS, Ong CT, Hookway TA, Guo C, Sun Y, et al. Architectural protein subclasses shape 3D organization of genomes during lineage commitment. Cell 2013; 153:1281-95; PMID:23706625; http://dx.doi.org/10.1016/j.cell.2013.04.053
6. Rao SS, Huntley MH, Durand NC, Stamenova EK, Bochkov ID, Robinson JT, Sanborn AL, Machol I, Omer AD, Lander ES, et al. A 3D Map of the Human Genome at Kilobase Resolution Reveals Principles of Chromatin Looping. Cell 2014; 159:1665-80; PMID:25497547; http://dx.doi.org/10.1016/j.cell.2014.11.021
7. Li L, Lyu X, Hou C, Takenaka N, Nguyen HQ, Ong CT, Cubenas-Potts C, Hu M, Lei EP, Bosco G, et al. Widespread rearrangement of 3D chromatin organization underlies polycomb-mediated stress-induced silencing. Mol Cell 2015; 58:216-31; PMID:25818644; http://dx.doi.org/10.1016/j.molcel.2015.02.023
8. Le Dily F, Bau D, Pohl A, Vicent GP, Serra F, Soronellas D, Castellano G, Wright RH, Ballare C, Filion G, et al. Distinct structural transitions of chromatin topological domains correlate with coordinated hormone-induced gene regulation. Genes Dev 2014; 28:2151-62; PMID:25274727; http://dx.doi.org/10.1101/gad.241422.114
9. Giorgetti L, Galupa R, Nora EP, Piolot T, Lam F, Dekker J, Tiana G, Heard E. Predictive polymer modeling reveals coupled fluctuations in chromosome conformation and transcription. Cell 2014; 157:950-63; PMID:24813616; http://dx.doi.org/10.1016/j.cell.2014.03.025
10. Van Bortle K, Nichols MH, Li L, Ong CT, Takenaka N, Qin ZS, Corces VG. Insulator function and topological domain border strength scale with architectural protein occupancy. Genome Biol 2014; 15:R82; PMID:24981874; http://dx.doi.org/10.1186/gb-2014-15-5-r82
11. Vietri Rudan M, Barrington C, Henderson S, Ernst C, Odom DT, Tanay A, Hadjur S. Comparative Hi-C Reveals that CTCF Underlies Evolution of Chromosomal Domain Architecture. Cell Rep 2015; 10:1297-309; PMID:25732821; http://dx.doi.org/10.1016/j.celrep.2015.02.004
12. Gomez-Marin C, Tena JJ, Acemel RD, Lopez-Mayorga M, Naranjo S, de la Calle-Mustienes E, Maeso I, Beccari L, Aneas I, Vielmas E, et al. Evolutionary comparison reveals that diverging CTCF sites are signatures of ancestral topological associating domains borders. Proc Natl Acad Sci U S A 2015; 112:7542-7; PMID:26034287; http://dx.doi.org/10.1073/pnas.1505463112
13. Pope BD, Ryba T, Dileep V, Yue F, Wu W, Denas O, Vera DL, Wang Y, Hansen RS, Canfield TK, et al. Topologically associating domains are stable units of replication-timing regulation. Nature 2014; 515:402-5; PMID:25409831; http://dx.doi.org/10.1038/nature13986
14. Sofueva S, Yaffe E, Chan WC, Georgopoulou D, Vietri Rudan M, Mira-Bontenbal H, Pollard SM, Schroth GP, Tanay A, Hadjur S. Cohesin-mediated interactions organize chromosomal domain architecture. EMBO J 2013; 32:3119-29; PMID:24185899; http://dx.doi.org/10.1038/emboj.2013.237
15. Arner E, Daub CO, Vitting-Seerup K, Andersson R, Lilje B, Drablos F, Lennartsson A, Ronnerblad M, Hrydziuszko O, Vitezic M, et al. Gene regulation. Transcribed enhancers lead waves of coordinated transcription in transitioning mammalian cells. Science 2015; 347:1010-4; PMID:25678556; http://dx.doi.org/10.1126/science.1259418
16. Lupianez DG, Kraft K, Heinrich V, Krawitz P, Brancati F, Klopocki E, Horn D, Kayserili H, Opitz JM, Laxova R, et al. Disruptions of topological chromatin domains cause pathogenic rewiring of gene-enhancer interactions. Cell 2015; 161:1012-25; PMID:25959774; http://dx.doi.org/10.1016/j.cell.2015.04.004
17. Guo Y, Xu Q, Canzio D, Shou J, Li J, Gorkin David U, Jung I, Wu H, Zhai Y, Tang Y, et al. CRISPR Inversion of CTCF Sites Alters Genome Topology and Enhancer/Promoter Function. Cell; 162:900-10; PMID:26276636; http://dx.doi.org/10.1016/j.cell.2015.07.038
18. Wang H, Maurano MT, Qu H, Varley KE, Gertz J, Pauli F, Lee K, Canfield T, Weaver M, Sandstrom R, et al. Widespread plasticity in CTCF occupancy linked to DNA methylation. Genome Res 2012; 22:1680-8; PMID:22955980; http://dx.doi.org/10.1101/gr.136101.111
19. Engel N, West AG, Felsenfeld G, Bartolomei MS. Antagonism between DNA hypermethylation and enhancer-blocking activity at the H19 DMD is uncovered by CpG mutations. Nat Genetics 2004; 36:883-8; PMID:15273688; http://dx.doi.org/10.1038/ng1399
20. Dixon JR, Jung I, Selvaraj S, Shen Y, Antosiewicz-Bourget JE, Lee AY, Ye Z, Kim A, Rajagopal N, Xie W, et al. Chromatin architecture reorganization during stem cell differentiation. Nature 2015; 518:331-6; PMID:25693564; http://dx.doi.org/10.1038/nature14222
21. Williamson I, Berlivet S, Eskeland R, Boyle S, Illingworth RS, Paquette D, Dostie J, Bickmore WA. Spatial genome organization: contrasting views from chromosome conformation capture and fluorescence in situ hybridization. Genes Dev 2014; 28:2778-91; PMID:25512564; http://dx.doi.org/10.1101/gad.251694.114
22. Seitan VC, Faure AJ, Zhan Y, McCord RP, Lajoie BR, Ing-Simmons E, Lenhard B, Giorgetti L, Heard E, Fisher AG, et al. Cohesin-based chromatin interactions enable regulated gene expression within preexisting architectural compartments. Genome Res 2013; 23:2066-77; PMID:24002784; http://dx.doi.org/10.1101/gr.161620.113
23. Zuin J, Dixon JR, van der Reijden MI, Ye Z, Kolovos P, Brouwer RW, van de Corput MP, van de Werken HJ, Knoch TA, van IWF, et al. Cohesin and CTCF differentially affect chromatin architecture and gene expression in human cells. Proc Natl Acad Sci U S A 2014; 111:996-1001
24. Lieberman-Aiden E, van Berkum NL, Williams L, Imakaev M, Ragoczy T, Telling A, Amit I, Lajoie BR, Sabo PJ, Dorschner MO, et al. Comprehensive mapping of long-range interactions reveals folding principles of the human genome. Science 2009; 326:289-93; PMID:19815776; http://dx.doi.org/10.1126/science.1181369