Identifying clusters of \textit{cis}-regulatory elements underpinning TAD structures and lineage-specific regulatory networks

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Cellular identity relies on cell-type–specific gene expression controlled at the transcriptional level by \textit{cis}-regulatory elements (CREs). CREs are unevenly distributed across the genome, giving rise to individual CREs and clusters of CREs (COREs). Technical and biological features hinder CORE identification. We addressed these issues by developing an unsupervised machine learning approach termed clustering of genomic regions analysis method (CREAM). CREAM automates CORE detection from chromatin accessibility profiles that are enriched in CREs strongly bound by master transcription regulators, proximal to highly expressed and essential genes, and discriminating cell identity. Although COREs share similarities with super-enhancers, we highlight differences in terms of the genomic distribution and structure of these \textit{cis}-regulatory units. We further show the enhanced value of COREs over super-enhancers to identify master transcription regulators, highly expressed and essential genes defining cell identity. COREs enrich at topologically associated domain (TAD) boundaries. They are also preferentially bound by the chromatin looping factors CTCF and cohesin, in contrast to super-enhancers, forming clusters of CTCF and cohesin binding regions and defining homotypic clusters of transcription regulator binding regions (HCTs). Finally, we show the clinical utility of CREAM to identify COREs across chromatin accessibility profiles to stratify more than 400 tumor samples according to their cancer type and to delineate cancer type–specific active biological pathways. Collectively, our results support the utility of CREAM to delineate COREs underlying, with greater accuracy than individual CREs or super-enhancers, the cell-type–specific biological underpinning across a wide range of normal and cancer cell types.

[Supplemental material is available for this article.]

More than 98% of the human genome consists of sequences lying outside of gene coding regions that harbor functional features, including \textit{cis}-regulatory elements (CREs) that are important in defining cellular identity by establishing lineage-specific gene expression profiles (Lupien et al. 2008; Heintzman et al. 2009; Ernst et al. 2011). CREs, such as enhancers, promoters, and anchors of chromatin interactions, are predicted to cover 20%–40% of noncoding sequences of the human genome (Kellis et al. 2014). Current methods to annotate CREs in biological samples include ChIP-seq for histone modifications (e.g., H3K4me1, H3K4me3, and H3K27ac) (Heintzman et al. 2007, 2009; Lupien et al. 2008; Ernst and Kellis 2010), chromatin binding protein (e.g., MED1, EP300, CTCF, and ZNF143) (Heintzman et al. 2007; Bailey et al. 2015), or chromatin accessibility assays (e.g., DNase-seq and ATAC-seq) (Thurman et al. 2012; Buenrostro et al. 2013). CREs are unevenly distributed across the genome, suggesting distinct biological underpinning to genomic coordinates based on CRE density, namely, between clusters of CREs (COREs) and individual CREs. Indeed, high CRE density, such as those reported as super-enhancers or stretch-enhancers, is associated to cell identity and is bound by transcription regulators with higher intensity than individual CREs (Hnisz et al. 2013; Whyte et al. 2013; Dowen et al. 2014; Boeva et al. 2017). In addition, such high-density CRE regions from cancer cells lie proximal to oncogenic driver genes (Lovén et al. 2013; Chipumuro et al. 2014; Northcott et al. 2014; Kron et al. 2017). Together, these features showcase the utility of classifying CREs into clusters versus individual CREs.

Here, we present a new methodology termed clustering of genomic regions analysis method (CREAM) relying on chromatin accessibility profiles, either from DNase-seq or ATAC-seq assays, as a unifying model to identify COREs in any cell type (Fig. 1). CREAM is a computational method relying on unsupervised machine learning that considers the distribution of distances between CREs in a given biological sample to systematically identify COREs consisting of at least two individual CREs. By conducting a comprehensive comparative study, we introduce CREAM as a new systematic way for the identification of COREs, outperforming other widely used CRE annotations, such as super-enhancers. We compared the enrichment of essential and highly expressed genes in the proximity of CREAM-identified COREs and individual CREs. We further compare the binding intensity of master transcription regulators between COREs and individual CREs. We
also show the utility of COREs in studying the three-dimensional structure of the genome. Finally, we report on the clinical value of identifying COREs in tumor samples to discriminate the cancer type and the biological underpinning specific to each sample.

Results

CREAM detects COREs from chromatin accessibility profiles

We developed CREAM as a new computational approach for the systematic identification of COREs. CREAM is designed to identify COREs from chromatin accessibility profiles through five iterative learning steps described in detail in the Methods section. Overall, these steps include the following: (1) grouping the individual CREs in clusters of varying number of individual CREs (referred to as Order); (2) identifying the threshold for the stitching distance between individual CREs within the clusters of the same Order; (3) identifying the maximum Order of COREs; (4) clustering individual CREs as COREs starting from the highest Order; and (5) filtering out low Order COREs with a stitching distance close to the corresponding stitching distance threshold of the same Order.

Applying CREAM across the DNase-seq data, aligned using human genome assembly GRCh37/hg19, from 102 cell lines available through the ENCODE Project Consortium (The ENCODE Project Consortium 2012) reveals between 1022 and 7597 COREs per cell line (Supplemental Fig. S1A), correlated with the total number of CREs identified in each cell line (Supplemental Fig. S1B). However, the fraction of CREs called within COREs is independent of the number of individual CREs (Supplemental Fig. S1C) and does not impact the median width of COREs across cell lines (Spearman’s correlation \(r < 0.25\)) (Supplemental Fig. S1D), supporting the specificity of CORE widths with respect to each biological sample irrespective of the total number of CREs. We further show the ability of COREs to classify samples according to their tissue of origin using the ENCODE Project Consortium cell lines. Our results specifically show that COREs identify the tissue of origin for the 78 DNase I profiles of the ENCODE Project Consortium cell lines with high accuracy (Matthews correlation coefficient [MCC] of 0.85 for tissues with four or more cell lines) (Supplemental Fig. S1E). In agreement, close to 40% of the 32,997 COREs found across the ENCODE Project Consortium cell lines are unique to one cell line, and only a very small number are shared across all cell lines (Supplemental Fig. S2A). Furthermore, even COREs common to >50% of cell lines (12% of all COREs found in the ENCODE Project Consortium cell lines) (Supplemental Fig. S2) are not enriched at housekeeping genes (P-value >0.05) (Hsiao et al. 2001). Collectively, these results emphasize the cell line specificity of COREs.

COREs are unique cis-regulatory units of biological significance

We next used the DNase-seq data from the ENCODE Project Consortium tier I cell lines (GM12878, K562, and H1-hESC) to further characterize the biological underpinnings of COREs versus individual CREs. We focused on the ENCODE Project Consortium tier I cell lines because of their extensive characterization (The ENCODE Project Consortium 2012), inclusive of expression profiles and DNA–protein interactions assessed by ChIP-seq assays, allowing for a comprehensive biological assessment of COREs identified across different cell lines.

We first assessed the signal intensity for chromatin accessibility at COREs versus individual CREs. Our results show that COREs have a higher average chromatin accessibility signal per base pair compared with that of individual CREs across the three tested cell lines (GM12878: fold change [FC] = 1.9; K562: FC = 8.4; H1-hESC: FC = 1.1) (Fig. 2A). We next examined the difference in the expression level of genes proximal to COREs versus those proximal to individual CREs. We found that COREs are proximal to genes expressed at higher levels than those near individual CREs in the GM12878, K562, and H1-hESC cell lines (Wilcoxon signed-rank test FDR <0.001; GM12878: FC = 4.6; K562: FC = 6.8; H1-hESC: FC = 1.3) (Fig. 2B). Up to 52%, 59%, and 39% of COREs overlap with active transcription start sites (TSSs) (TSSs harboring peaks of chromatin accessibility) in the GM12878, K562, and H1-hESC cell lines, respectively (Supplemental Fig. S2B). The association of COREs compared with individual CREs with highly expressed genes remains significant (FDR < 0.05) even when focusing on COREs distal to TSS (up to ±25 kb away from the TSSs; Supplemental Fig. S3), although differences in the expression of genes proximal to COREs and individual CREs decreases with increasing distance (Spearman’s correlation \(\rho < -0.8\); Fig. 2C). Hence, COREs are in proximity of genes with higher expression with respect to genes proximal to individual CREs irrespective of the distance and overlap between the CREs and gene TSSs.

We next assessed the relevance of COREs versus individual CREs in bookmarking genes essential for growth. For this, we
CREAM identifies COREs bound by master transcription regulators

Transcription regulators (TRs) bind CREs to modulate the expression of cell-type-specific gene expression patterns. Quantifying the binding intensity of transcription regulators over COREs in the GM12878, K562, and H1-hESC cell lines reveals that >20% of ChIP-seq data of transcription regulators (GM12878: 92/237; K562: 256/325; H1-hESC: 24/119) show binding intensity higher over COREs compared with individual CREs when normalizing the ChIP-seq signal over COREs to the size of each CORE (FC > 2, FDR < 0.001) (Fig. 3A). The higher enrichment of TR binding intensity in COREs can be also seen using COREs excluding the CRE-free gaps (Supplemental Fig. S4A) regardless of whether COREs overlap active TSSs (TSSs harboring peaks of chromatin accessibility) or not (Supplemental Fig. S4B). This higher transcription regulator binding intensity at COREs is showcased in GM12878 by the master transcription regulators TCF3 and EB1 (Somassundaram et al. 2015). Specifically, we observed a greater than threefold difference in binding intensity for TCF3 and EB1 in the GM12878 cell line over COREs compared with individual CREs (Fig. 3B), exemplified at the CORE proximal to the ZFAT gene (Fig. 3C). Similarly, the master transcription regulators GABPA and CREB1 (Shankar et al. 2005; Yang et al. 2013) bind with a more than threefold greater intensity over COREs compared with individual CREs in the K562 cell line (Fig. 3B), exemplified at the CORE overlapping the LMBRI, NOM1, and MXN1 genes (Fig. 3C). Finally, in the H1-hESC cell line, the master transcription regulators NANOG and MYC (Pan and Thomson 2007) bind with higher intensity at COREs (FC > 1.2, FDR < 0.001) (Fig. 3B) in the H1-hESC cell line, exemplified at the HOXA locus CORE (Fig. 3C).

CTCF- and cohesin-enriched COREs map to topologically associated domain boundaries

Beyond COREs, the human genome can be partitioned in various clusters including those based on contact frequencies between distal genomic coordinates that define topologically associated domains (TADs) (Edelmann et al. 2015). To assess the relation between COREs and TADs, we integrated the distribution of COREs with TADs reported from Hi-C data in the GM12878 and K562 cell lines (Rao et al. 2014). Our analysis reveals higher fraction of COREs compared with individual CREs at TAD boundaries (permutation test FDR < 0.001) (Fig. 4A,B; Supplemental Fig. S5A). Similar results are seen in the HeLa, HMEC, HUVEC, and NHEK cell lines (Supplemental Fig. S5B; Rao et al. 2014; Edelmann et al. 2015).

combined the CRISPR/Cas9 gene essentiality screen data reported in the K562 cell line (Wang et al. 2015) with CORE identification from the K562 cell line, revealing the enrichment of gene essential for growth proximal to COREs (FDR < 0.001 using permutation test) (Fig. 2D). This is exemplified at the BCR gene that is the most essential gene and proximal to a CORE in K562 chronic myelogenous leukemia (CML) cell line (Fig. 2D), positive for the BCR-ABL gene fusion reported in CML (Ren 2005). By extending our analysis to essentiality scores from other cell lines tested by Wang et al. (2015), we show that the essentiality score of genes proximal to K562 COREs is less in the KBM-7, Jiyoye, and Raji cell lines compared with the K562 cell line (FDR < 0.001) (Fig. 2E). We further show that the expression of genes essential for growth in K562 proximal to COREs is higher than the expression of essential genes associated with individual CREs (FDR < 0.001) (Fig. 2F). These results support the cell-type-specific nature of COREs and their association with essential genes and argue in favor of COREs accounting for a greater regulatory potential relevant to cell type essentiality than individual CREs.

Figure 2. Comparison of genomic characteristics of the COREs identified by CREAM versus individual CREs in the GM12878, K562, and H1-hESC cell lines. (A) Distribution of DNase I signal intensity in individual CREs and COREs (signal per base pair). (B) Expression level of genes in 10-kb proximity of individual CREs or COREs. (****) P-value < 0.0001. (C) Median expression of genes according to distance to the closest individual CRE (gray) or CORE (red). (D) Volcano plot of significance (FDR) and effect size (essentiality score) of genes in proximity of CREAM-identified COREs in the K562 cell line (red indicates significant fold change; gray, insignificant fold change). (E) Essentiality score from K562, KBM-7, Jiyoye, and Raji cell lines for genes at nominal (±10 kb) to COREs identified by CREAM in the K562 cell line. (****) P-value < 0.0001 using Wilcoxon signed-rank test. (F) Expression level of essential genes associated with individual CREs versus COREs. (***) P-value < 0.01.
Together, this suggests that COREs are preferentially found at TAD boundaries. CTCF, cohesin (RAD21 and SMC3), YY1, and the ZNF143 transcription regulators preferentially bind chromatin at anchors of chromatin interactions, inclusive of TAD boundaries (Heidari et al. 2014; Rao et al. 2014; Bailey et al. 2015; Weintraub et al. 2017). We therefore assessed whether these transcription regulators were enriched within COREs at TAD boundaries based on their ChIP-seq signal intensity. CTCF and RAD21 were preferentially enriched within COREs compared with individual CREs restricted to TAD boundaries in both the GM12878 and K562 cell lines (FC > 1.5, FDR < 0.001 for CTCF and RAD21; FC > 1.7, FDR < 0.001 for SMC3 in GM12878 and K562 respectively) (Fig. 4D). ZNF143 also preferentially occupied TAD-boundary COREs as opposed to intra-TAD COREs but only in the K562 cell line (FC = 1.42, FDR < 0.001) (Fig. 4D). We observed lesser differences in the binding intensity of YY1 at TAD-boundary COREs versus intra-TAD COREs in the GM12878 and K562 cell lines (FC < 1.25 in both cell lines) (Fig. 4D). Extending this analysis to the remaining ChIP-seq data for transcription regulators in the GM12878 and K562 cell lines (The ENCODE Project Consortium 2012) revealed 69% and 35% of transcription regulators with increased binding intensity at TAD-boundary COREs versus intra-TAD COREs but with low effect size in the GM12878 and K562 cell lines, respectively (FC > 1, FDR < 0.001) (Fig. 4D).

The enrichment of CTCF and cohesin within COREs at TAD boundaries led us to assess if they were themselves forming homotypic clusters of transcription regulator binding regions (HCTs) (Gotea et al. 2010) at TAD boundaries. Using CREAM on the 86 and 98 ChIP-seq data from the GM12878 and K562 cell lines, respectively, identified 41 and 59 transcription regulators in each cell line forming at least 100 HCTs (Supplemental Table S1). Comparing the distribution of HCT at TAD boundaries versus intra-TADs revealed that >50% of CTCF, RAD21, SMC3, and ZNF143 HCTs lie at TAD boundaries (Fig. 4E), exemplified at the MYC and BCL6 gene loci (Fig. 4F). This contrasts with other transcription regulators, such as SP1 and GATA2 with <10% of HCTs mapping to TAD boundaries in the GM12878 and K562 cell lines, respectively, identified 41 and 59 transcription regulators in each cell line forming at least 100 HCTs (Supplemental Table S1). Taken together, these results suggest that clusters of CTCF and cohesin binding regions establishing HCTs are preferentially found at TAD boundaries.

COREs and super-enhancers are two distinct biological features of cells

Similar to COREs, super-enhancers were introduced as high-signal intensity regions identified from ChIP-seq data from features, such as H3K27ac or MED1, typical of a subset of CREs, including promoters and enhancers (Hnisz et al. 2013; Lovén et al. 2013; Vahedi et al. 2015). Although the concept of clusters of CREs...
Figure 4. Arrangement of COREs and individual CREs with respect to TAD boundaries. (A) Schematic representation of TAD boundaries and intra-TAD regions (25-kb Hi-C resolution). (B) Comparison of fraction of COREs and individual CREs from DNase-seq that lie at TAD boundaries with increasing distance from TAD-boundary cutoffs in the GM12878 and K562 cell lines. (C) Enrichment of TR binding intensities within COREs over individual CREs that lie in proximity of TAD boundaries (±10 kb) versus COREs and CREs farther away from TAD boundaries (intra-TAD elements) in the GM12878 or K562 cell line. (D) Enrichment of TR binding intensity in COREs proximal to TAD boundaries (±10 kb) versus intra-TAD domains. (E) Fraction of HCTs (purple) and individual TR binding regions (gray) at TAD boundaries (±10 kb). The total number of individual binding regions for each TR in the GM12878 and K562 cell lines is also reported (orange). (F) Examples of HCTs for CTCF, RAD21, SMC3, and ZNF143 at the TAD boundary for the MYC and BCL6 genes (10-kb Hi-C resolution).
was introduced before super-enhancers (de Laat and Grosveld 2003; Gautlont et al. 2010; Song et al. 2011), the computational method developed for super-enhancer calling, known as ROSE (Hnisz et al. 2013; Lovén et al. 2013; Whyte et al. 2013), accelerated the inclusion of super-enhancer identification across numerous studies. We therefore comprehensively compared CORE identification by CREAM with super-enhancer mapping from ROSE using the data from the GM12878, K562, and H1-hESC cell lines.

Our comparison of super-enhancers, identified either by ROSE or its latest version (ROSE2) (Stratton et al. 2016; https://github.com/linlabbcm/rose2), with COREs revealed limited overlap in all the three cell lines (Jaccard index <0.5) (Fig. 5A). Moreover, the pathway enrichment analysis based on genes within 10 kb of COREs or super-enhancers shows higher enrichment of phenotypic-specific pathways for COREs. For instance, enrichment for the B CELL RECEPTOR SIGNALING PATHWAY term is 2.6-fold more significant based on COREs as opposed to super-enhancers found in the lymphoblastoid GM12878 cell line (Fig. 5B). Similarly, the CHRONIC MYELOID LEUKEMIA term is 3.07-fold more enriched in genes proximal to COREs compared with super-enhancer in the chronic myeloid leukemia K562 cell line (Fig. 5B).

Finally, the WNT SIGNALING PATHWAY term is 2.36-fold more enriched in genes proximal to COREs compared with super-enhancers in the H1 human embryonic stem cell line (Fig. 5B). We further compared the structure of COREs and super-enhancers according to their proportion reported to harbor two or more CREs. Although all COREs consisted of at least two CREs, between 75% and 90% of super-enhancers identified by ROSE were composed of at least two CREs in the GM12878, K562, and H1-hESC cell lines (Fig. 5C). This number plummets to <65% for super-enhancers called by ROSE2 in these same cell lines (Fig. 5C). This argues for a greater similarity between COREs and super-enhancers identified by ROSE than ROSE2. We next compared the relationship between gene expression versus COREs and super-enhancers. Our results reveal the higher expression of genes located in proximity to both COREs and super-enhancers, called either using ROSE or ROSE2, as opposed to genes exclusively proximal to COREs or super-enhancers in the GM12878 and K562 cell lines (Fig. 5D). Similarly, in the H1-hESC cell line, genes commonly assigned to COREs and super-enhancers called by ROSE show higher expression compared with genes assigned uniquely to either COREs or super-enhancers (FDR <0.001) (Fig. 5D), but this does not apply to super-enhancers called by ROSE2, in which CREAM alone serves to identify COREs near genes with the highest level of expression (FDR <0.001) (Fig. 5D).

Moreover, the expression of CORE-specific genes was higher in the GM12878 and K562 cell lines compared with genes exclusively in proximity of ROSE or ROSE2 super-enhancers (FDR < 0.001) (Fig. 5D). Regarding H1-hESC, the expression of CORE-specific genes was higher than ROSE2-specific genes (FDR <0.001) (Fig. 5D) but lower than ROSE-specific genes (FDR <0.001; Fig. 5D). Expanding our analysis to genes essential for growth in the K562 cell lines revealed that genes located in proximity of both COREs and super-enhancers have the highest enrichment for essential genes, followed by genes only proximal to COREs and, finally, genes only proximal to super-enhancers (Fig. 5E). Collectively, these results show a level of similarity between COREs and super-enhancers and also highlight differences in which COREs are more associated with biological functions than super-enhancers. This argues that COREs identified using CREAM are a more precise reflection of cellular identity and function.

As a final comparison, we assessed the enrichment of transcription regulators according to their ChIP-seq profiles within COREs versus super-enhancers. Our analysis reveals that >60% of transcription regulators are enriched in COREs compared with ROSE-super-enhancers in the GM12878 and H1-hESC cell lines (FC > 2 and FDR < 0.001) (Fig. 5F). In the K562 cell line, >30% of transcription regulators are more enriched in COREs compared with ROSE-super-enhancers (FC > 2 and FDR < 0.001) (Fig. 5F). In contrast, <2% of transcription regulators are more enriched in ROSE-super-enhancers compared with COREs in any of the three cell lines (Fig. 5F).

Collectively, these results support the unique biological nature of COREs compared with super-enhancers toward chromatin looping factors and TAD boundaries, of relevance to the three-dimensional organization of the genome.

Clinical utility of CREAM to identify COREs discriminating tumor type and underpinning biological pathway

CRE identification on the human genome assembly GRCh38/hg38 was recently completed through ATAC-seq assays in 400 human tumor samples from 23 different cancer types part of The Cancer Genome Atlas (TCGA) (Corces et al. 2018). Using the k-nearest neighbor method (k = 3) on the COREs identified by CREAM classified these TCGA ATAC-seq profiles according to their tumor type (MCC = 0.86) (Fig. 6A). Out of 22 cancer types with more than four patient samples with available ATAC-seq profiles, 17 had balanced accuracy of >85% (Fig. 6A). We found that, in patient tumor ATAC-seq profiles, COREs were located in proximity of genes with higher expression than individual CREs (Fig. 6B) and were overrepresented in 49 out of 50 hallmark genes of cancer gene sets (FDR < 0.05) (Fig. 6C; Liberzon et al. 2015). The TNF-α SIGNALING VIA NFκB hallmark gene set was enriched for almost all of the TCGA samples, whereas other hallmark gene sets were tissue specifically enriched, such as the ANDROGEN RESPONSE hallmark gene set enriched in prostate adenocarcinoma (PRAD) tumor samples (Fig. 6C). Altogether our results show the potential utility of COREs in clinical setting to discriminate cancer types and identify hallmark gene sets within each tumor sample of biological relevance.

Discussion

Although the concept that CREs are not all equal is well established, their classification into clusters is recent and warrants the development of strategies for their classification according to the various approaches developed to map CREs. Here, we developed CREAM as the first unsupervised machine learning method providing a systematic approach to set the filters through an iterative learning process to identify COREs from chromatin accessibility profiles generated in any cell type. We show that CREAM identifies COREs that have higher transcription regulator binding intensity
Figure 5. Comparison of CREAM-identified COREs and super-enhancers of the GM12878, K562, and H1-hESC cell lines. (A) Similarity of COREs and super-enhancers based on their genomic loci overlap. (B) Top five enriched biological pathways using genes in 10-kb proximity of the identified COREs and super-enhancers in each one of the GM12878, K562, and H1-hESC cell lines. (C) Percentage of COREs and super-enhancers containing two or more individual CREs. (D) Expression of genes in 10-kb proximity of both COREs and super-enhancers or exclusively in proximity of COREs or super-enhancers. (E) Enrichment of essential genes among genes in proximity of both COREs and super-enhancers or exclusively in proximity of COREs or super-enhancers. (F) Enrichment of TR binding intensity from ChIP-seq data in COREs identified by CREAM versus super-enhancers. Volcano plots represent $-\log_{10}(FDR)$ versus $\log_{2}(FC)$ in ChIP-seq signal intensities. Each dot is one TR (colored indicates significant FC; gray, insignificant FC). The barplots show how many TRs have higher signal intensity in COREs or super-enhancers (FDR < 0.001 and $\log_{2}(FC) > 1$). FC is defined as the ratio between the average signal per base pair in COREs versus super-enhancers. (G) Distribution of ChIP-seq signal intensity of CTCF at COREs and super-enhancers in 10-kb proximity of TAD boundaries.
and that are enriched proximal to genes essential for growth compared with individual CREs. CREAM-identified COREs also classify cell types according to their tissue of origin, discriminating normal from cancer cells. These results support the utility of CREAM for reporting COREs from chromatin accessibility data of biological significance.

We also assessed the biological relevance of COREs with regards to the three-dimensional organization of the genome by comparing their distribution with regard to TADs. Our results show that COREs are enriched compared with individual CREs at TAD boundaries. These COREs are preferentially bound by a limited number of transcription regulators, namely, CTCF, the cohesin complex (RAD21, SMC3), and, to a lesser extent, ZNF143. These are transcription regulators previously shown to regulate contact frequencies between distal genomic coordinates defining the three-dimensional organization of the genome.
Step 1: grouping of individual CREs throughout the genome

CREAM initially groups neighboring individual CREs throughout the genome. Each group can have different number of individual CREs. Then it categorizes the groups based on their included CRE numbers. We defined Order (O) for each group as its included CRE number. In the next step, CREAM identifies the maximum allowed distance between individual CREs for calling a group as CORE of a given O.

Step 2: maximum window size identification

We defined maximum window size (MWS) as the maximum allowed distance between individual CREs included in a CORE. For each O, CREAM estimates a distribution of window sizes as the maximum distance between individual CREs in all groups of that O within the genome. Afterward, MWS will be identified based on the low stringent outlier threshold as follows:

\[ \text{MWS} = Q1(\log(\text{WS})) - 1.5 \times IQR(\log(\text{WS})) \]

where MWS is the maximum allowed distance between neighboring individual CREs within a CORE. Q1(log[WS]) and IQR(log[WS]) are the first quartile and interquartile of distribution of window sizes (Fig. 1).

Step 3: maximum Order identification

After determining MWS for each Order of COREs, CREAM identifies the maximum O (O_{max}) for the given sample. Increasing the O of COREs results in the gain of information within the clusters, allowing the individual CREs to have further distance from each other. Hence, starting from COREs of O = 2, the O increases up to a plateau at which an increase of O does not result in an increase in MWS. This threshold is considered as the O_{max} for COREs within the given sample.

Step 4: CORE calling

CREAM starts to identify COREs from the O_{max} down to O = 2. For each O, it calls groups with window size less than MWS as COREs. As a result, many COREs with lower Os are clustered within COREs with higher Os. Therefore, remaining lower O COREs, for example, O = 2 or 3, have individual CREs with a distance close to MWS (Fig. 1). These clusters could have been identified as COREs because of the initial distribution of MWS derived mainly by COREs of the same O that are clustered in COREs of higher Os. Hence, CREAM eliminates these low O COREs as follows.

Step 5: minimum Order identification

COREs that contain individual CREs with a distance close to MWS can be identified as COREs owing to the high skewness in the initial distribution of MWS. To avoid reporting these COREs, CREAM filters out the clusters with (O < O_{min}) that do not follow monotonic increase of maximum distance between individual CREs versus O (Fig. 1). CREAM starts from the lowest order (O = 2) and checks changes of (MWS – median[WS]) / median(WS), where WS is the distribution of maximum distance between individual CREs within COREs of that order. Then CREAM filters out called COREs with Order = 2 up to the point at which this parameter, (MWS – median[WS]) / median(WS), is decreasing by increasing order.

Association with genes

A gene is considered associated with a CRE or a CORE if found within ±10-kb window from each other. This distance was chosen to avoid a false-positive association of elements with gene TSSs based on previous reports (Sanyal et al. 2012). Expressions of genes with respect to distance of COREs and individual CREs with gene TSSs were conducted for different distances from ±1-kb up to ±25-kb windows as suggested by Sanyal et al. (2012).

Association with essential genes

The number of genes that are in ±10-kb proximity of COREs and are essential in the K562 cell line are identified (Wang et al. 2015). This number is then compared with the number of essential genes in 10,000 randomly selected (permuted) genes, among the genes included in the essentiality screen. This comparison is used to compute FDR, as the number of false discoveries in permutation test, and z-score regarding the significance of enrichment of essential genes among genes in ±10-kb proximity of COREs identified for the K562 cell line.

Gene expression comparison

RNA sequencing profiles of the GM12878, K562, and H1-hESC cell lines, available in the ENCODE Project Consortium database (The ENCODE Project Consortium 2012), are used to identify expression of genes in proximity of individual CREs and COREs. The expression of genes is compared using Wilcoxon signed-rank test.
Gene expression enrichment in TCGA

The expression of genes associated with COREs of each tumor sample in TCGA was compared with the expression of 100 randomly selected gene sets, with the same number of genes. The Z-score is calculated considering the null distribution generated relying on the average gene expression in the random gene sets. The P-values were calculated by comparing the expression of genes associated with COREs with genes associated with individual CREs using Wilcoxon signed-rank test.

Pathway enrichment analysis

A hypergeometric test was used to identify P-values for enrichment of hallmark gene sets using the dhyper function in the stats R package. CORE-associated genes for each sample and a catalog of genes associated with peaks were used as the query and background gene lists, respectively.

Housekeeping genes

The list of genes within the HSIAO_HOUSEKEEPING_GENES gene set (Hsiao et al. 2001) was used as the housekeeping genes.

Transcription regulator and input signal binding enrichment

bedGraph files of ChIP-seq data of transcription regulators are overlapped with the identified COREs and individual CREs in the GM12878, K562, and H1-hESC cell line using BEDTools (version 2.23.0) (Quinlan and Hall 2010). The resulting signals were summed over all the individual CREs or COREs and then normalized to the total genomic coverage of individual CREs or COREs, respectively. These normalized transcription regulator binding intensities are used for comparing TR binding intensity in individual CREs and COREs (Fig. 4). A Wilcoxon signed-rank test is used for this comparison.

Similar analysis is used, for enrichment of transcriptional regulators, to get overlap of DNase I signal data of the cell lines within individual CREs or COREs. The overlapped signal then normalized to the size of COREs and individual CREs. The distributions of these normalized signal per base within COREs and individual CREs were then compared for a given cell line.

We included the DNase I, ChIP-seq, and gene expression profiles available from all three tier 1 cell lines from the ENCODE Project Consortium (GM12878, K562, and H1-hESC) to provide a comprehensive analysis of COREs versus biochemical measurements across a diverse collection of cell types, acknowledging that differences in the significance in trends across cell lines could arise from cell-type-specific biology or variability in the quality of data between cell types.

Sample similarity

Similarity between two samples from the ENCODE Project Consortium or TCGA data sets was identified relying on the Jaccard index for the commonality of their identified COREs throughout the genome. Then this Jaccard index is used as the similarity statistics in a three-nearest-neighbor classification approach. We assess the performance of the classification using leave-one-out cross-validation. We used Matthews correlation coefficient for performance of the classification model (Smirnov et al. 2016). The phenotype of each tissue is considered as a class, and the obtained vector is used to calculate MCC using the implemented MCC function in PharmacoGx package in R (Smirnov et al. 2016). In this classification scheme, we considered the phenotype of the closest sample to an out of pool sample as its phenotype.

Multiple hypothesis correction

P-values were corrected for multiple hypothesis testing using the Benjamini-Hochberg procedure (McDonald 2009).

Software availability

CREAM is publicly available as an open source R package (https://cran.r-project.org/doc/FAQ/R-FAQ.html) on the Comprehensive R Archive Network (https://CRAN.R-project.org/package=CREAM) and as Supplemental Code.

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References

Bailey SD, Zhang X, Desai K, Aid M, Corradin O, Lari RC-S, Akhtar-Zaidi B, Scachen PC, Haibe-Kains B, Lupien M. 2015. ZNF143 provides sequence specificity to secure chromatin interactions at gene promoters. Nat Commun 6: 6186. doi:10.1038/ncomms7186

Boeva V, Louis-Brennetot C, Peltier A, Durand S, Pierre-Eugène C, Raynal V, Etchevers HC, Thomas S, Lemine A, Daudigeos-Dubus E, et al. 2017. Heterogeneity of neuroblastoma cell identity defined by transcriptional circuitries. Nat Genet 49: 1408–1413. doi:10.1038/ng.3921

Buenrostro JD, Giresi PG, Zaba LC, Chang HY, Greenleaf WJ. 2013. Transposition of native chromatin for fast and sensitive epigenomic profiling of open chromatin, DNA-binding proteins and nucleosome position. Nat Methods 10: 1213–1218. doi:10.1038/nmeth.2688

Chipumuro E, Marco E, Christensen CL, Kwiatkowski N, Zhang T, Hatheway CM, Abraham BJ, Sharma B, Yeung C, Altabet A, et al. 2014. CDK7 inhibition suppresses super-enhancer-linked oncogenic
transcription in MYCN-driven cancer. Cell 159: 1126–1139. doi:10.1016/j.cell.2014.10.024
Corces MR, Granja JM, Shams S, Louie BH, Seoane JA, Zhou W, Silva TC, Groeneveld C, Wong CK, Cho SW, et al. 2018. The chromatin accessibility landscape of primary human cancers. Science 362: eaav1898. doi:10.1126/science.aav1898
de Laat W, Grosveld F. 2003. Spatial organization of gene expression: the active chromatin hub. Chromosoma Res 11: 447–459. doi:10.1023/A:1024922626762
Dowen JM, Fan ZP, Hnisz D, Ren G, Abraham BJ, Zhang LN, Weintraub AS, Schuimers J, Lee TI, Zhao K, et al. 2014. Control of cell identity genes occurs in insulated neighborhoods in mammalian chromosomes. Cell 159: 374–387. doi:10.1016/j.cell.2014.09.030
Ea V, Baudement M-O, Lesne A, Forné T. 2015. Contribution of topological domains and loop formation to 3D chromatin organization. Genes (Basel) 6: 734–750. doi:10.3390/genes6030734
The ENCODE Project Consortium. 2012. An integrated encyclopedia of DNA elements in the human genome. Nature 489: 57–74. doi:10.1038/nature11247
Ernst J, Kellis M. 2010. Discovery and characterization of chromatin states for systematic annotation of the human genome. Nat Biotechnol 28: 817–825. doi:10.1038/nbt.1662
Ernst J, Kheradpur P, Mikkelsen TS, Shores N, Ward LD, Epstein CB, Zhang X, Wang L, Isner R, Coyne M, et al. 2011. Mapping and analysis of chromatin signatures in nine human cell types. Nature 473: 43–49. doi:10.1038/nature09906
Gauton KJ, Nammo T, Pasqualli L, Simon JM, Giresi PG, Fogarty MP, Panhuis TM, Mieczkowski P, Secchi A, Bosco D, et al. 2010. A map of open chromatin in human pancreatic islets. Nature 265: 255–259. doi:10.1038/n.530
Gotea V, Visel A, Westlund JM, Nobrega MA, Pennacchio LA, Ovcharenko I. 2010. Homotypic clusters of transcription factor binding sites are a key component of human promoters and enhancers. Genome Res 20: 565–577. doi:10.1101/gr.104471.109
Heidari N, Phanstiel DL, He C, Grubert F, Jahnabani F, Kasowski M, Zhang MQ, Snyder MP. 2014. Genome-wide map of regulatory interactions in the human genome. Genome Res 24: 1905–1917. doi:10.1101/gr.176866.114
Heintzman ND, Stuart RK, Hon G, Fu Y, Ching CW, Hawkins RD, Laurencot LO, Van Calcar S, Qu C, Ching KA, et al. 2007. Distinct and predictive chromatin signatures of transcriptional promoters and enhancers in the human genome. Nat Genet 39: 311–318. doi:10.1038/ng1966
Heintzman ND, Hon GC, Hawkins RD, Kheradpour P, Stark A, Harp LF, Ye Z, Lee IK, Stuart RK, Ching CW, et al. 2009. Histone modifications at human enhancers reflect global cell-type-specific gene expression. Nature 459: 108–112. doi:10.1038/nature07829
Hnisz D, Abraham BJ, Lee TI, Lau A, Saint-André V, Sigova AA, Hoke HA, Young RA. 2013. Super-enhancers in the control of cell identity. Cell 153: 307–321. doi:10.1016/j.cell.2013.09.053
Hsiao LL, Dangond F, Yoshida T, Hong R, Jensen RV, Misra J, Dillon W, Lee JT, Sanborn AL, Machol I, Omer AD, Lander ES, et al. 2014. A 3D map of the human genome at kibbase resolution reveals principles of chromatin looping. Cell 159: 1665–1680. doi:10.1016/j.cell.2014.11.021
Ren R. 2005. Mechanisms of BCR-ABL in the pathogenesis of chronic myelogenous leukemia. Nat Rev Cancer 5: 172–183. doi:10.1038/nrc1567
Sanyal A, Lajoie BR, Jain G, Dekker J. 2012. The long-range interaction landscape of gene promoters. Nature 489: 109–113. doi:10.1038/nature11279
Shankar DB, Cheng JC, Kinjo K, Federman N, Moore TB, Gill A, Rao NP, Landev AM, Sakamoto KM. 2005. The role of CREB as a proto-oncogene in hematopoiesis and in acute myeloid leukemia. Cancer Cell 7: 351–362. doi:10.1016/j.ccr.2005.02.018
Smirnov P, Saikishian Z, El-Hachem N, Wang D, She A, Olsen C, Freeman M, Selby H, Gendoo DM, Grossmann P, et al. 2016. PharmacogX: an R package for analysis of large pharmacogenomic datasets. Bioinformatics 32: 1244–1246. doi:10.1093/bioinformatics/btv723
Somasundaram R, Prasad MAJ, Underbjerg S, Vignardsson M. 2015. Transcription factor networks in B-cell differentiation link development to acute lymphoid leukemia. Blood 126: 144–152. doi:10.1182/blood-2014-12-757688
Song L, Zhang Z, Grasfeder LL, Boyle AP, Giresi PG, Lee BK, Sheffield NC, Graf S, Huss M, Keefe D, et al. 2011. Open chromatin defined by DNasel and FAIRE identifies regulatory elements that shape cell-type identity. Genome Res 21: 1757–1767. doi:10.1101/gr.112541.111
Stratton MS, Lin CY, Anand P, Tanant PD, Ferguson BS, Wickers ST, Ambarkar AV, Sucharov CC, Bradner JE, Haldar SM, et al. 2016. Signal-dependent recruitment of BRD4 to cardiomyocyte super-enhancers is suppressed by a microRNA. Cell Rep 16: 1366–1378. doi:10.1016/j.celrep.2016.06.074
Thurman RE, Rynes E, Humbert R, Vierstra J, Maurano MT, Haugen E, Sheffield NC, Stengachis AB, Wang H, Vernot B, et al. 2012. The accessible chromatin landscape of the human genome. Nature 489: 75–82. doi:10.1038/nature11232
Vahedhi G, Kanno Y, Furumoto Y, Jiang K, Parker SCJ, Erdos MR, Davis SR, Roychoudhuri R, Restifo NP, Gadina M, et al. 2015. Super-enhancers delineate disease-associated regulatory nodes in T cells. Nature 520: 558–562. doi:10.1038/nature14154
Wang T, Bisroey K, Hughes NW, Krupczak KM, Post Y, Wei JJ, Lander ES, Sabatini DM. 2015. Identification and characterization of essential genes in the human genome. Science 350: 1096–1101. doi:10.1126/science.aac7041
Weintraub AS, Li CH, Zamudio AV, Sigova AA, Hambri BM, Day DS, et al. 2014. GABP transcription factor is required for development and genomic features. Cell 159: 307–321. doi:10.1016/j.cell.2013.03.035
Yang Z-F, Zhang H, Ma L, Peng C, Chen Y, Wang J, Green MR, Li S, Rossmann AG. 2013. GARP transcription factor is required for development of chronic myelogenous leukemia via its control of PRKD2. Proc Natl Acad Sci USA 110: 2312–2317. doi:10.1073/pnas.1212904110
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