Computational Identification of Active Enhancers in Model Organisms

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Abstract As a class of cis-regulatory elements, enhancers were first identified as the genomic regions that are able to markedly increase the transcription of genes nearly 30 years ago. Enhancers can regulate gene expression in a cell-type specific and developmental stage specific manner. Although experimental technologies have been developed to identify enhancers genome-wide, the design principle of the regulatory elements and the way they rewire the transcriptional regulatory network tempo-spatially are far from clear. At present, developing predictive methods for enhancers, particularly for the cell-type specific activity of enhancers, is central to computational biology. In this review, we survey the current computational approaches for active enhancer prediction and discuss future directions.

Introduction

Gene transcription is regulated by a series of accurately orchestrated interactions between transcription factors (TFs) and cis-regulatory DNA elements, e.g., promoters and enhancers [1]. Enhancers are often found in non-coding regions of a genome and generally distal to their target promoters. The first characterized enhancer was a DNA segment that markedly increased the transcription of the β-globin gene in a transgenic assay in the SV40 tumor virus genome about 30 years ago [2]. Nonetheless, global identification of enhancers and their activities remains challenging, since enhancers can activate transcription regardless of their location or orientation [3]. The development of computational enhancer recognition approaches has been greatly facilitated by the massive amount of genomic data available owing to the rapid advances in sequencing technologies in recent years. Early algorithms were developed largely based on evolutionary constraints with the assumption that highly conserved non-coding regions should have functional potential [4]. However, conservation by itself is not sufficient to confer cell-type specific enhancer activities, suggesting that additional (e.g., epigenetic) information is required for accurate prediction. Genome-wide maps of chromatin marks have been used to show that active enhancers are likely to be associated with certain characteristic chromatin signatures, e.g., monomethylation of histone H3 at lysine residue 4 (H3K4me1) [5]. But, Bonn et al. reported that H3K4me1 is...
distributed similarly between mesodernally active and inactive enhancers, indicating that the placement of H3K4me1 is not cell type specific during embryonic development [6]. Hitherto, to the best of our knowledge, there is no evidence that active enhancers should necessarily exhibit the same single or a combination of epigenetic marks across all the cell types [7]. Therefore, it is necessary to select optimal combinations of epigenetic marks to predict when and where an enhancer is active [8–11]. In this review, we first survey the most commonly adopted strategies in enhancer recognition and then discuss potential future directions.

The principle of enhancer recognition

Enhancers may be characterized by quantitative measures, termed features, associated with the underlying DNA sequences. In principle, an enhancer recognition algorithm utilizes informative and discriminative features as input to discriminate enhancers from non-enhancers, ideally from other non-enhancer cis-regulatory elements. Algorithms and features are both important. We therefore will discuss them separately.

Features can be briefly classified into three categories, namely comparative genomic features, TF binding related genetic features and epigenetic features (Figure 1). Comparative genomic features mainly refer to the conservation scores calculated by comparing the genome sequences of different species. The predictive power of comparative genomic features stems from the fact that functional genome regions (e.g., enhancers) are subjected to negative selection [12,13]. TF binding related genetic features use quantitative scores presumably representing the TF binding affinity at the DNA sequence of interest. The DNA binding sites of a given TF are usually determined by the DNA nucleotide sequence and the binding affinity between the TF and the DNA sequence [14–16]. It is believed that TFs are the actual operators for enhancer regulatory activities [17], which may explain why TF binding related genetic features are predictive. Direct measurement of the binding affinity between a TF and DNA sequence is not easy. However, the binding affinity can be inferred indirectly, either by experimentally measuring frequency of TF binding events, such as chromatin immunoprecipitation (ChIP) [18], or by calculating the similarity of the DNA sequences with a known TF binding motif [19,20]. The epigenetic feature mainly includes the level of histone modifications and of DNA methylation. Recent experimental evidence supports the association of several histone modifications with enhancer activity. The histone modification levels thus have served as features to predict active enhancers in humans [21,22]. Researchers also attempt to seek optimum combinations of these features for whole-genome prediction of active enhancers [5,9–11] (Table 1). Obviously, not all the aforementioned features are equally important for active enhancer prediction. The level of some dominant features showed strong correlation with enhancer activity [5,23], although the nature of the relationship between the features and enhancer states is poorly understood. Further development of superior predictive methods can not only help us to reveal such structure, but also help to improve sensitivity and specificity of the predictions.

Algorithms for enhancer recognition can be roughly divided into two groups. One group comprises probabilistic graphical models which describe the generative process of specific signals, such as Bayesian networks (BNs) [24] and hidden Markov models (HMMs) [25]. The other group employs

Figure 1  Features used in enhancer prediction algorithms
The comparative genomic features are usually generated from comparison between DNA sequences in closely-related species. TF binding features result from two sources, one from known TF binding motifs and the other from ChIP experiments. Epigenetic features can be measured by various technologies. See the main text for more details.
Table 1 Features of computational methods for enhancer prediction

| Feature                      | Method                      | Ref |
|------------------------------|-----------------------------|-----|
| Comparative genomic features | Aparicio’s method [4]       |     |
|                              | Visel’s method (2008) [30]  |     |
|                              | Chen’s method [8]           |     |
|                              | Yip’s method [50]           |     |
| Sequence-based TF binding related features | Narlikar’s method [65] |     |
|                              | Chen’s method [8]           |     |
|                              | Lee’s method [44]           |     |
|                              | Yip’s method [50]           |     |
| Experiment-based TF binding related features | Visel’s method (2009) [46] |     |
|                              | Zinzen’s method [67]        |     |
|                              | May’s method [48]           |     |
|                              | Chen’s method [8]           |     |
| Epigenetic features          | Heintzman’s method [5]      |     |
|                              | Won’s method [11]           |     |
|                              | Firpi’s method [10]         |     |
|                              | SEGWAY [69]                 |     |
|                              | Kharchenko’s method [60]    |     |
|                              | He’s method [23]            |     |
|                              | Ernst’s method [61]         |     |
|                              | ChromaGenSVM [9]            |     |
|                              | Yip’s method [50]           |     |
|                              | Chen’s method [8]           |     |
|                              | Bonn’s method [6]           |     |

Note: More than one type of features were employed to build enhancer recognition model in some studies. For example, Chen et al. used all four types of features to develop active enhancer recognition model [8].

Transcriptional factor binding related genetic features

Transcriptional factor binding related genetic features can be roughly classified into two groups. One group includes quantitative scores of similarity to a known TF binding motif, representing the TF binding affinity to the DNA segments (sequence-based TF binding related genetic features). The other group includes experimental measurements of TF binding frequency, which also presumably represents TF binding affinity (experiment-based TF binding related genetic features).

The sequence-based TF binding related genetic features comprise individual TF binding and the enrichment of modular combinations of TF binding. Measuring TF binding affinities is not an easy task experimentally; however, it can be approached from the nucleotide preferences at each sequence position [20], e.g., position weight matrix (PWM). PWM describes the probability of observing the respective nucleotides A, C, G, and T in each position of a sequence motif. It has been found that there is a strong correlation between PWM scores and the TF binding affinity [15,16,20]. PWMs for known TFs have been cataloged in databases [34,35]. These matrices enable people to assign a quantitative score to any sequence to evaluate the binding affinity of the specific TF at that sequence (Figure 1). In vertebrates, functional TF binding sites are usually clustered into a modular structure, which motivates researchers to seek cis-regulatory modules (CRMs) as the advanced predictive features for cis-regulatory element recognition [36,37]. The CRM features are often calculated as the likelihood of the CRM in a given genome context [38]. For example, MSCAN value measures the statistical significance of the appearance of potential combinatorial TF binding sites [39]. All the TF binding sites are represented by PWM scores and MSCAN returns the significance of the CRM. A similar strategy is adopted in MCAST [40].

To further improve the performance, additional phylogenetic footprinting is employed to align interested orthologous DNA sequences to define a conserved region and then the significance of the CRM is calculated in the regions. For example, EEL approach was used to scan a given pair of orthologous sequences to identify conserved TF binding sites, and, then EEL scores were calculated by considering both distances and differences in the angles between adjacent binding sites [41]. Another example, MorphMS, implemented a pair-HMM statistical alignment between two species [42]. A first order Markov network with three states (match, deletion and insertion) was implemented and emits two strings, one for each species. The string emitted in the match state was chosen by another probabilistic process, which models the arrangement of binding sites and non-binding (“background”) sites by PWM. Then, two log likelihood ratio (LLR) scores were reported. The two scores (LLR1 and LLR2) compare the likelihood of a sequence under the MORPH model to the likelihood of the sequences under null models. The null model used in LLR1 only considers background PWM, while the null model for LLR2 assumes that the two orthologous sequences were generated independently.

Besides the similar strategy used in MorphMS, another algorithm EMMA incorporates gains and losses at binding
site, a process that is believed to be an important part of CRM evolution [43]. However, the computational cost increases exponentially with the number of TFs considered. One alternate choice for this type of sequence features is \( k \)-mer profile, which is the frequency of all possible \( k \)-mer (putative motifs with length of \( k \)) in a given sequence region [44]. The profile measures how likely the \( k \)-mers in one enhancer would be found in another independently-generated sequence. Using such \( k \)-mer features, Leung and Eisen developed a profile similarity between pairs of sequences to detect novel enhancers [45]. However, the search space is growing exponentially with \( k \).

The sequence-based TF binding related genetic features alone are not sufficient for active enhancer recognition. First, most of the features are conserved TF binding sites, while many enhancer elements are not conserved. For example, in Drosophila, the cone-specific Pax2 enhancer carries barely-conserved TF binding sites, which have been shown to possess similar enhancer functions in transgenic assays [31]. Similarly, a large proportion of a 40 kb region in the Phox2b locus showed regulatory activity by transgenic assay in zebrafish, while only 29–61% identified regulatory sequences were conserved [32]. Second, in any given tissue, only a subset of enhancers is active. This tissue-specific activity may result from a tissue-specific combination of binding TFs or from regulation at the epigenetic level.

TF binding in given tissues or cell types can be experimentally measured, which gives experiment-based TF binding related genetic features. For example, data from chromatin immunoprecipitation followed by massively parallel DNA sequencing (ChIP-seq) technology precisely provide binding loci for the TFs under the given conditions [18]. Visel et al. mapped the genome-wide occupancy of p300 in three cell lines by ChIP-seq. Using transgenic mouse assay, they show that p300 binding sites are predictive for enhancer activity in the cell types examined [46]. Similarly, CREBBP-bound enhancers also show environment-dependent activity in neurons [47], or in transgenic mouse enhancer assays [48]. Recently, ENCODE project has generated high-throughput sequencing (ChIP-seq or ChIP-chip) data sets for 119 distinct transcription factors over five main cell lines [49]. These experimental results have been used for enhancer recognition [50].

### Epigenetic features

Epigenetic features consist of chromatin structure, histone modifications, DNA-methylation levels and non-coding RNAs. In this review, we mainly focus on the first two types of epigenetic features, since other features have been reviewed elsewhere (such as [51]). Chromatin structure controls DNA accessibility of TFs to enhancer or other regulatory elements. DNA accessibility can be inferred as DNase I hypersensitivity [52,53] or by Formaldehyde-Assisted Isolation of Regulatory Elements (FAIRE) technology [54]. The regions detected by DNase I or FAIRE are associated with all known classes of active DNA regulatory elements, including enhancers [55]. For example, Wiench et al. found that CpG methylation at glucocorticoid receptor (GR) -associated DNase I hypersensitive sites was a cell type-specific event and suggested that these sites could be a unique class of active enhancers [56]. Comparing DNase I-seq and FAIRE-seq data in seven human cell types indicated that data from these two assays were not fully overlapping [57]. DNase I tended to find the regions around transcriptional start sites, while FAIRE was more sensitive in detecting distal regulatory elements. Notably, neither DNase I nor FAIRE hypersensitive sites detected in one cell type are sufficient to demonstrate their enhancer state, as many other regulatory element sites, such as repressors or insulators, are also DNase I or FAIRE hypersensitive [57]. Therefore, DNase I or FAIRE hypersensitivity data should be regarded as a necessary but not sufficient input for active enhancer prediction.

In addition to DNA accessibility, the presence of characteristic histone modifications is another sign for the activity of enhancers, e.g., elevated H3K4 monomethylation (H3K4me1) levels and depleted H3K4 trimethylation (H3K4me3) levels have been correlated with enhancer activity [5]. Further experiments showed that active enhancers marked by H3K4me1 in ES cells are also flanked by H3K27 acetylation (H3K27ac), while regions marked by H3K27 trimethylation (H3K27me3) are associated with early developmental genes which are poised in ES cells [58,59]. In another study, however, Bonn et al. found that H3K4me1 was distributed similarly between mesodermally active and inactive enhancers, indicating that the placement of H3K4me1 is not completely cell type specific during embryonic development [6]. Instead, they found a conditional link between the presence of H3K79me3, H3K27ac marks and enhancer activity.

Although the histone modification patterns mentioned above showed promising potential for enhancer activity prediction in certain cell types, the general pattern of histone modifications for prediction still remains elusive. In human CD4 T cells, 39 histone modification types have been mapped and several histone mark combinations showed correlation with enhancers, yet no single mark is associated with more than 40% of enhancers [7]. Integrating more epigenetic marks may render a more reliable, robust and precise model to capture active enhancers. Several attempts have been made [5,9–11]. One such attempt employed 10-fold cross-validation for all possible combinations of six histone modification marks to predict p300 binding sites, and found that enrichment of H3K4me1 and depletion of H3K4me3 is the most predictive combination for p300 binding [5]. Many more sophisticated computational technologies have also been applied to search for optimal combinations for active enhancers. For example, Won et al. coupled HMM with simulated annealing to search for the most informative combination of histone modification marks [11]. In Drosophila, Kharchenko and coworkers found that active enhancers lack H3K4me3 and are enriched for H3K4me1, H3K27ac and H3K18ac [60]. Similarly, Chrom-HMM labeled active enhancers with the H3K4me1, H3K4me2 and H3K27ac signature [61]. In a vast collection of epigenetic marks (20 histone methylations and 18 histone acetylations), genetic algorithms indicated that the most predictive histone modification signals within enhancers are H3K4me1 and H3K4me3 [9]. A similar pattern was also extracted from nearly 40 ENCODE histone modifications by using fisher discriminate analysis [10].

The features we discussed above can also be roughly classified into two classes, based on the prediction power for enhancer activity. One class of features represents the potential of a locus to be an enhancer, e.g., comparative genomic features or sequence-based TF binding related genetic features, because
the features describe the static DNA sequence characteristics which are shared by almost all cells in an organism. The other class of features, e.g., experiment-based TF binding related genetic features or epigenetic features, further indicates enhancer activity of the loci in a given tissue or cell type. These features are the actual measurement of cellular or molecular activities that had already been associated with enhancer activity in living cells. For example, when Visel and colleagues compared the evolutionary conservation score and p300 binding sites, they found that only 47% (246 out of 528) of conserved enhancer candidates were active in a transgenic mouse assay, whereas 87.7% of p300 binding sites were reproducibly active in the same transgenic assay [46]. Heintzman et al. exhaustively searched all combinations of six different histone modification marks, and identified the optimal combinations of H3K4me1 and H3K4me3 [5]. Despite the fine performance of this simple model, the best predictive power in one dataset does not guarantee its performance in another. Moreover, an ever increasing number of features would challenge these simple methods. This is not only because of the inter-correlations between the features, but also because of the difficulties in interpreting the relative importance of each feature. A class of computational technology, named feature selection, has been applied to solve such problems [64]. For example, Narlikar et al. built a linear regression model to identify active enhancers in heart based on 727 sequence features including 721 TF binding related genetic features [65]. The LASSO linear regression method was then applied to find features relevant to enhancer activity and 45

Figure 2  Flow scheme of model building
To improve model interpretability and reduce overfitting, sophisticated computational strategies implement feature selection algorithm to select a subset of relevant features for model building. Then, appropriate classification model is employed to differentiate active enhancers from non-enhancers. Generally, there are two major classification models. The first is the discriminative models which find the optimal classification border in the feature space (lower left panel). The other one is the probabilistic graphical models that try to model the joint distribution of states and associated features with graph (lower right panel). ANN, artificial neutral network; BN, Bayesian network; HMM, hidden Markov model; SVM, support vector machine.
| Category                        | Method               | Operational model                                             | Positive predictive value (%) | Note                                                                                             | Ref         |
|--------------------------------|----------------------|----------------------------------------------------------------|----------------------------|--------------------------------------------------------------------------------------------------|-------------|
| Discriminative model           | Heintzman’s method   | Thresholds of histone modification profiles                    | 39.5                       | Mapped to distal p300 binding sites in HeLa cells                                               | [5]         |
|                                | Visel’s method (2009)| Thresholds of p300 binding profiles                            | 87.7                       | With reproducible enhancer activity in transgenic mouse                                          | [46]        |
|                                | Narlikar’s method    | Linear regression                                              | 62                         | With reproducible enhancer activity in vivo in mouse and zebrafish                               | [65]        |
|                                | Zinzen’s method      | Support vector machine                                         | 71.4                       | With reproducible enhancer activity in transgenic Drosophila                                     | [67]        |
|                                | Firpi’s method       | Time-delay neural network                                       | 66.3                       | Overlapped with p300 binding sites, Dnase I hypersensitivity sites or TRAP220 binding sites in  | [10]        |
|                                | Lee’s method         | Support vector machine                                         | 74.5                       | Overlapped with Dnase I hypersensitive enhancers in embryonic mouse whole brain cells            | [44]        |
|                                | ChromaGenSVM         | Support vector machine                                         | 57                         | Overlapped with p300 binding sites, Dnase I hypersensitivity sites or TRAP220 binding sites in  | [9]         |
|                                | Won’s method         | Hidden Markov model                                             | 54.8                       | Overlapped with p300 binding sites, Dnase I hypersensitivity sites or TRAP220 binding sites in  | [11]        |
|                                | Bonn’s method        | Bayesian network                                               | 78                         | Overlapped with previously identified TF binding sites in Drosophila                             | [6]         |
|                                | Other                | Multinomial logistic                                           | 83                         | Overlapped with at least one TF peak from 7 mouse embryonic stem cell ChIP-seq datasets          | [8]         |
|                                | Yip’s method         | Random forest                                                  | 67                         | With enhancer activity in vivo in mouse and medaka fish (28/42)                                  | [50]        |

*Note:* The performance shown here is the reported performance compared to experimental results. The positive predictive value (percentage) was calculated as follows: positive predictive value = true positive/(true positive + false positive).
features were assigned nonzero weights. The accuracy of 92% was achieved in distinguishing heart enhancers from a large pool of random noncoding sequences.

Recently, more sophisticated methods have been implemented to find the optimal classification border in the feature space. Typical methods include ANNs and SVMs. A neural network is a parallel system, capable of resolving paradigms like enhancers which are critical in the regulation of enhancer–promoter interactions. Polymer models are valuable tools in 3D chromatin structure study, to reveal the principle of enhancer activity. This BN model achieved better performance (AUC = 0.82), compared to the aforementioned NBc model.

Conclusion and outlook

Enhancers are regulatory DNA elements that can activate transcription largely independent of their location or orientation. Often, enhancers regulate gene expression in a tissue-specific manner and play important roles in cell differentiation [17]. In this review, we have described the general computational strategies for enhancer prediction. It has been suggested that H3K4me1 and p300 binding signatures are the most predictive features for active enhancer recognition [5,46], however, this notion may be disputed by new data. For example, a recent study found that H3K79me3 and H3K27ac, instead of H3K4me1, are predictive for cell type specific enhancer activity during embryonic development [6]. Recently, a more complicated picture, which involves nuclear organization, chromatin structure and non-coding RNAs, is emerging for enhancer activation. Accumulating data suggested that the insulators are critical in the regulation of enhancer–promoter interaction which is believed to be accomplished by long-range inter- or intra-chromosomal chromatin interactions [70].

From the perspective of computational biology, the field of enhancer research is now moving toward the modeling of 3D chromatin structure in nuclei, to reveal the principle of enhancer–promoter interactions. Polymer models are valuable tools in 3D chromatin structure study, e.g., the dynamic random loop model [71] and the fractal globular model [72]. To understand enhancers in the context of gene regulatory networks, it is necessary to integrate data from ultra-heterogeneous data sources in this “big data” era. For example, enhancer transcribed RNAs (eRNAs) were recently found prevalent at enhancer loci [47]. Some of such non-coding RNAs even act like enhancers [73]. Therefore, the integration of RNA-seq data is essential for a model which aims to understand eRNA associated enhancer activity.

Competing interests

The authors have declared that no competing interests exist.

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