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RESEARCH HIGHLIGHT

Mapping enhancer and promoter interactions

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Histone modifications have previously been leveraged to map the locations of candidate enhancer and promoters in one dimensional genomic space. Chepelev *et al.* report the first Chromatin Interaction Analysis with Paired-End Tag (ChIA-PET) sequencing of a specific histone modification to systematically map long-range physical interactions among enhancer and promoters, yielding insights into the nature of these interactions.

Within the vast sequence of the human genome are encoded enhancer elements, which influence the expression level of genes. The detection of enhancers and prediction of their cell types of activity from primary DNA sequence is extremely challenging. However, enhancers are known to be associated with distinct patterns of histone modifications in cell types in which they are active [1]. Substantial progress has been made in systematically mapping histone modifications, and from that data computationally predicting candidate enhancers in multiple cell types [2, 3].

Disease-associated variants are known to occur in candidate enhancer regions [3, 4] and the ability to accurately identify the target genes of enhancers will likely become key to understanding their effects. While enhancers can be associated with genes based on the nearest gene or absolute genomic distance, these approaches are heuristic and additional evidence is needed for more confident associations. The co-variation of gene expression and enhancer-associated histone modifications across cell types has been used in associating enhancers with their gene targets [3]. The association of specific genetic variants with differential target gene expression levels as determined through expression quantitative trait loci (eQTL) studies [5] is another such avenue. However, these approaches only offer indirect evidence, and are generally underpowered to detect distal interactions.

Chromatin confirmation capture-based protocols provide direct evidence for physical interactions (recently reviewed in de Wit & de Laat, 2012 [6]). The original 3C protocol tests interactions involving two pre-specified sequences [7]. An extension of the approach, Hi-C [8], can detect interactions between any pair of genomic locations. However, the detected interactions were not specific to enhancer or promoter interactions, and at the sequencing depth used were at a lower resolution (1 Mb) than necessary to reliably observe individual enhancer-promoter interactions.

By contrast, ChIA-PET [9] maps genome-wide interacting locations involving a specific immunoprecipitated protein that is used as a bait in the assay. The first two published data sets applying this method interrogated interactions involving the transcription factor estrogen receptor-α [9] in the breast cancer MCF-7 cells and the insulator-binding protein CTCF[10] in mouse embryonic stem cells. However, these proteins are only expected to be involved in a subset of enhancer-promoter interactions in these cells, and thus many enhancer-promoter interactions would be missed in these ChIA-PET data sets.

Recently two studies have applied ChIA-PET with baits that would be expected to more systematically capture enhancer-promoter interactions. One study used RNA Polymerase II [11] and the study by Chepelev *et al.* [12], published in *Cell Research* used histones with the H3K4me2 modification. H3K4me2 was strategically selected as the histone modification because of its general association with both promoter and enhancer regions [13].

Chepelev *et al.* report a total of 6 520 long-range interactions (> 20 kb) involving H3K4me2 in $CD4^+$ T cells using ChIA-PET. Of these interactions, 2 373 involved enhancer-promoter interactions. Interestingly, a substantial number of the detected interactions, 3 669, were distal promoter-promoter interactions, a finding consistent with results using RNA Polymerase II to detect interactions [11]. The remaining 478 interactions were reported to be enhancer-enhancer interactions.

A global analysis of the interactions found that 9% of enhancers with long-range interactions interact with multiple promoters and 25% of interacting

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promoters have more than one long-range enhancer interaction. Genes with interacting enhancers were on average higher expressed than those without, and gene expression levels were reported to positively correlate with the number of interacting enhancers. Genes associated with promoter-promoter interactions were found to be significantly more likely to be co-expressed in a tissue-specific manner.

To gain insights into regulators potentially involved in the interactions, the authors analyzed the overlap of interacting enhancers with transcription factor binding sequence motifs and binding locations of the histone acetyltransferase p300, associated with enhancer activity [14], and the insulator-binding CTCF. Surprisingly, only 14% of the enhancers had p300 peaks while 23% had peaks for CTCF. There is increasing evidence though for CTCF's role in mediating long-range chromatin interactions [10]. Motif analysis of the enhancers found enrichment for binding sites of a number of regulators of known importance in T-cells.

The data from Chepelev *et al.* also revealed insights into higher level chromatin organization. The data supported that chromosomes can be divided into active regions containing frequent interactions, and more silent regions generally lacking interactions. Interestingly, there was also evidence supporting very long-range interactions among distal active regions, suggesting that these regions may in three-dimensional space, be in closer proximity than would be expected based on their one-dimensional genomic positions, consistent with a previously proposed model [8].

The number of enhancers and promoters with interactions detected is substantially fewer than the number of previously predicted active enhancers or promoters in a cell type [2, 3]. For technical reasons, interactions occurring within 20 kb were not considered and it is therefore possible that many promoters and enhancers interact exclusively within this distance. Other approaches for associating enhancer with target genes are more effective for predicting short-range interactions, emphasizing the need for methods that can integrate ChIA-PET data to form a more comprehensive set of predictions. Future refinements of the experimental protocol that improve sensitivity would have the potential to reveal additional intrachromosomal as well as interchromosomal interactions, the latter of which could not be confidently mapped by the authors.

Going forward it will be important to compare interaction maps derived using ChIA-PET with general marks such as H3K4me2 with those produced with sequence-specific transcription factors to confidently establish the comprehensiveness of the detected long-range regulatory interactions. As enhancers can be highly cell type specific [2, 3], having interaction maps in additional cell types and conditions will be a valuable resource. Such interaction maps would likely yield insights into the dynamics of gene regulation and into the mechanisms by which individual distal regulatory variants act to confer disease risk [11]. Genomics has been largely centered on a one-dimensional representation of the genome, but this work by Chepelev *et al.* [12] and recent work by others represent important steps towards mapping and understanding the higher dimensional nature of the genome.

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