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Review

Enhanced Identification of Transcriptional Enhancers Provides Mechanistic Insights into Diseases

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Enhancers are distal *cis*-regulatory DNA elements that increase the expression of target genes. Various experimental and computational approaches including chromatin signature profiling have been developed to predict enhancers on a genome-wide scale, although each method has its advantages and disadvantages. Here we overview an emerging method to identify transcribed enhancers at exceedingly high nucleotide resolution based on enhancer RNA transcripts captured by Cap Analysis of Gene Expression (CAGE) technology. We further argue that disease-causative regulatory mutations at enhancers are increasingly recognized, emphasizing the importance of enhancer identification in functional and clinical genomics including, but not limited to, genome-wide association studies (GWASs) and cancer genomics studies.

Trends

Various experimental and computational approaches have been developed to predict enhancers on a genome-wide scale, although each method has its advantages and disadvantages.

Cap analysis of gene expression (CAGE) identifies transcribed enhancers at exceedingly high nucleotide resolution by detecting enhancer RNAs (eRNAs).

Disease-associated SNPs and recurrent somatic cancer mutations are identified within enhancers. These variants might alter enhancer activities and contribute to pathogenesis, highlighting the importance of enhancer identification in various clinical settings.

Regulation of Gene Expression by Enhancers

Spatiotemporal control of gene expression is of critical importance for cellular differentiation, organogenesis, and homeostasis in multicellular organisms and dysregulation of gene expression is linked to many diseases (reviewed in [1,2]). Regulation of gene expression is a multilayered process [3] and an initial step is the synthesis of RNAs. Besides core promoter sequences in the immediate proximity of transcription start sites (TSSs), which recruit general transcription factors (TFs) and initiate RNA polymerase II (RNAPII)-mediated transcription, other *cis*-acting elements such as proximal promoters, enhancers, and silencers cooperatively modulate basal transcription from the core promoters (reviewed in [4]). Among these, enhancers are small segments of promoter-distal *cis*-regulatory DNA regions that significantly enhance the expression of target genes independent of location or orientation with respect to the target genes (reviewed in [5,6]).

An enhancer was first described in the genome of simian virus 40 (SV40) in 1981 by Banerji *et al.* [7] and Moreau *et al.* [8], where a 72-bp repeated sequence located upstream of the SV40 early region significantly increased the ectopic expression of a reporter gene. Remarkably, a non-viral enhancer was discovered in 1983 within a mouse immunoglobulin heavy chain gene [9–11], followed by studies documenting many enhancers in various organisms. Thus, an enhancer was originally defined by its functionality in enhancing the transcription of target genes.

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Enhancers contain binding sites for TFs that interact with coactivators including the histone acetyltransferases p300/CREB-binding protein (CBP) (reviewed in [12,13]). An enhancer is then brought into close proximity with its target promoter through chromatin looping, facilitated by Mediator, which associates with cohesin and the cohesin loading factor [14]. Regions bound by TFs are typically depleted of nucleosomes and are sensitive to cleavage by deoxyribonuclease I (DNase I) [15]. These nucleosome-depleted regions (NDRs) are flanked by regions marked with specific histone modifications such as histone H3 lysine 4 monomethylation (H3K4me1) and H3K27 acetylation (H3K27ac). H3K4me1 is associated with inactive, poised, and active enhancers whereas H3K27ac is more specifically associated with active enhancers (reviewed in [16,17]).

Super-enhancers have been proposed to describe groups of putative enhancers clustering in close genomic proximity. These can span exceptionally large genomic regions with strong enrichment for the binding of TFs and Mediator [18–20]. Intriguingly, super-enhancers are often located near genes related to cell type-specific function and are enriched for sequence motifs of cell type-specific master TFs. However, the term super-enhancer has been used in many studies without a clear definition (reviewed in [21]).

Various Approaches to Identify Enhancers

With an increasingly clearer picture of how enhancers work, various computational and experimental approaches have been developed to identify enhancers (Table 1).

Comparative genomic analyses on conserved noncoding sequences or TF-binding motifs successfully identified a fraction of novel enhancers [22–24] (reviewed in [25,26]). Candidate enhancer elements together with their *in vivo* validation experiments are available through VISTA Enhancer Browser[i] [27]. With the advent of next-generation sequencing technology, many high-throughput experimental approaches were developed to predict enhancers on a genome-wide scale. ChIP-seq of TFs predicts a subset of putative enhancers (reviewed in [13]) whereas ChIP-seq of p300 covers enhancers more ubiquitously [28]. High-throughput profiling of DNase I hypersensitive sites (DHSs) allows identification of enhancers [15], although DHSs also include other regulatory DNA regions such as promoters, insulators, and silencers. More recently, an assay for transposase-accessible chromatin using sequencing (ATAC-seq) was developed as a rapid and sensitive alternative method for examining chromatin accessibility [29] (reviewed in [30]). In addition, ChIP-seq of histone modifications enables genome-wide prediction of putative enhancers and combinatorial analysis of distinct histone marks allows determination of enhancer activation (reviewed in [16,17]).

The Encyclopedia of DNA Elements (ENCODE) Project has contributed enormously to building a comprehensive map of functional elements in the human genome[ii] [31] (Box 1). They have provided 457 ChIP-seq datasets on more than 119 TFs in many human cell lines and these data are available on Factorbook[iii] [32]. Furthermore, Ernst *et al.* annotated 15 chromatin states by ChIP-seq of nine distinct histone modifications across nine cell types. They defined regions with high H3K4me1 and H3K27ac levels as strong enhancers and regions with high H3K4me1 but low H3K27ac levels as weak or poised enhancers [33].

The term enhancer is ambiguously defined and different methods identify ‘enhancers’ by capturing different features or aspects (e.g., NDRs, TF-binding sites, histone marks surrounding NDRs). Moreover, each method has its advantages and disadvantages. NDRs, which can be identified by DNase-seq or ATAC-seq, are observed at diverse types of regulatory elements in addition to enhancers [30]. Similarly, TFs bind to a broad spectrum of regulatory elements [13]. In addition, ChIP-seq data suffer from the intrinsic issue of limited base resolution. This issue arises from sonication-based chromatin fragmentation followed by size selection of 200–400-bp

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