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Enhancer, epigenetics, and human disease

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Enhancers encode a huge body of information to determine the precise tissue specific gene expression pattern during normal development. Nowadays, enhancers are also considered as key players in directing disease transcriptional program during pathogenesis. New genomic technologies allow the identification, functional characterization and manipulation of enhancers. The advances in the transcriptional enhancer field hold great promise in linking developmental or disease phenotypes to genetic variants and promoting precision medicine.

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Introduction

Enhancers are regulatory DNA sequences widely dispersed throughout the genomes. The first discovery of promoter activation by remote *cis*-DNA element was made by Schaffner and colleagues in 1981 [1••]. The small piece of 'enhancer' was demonstrated to be functional in gene activation even located thousands of base pairs away from either side of the transcription start site (TSS), irrespective of its orientation [1••,2,3]. By conventional genetics and biochemistry screening approaches, this type of cis-regulatory element was later also found in eukaryotic genomes to determine when and where a gene should be turned on [4••,5••,6]. Based on these observations, enhancers are transcriptional factor binding regions distal to TSSs but able to remotely stimulate target gene expression in a spatiotemporal specific manner.

Precise regulation of spatiotemporal gene expression patterns by enhancers during development endows metazoans with diverse functional cell types to complete life cycle. Owing to rapidly developing human cancer and diseases genomics, it has been gradually realized that aberrant gene expression led by faulty enhancer function is one of the main drivers in the pathogenesis of diseases including cancers. Here we give an overview about enhancer associated chromatin signatures and the machineries involved in the dynamic deposition, conversion or removal of these chromatin modifications. We discuss current understanding about the functional modes of enhancer bound factors in mediating enhancer-promoter communication. We also describe recent advances in allele specific enhancers and their implications in therapeutic decision making.

Chromatin signatures of enhancers

DNA at enhancers, as at other cis-regulatory elements including promoters, is depleted of nucleosomes and hypersensitive to DNase treatment [7]. Recent genome wide studies using the powerful next generation sequencing (NGS) platform have revealed that nucleosomes binding to enhancer flanking regions usually bear certain chromatin modification signatures. Histone H3 lysine 4 monomethylation (H3K4me1) is typically enriched in the vicinity of enhancers, whereas H3K4 trimethylation (H3K4me3) is predominantly high in the surrounding regions of active promoters [8]. However, the two histone modification marks are not mutually exclusive in the genome. H3K4me3 is also detectable over the enhancer regions, and its level was found to correlate with enhancer activity [9,10]. Therefore, a local ratio of H3K4me1 to H3K4me3 serves as a more reliable indicator in enhancer prediction. Additionally, active enhancers and promoters are also occupied by H3K27 acetylation (H3K27ac) [11•,12•]. H3K27 trimethylation (H3K27me3), which cannot coexist with H3K27ac on the same histone H3, labels the repressed or poised regulatory regions [12•]. Many other histone modifications, including acetylation on Histone H3 lysine 9 or 18, and phosphorylation on Histone H3 serine 10 or 28, have been also demonstrated to be associated with enhancers [13–15].

Chromatin modifiers of enhancers

Genes primed for future activation will be initially bookmarked by pioneer transcription factors at the nucleosome-occluded enhancer regions [16,17]. Pioneer factors, through recruiting nucleosome remodelers, can locally create nucleosome free region (NFR), decompact chromatin and facilitate the sequential binding of other transcriptional regulators, including various epigenetic machineries [16]. Distinct chromatin signatures of enhancers are established through interplay among these epigenetic machineries. Trr or MLL3/4 complexes in complex proteins

associated with set1 (COMPASS)-like family are required for the maintenance of H3K4me1 at majority of enhancers [18–20]. The unique subunit of Trr or MLL3/4 complexes, the H3K27 demethylase UTX, may be involved in the removal of the inactive enhancer mark H3K27me3, which is deposited by Polycomb repressive complex 2 (PRC2) *via* the methyltransferase activity of EZH2 [18,21,22]. The transition from poised enhancer to active enhancer also requires the histone acetyltransferases CBP and p300 to set up H3K27ac surrounding the enhancer element [8,21,23]. The H3K4 demethylase KDM1A, also known as LSD1, functions possibly together with the nucleosome remodeling and histone deacetylation (NuRD) complex in removing H3K4me1 and H3K27ac and decommissioning enhancers during stem cell differentiation [24,25]. Genes encoding the histone modifiers are frequently mutated in various types of cancers, which has been reviewed elsewhere [26–29].

Histone modifications catalyzed by various epigenetic modifiers reflect the influence of these transcriptional regulation related modifiers on local chromatin environment and gene activity. However, most of the modifiers are able to act on multiple substrates, including both histone and non-histone proteins, and also involved in other cellular processes [30]. For example, protein stability and transcriptional activity of the tumor suppressor p53 are dynamically regulated through its methylation turnover controlled by these modifiers including LSD1 [31]. Therefore, the contribution from the histone modifications *per se* on enhancer activity and transcription in metazoans is not well understood from genetic manipulation of the histone modifiers. In addition, major histones are encoded by multiple copy genes in most organisms. It is technically challenging to replace all the wide type copies with the modification target residue mutants.

Inspired by the discovery that single allele mutation of Histone H3 lysine 27 to methionine (H3K27M) in human diffuse intrinsic pontine gliomas (DIPGs) can inhibit H3K27 methylation *in vivo* [32], point mutation of the modification target residue on histone proteins has been recently employed to investigate the function of histone modifications [33]. Intriguingly, these histone mutants were found to mainly affect the activity of histone modifiers [32–34]. Furthermore, interpreting the data obtained from histone lysine to methionine mutants remains challenging as some of the residues, such as lysine 4 and lysine 9 of Histone H3, are target residues of multiple modifications, including mono-methylation, di-methylation and tri-methylation.

Enhancer–promoter communication

Current researches based on chromatin conformation capture derived assays and fluorescence *in situ* hybridization (FISH) favor the model that enhancers regulate the transcription from the target promoters through long range chromatin looping, which brings enhancers and promoters into close physical proximity [35–40]. Architectural proteins CTCF and cohesin are involved in the gene regulatory chromatin interaction process [41–45]. CTCF is an insulator protein blocking enhancer–promoter interaction. Beyond the renowned function in sister chromatid cohesion, cohesin also regulates transcription [41]. Together with the Mediator complex at enhancers, cohesin facilitates the interaction between enhancer and promoter [46•]. The Hi-C data has suggested that eukaryotic genomes are partitioned into different topologically associating domains (TADs), within each of which chromatin interactions in regulating gene expression are highly frequent [47,48]. Together with CTCF at the borders of TADs, cohesin helps maintain the relatively insulated chromatin domain. Recent chromatin interaction analysis by paired-end tag sequencing (ChIA-PET) studies have further demonstrated on a genome-wide scale that cohesin associated CTCF-CTCF loops are required for forming the relatively insulated TADs, which constrain cohesin-mediated enhancer–promoter interaction within them [49–51]. Additionally, the orientation of CTCF binding sites (CBSs) is critical in establishing specific chromatin interaction in vertebrates [52–54]. CTCF-CTCF loops preferentially form between CBSs that occur in a convergent orientation [55–57]. Inversion of specific CBSs by CRISPR mediated genome editing leads to reconfiguration of the topology of chromatin and transcription change [52,53].

Regulation of RNA polymerase II by enhanceosome

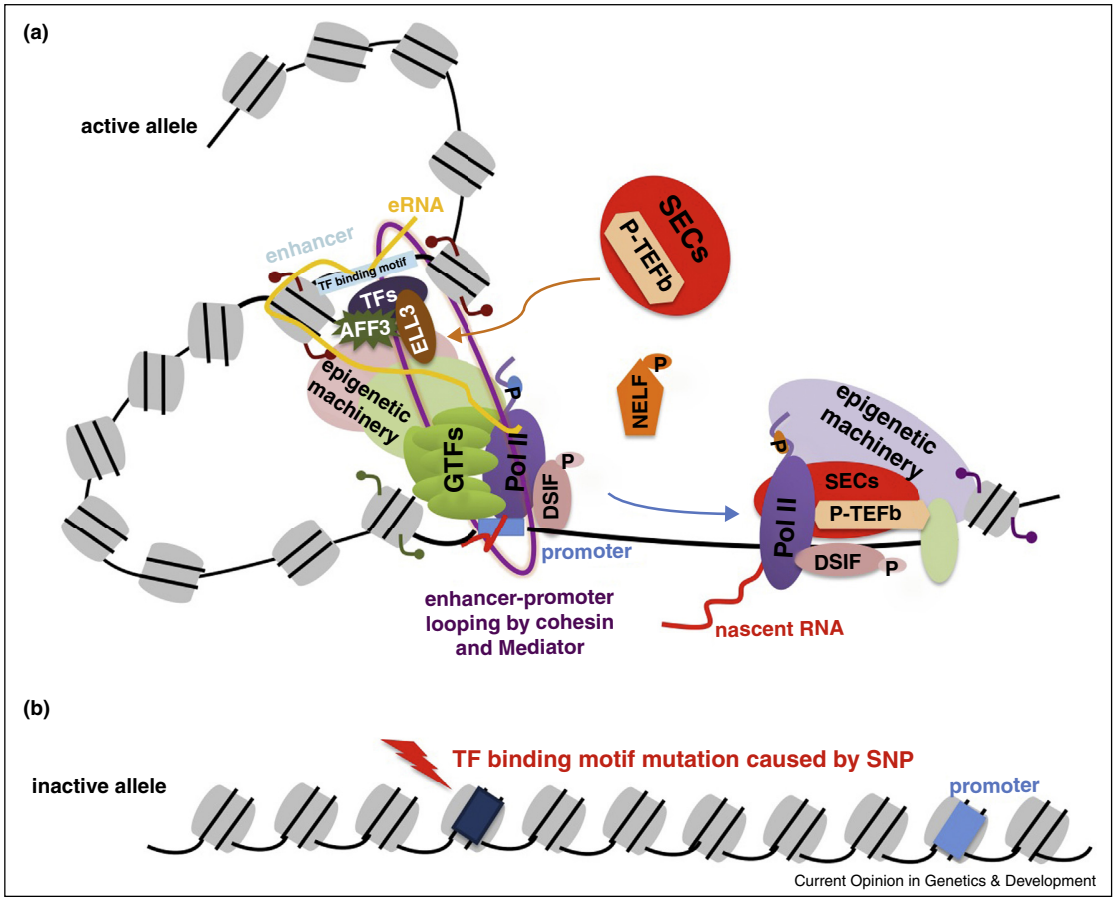
Transcription by RNA polymerase II (Pol II) is highly regulated at several steps, including establishment of preinitiation complex (PIC), promoter-proximal pausing, and entry into productive elongation stage [58,59]. In addition to opening up the local chromatin through sequential binding of pioneer factors, nucleosome remodelers and chromatin modifiers, enhancers also regulate the behavior of RNA Pol II [16,60,61•,62]. The RNA Pol II elongation factor ELL3 was found specifically occupying enhancers at different stages in mouse embryonic stem cells [61•]. ELL3 is required for the establishment or stability of paused Pol II at a group of developmental genes close to ELL3 bound poised enhancers. ELL3 itself is a component of the Super Elongation Complex (SEC) [63,64]. Upon differentiation signal stimulation, ELL3 is able to recruit the AFF4 centered SEC to its target genes and activate their expression [61•,65]. The function of ELL3 on paused Pol II has been demonstrated to rely on cohesin. The depletion of cohesin leads to less efficient transition from paused Pol II to elongating Pol II at most of genes in Drosophila cultured neuronal cells [66]. Similarly, in human Cornelia de Lange syndrome (CdLS) which is caused by mutations of genes involved in cohesin pathway, Pol II occupancies at both promoters and gene bodies are impaired [67]. Factors like ELL3 and cohesin

within the enhanceosome might regulate the processivity of Pol II at different stages, at least in part through SEC (Figure 1). This is further substantiated by the recent finding that AFF4 mutations within the AFF family member homology domain caused CHOPS syndrome (Cognitive Impairment, Coarse Facies, Heart Defects, Obesity, Pulmonary Involvement, Short Stature and Skeletal Dysplasia), which mirror both the phenotypes and the transcriptome of CdLS [68]. In CHOPS syndrome as well as CdLS, AFF4, cohesin and RNA Pol II genomic distribution were altered, suggesting that the SEC and cohesin are involved in the pathogenesis of CHOPS through enhancer's function to elongation.

Transcription of active enhancers

On the other hand, transcription by Pol II has been observed from the regions associated with active enhancer marks. The transcripts from enhancers are named as enhancer RNAs (eRNAs) [69•,70•,71•]. In general, eRNAs are non-coding, non-spliced and unstable [72]. Although eRNAs are lowly expressed, their expression level are usually positively correlated with the level of mRNA transcripts from neighboring genes [69•]. In a recent study it has been demonstrated that the Integrator complex is recruited to enhancers and required for the biogenesis of eRNAs [73]. Disruption of the Integrator complex diminishes both the induction of eRNA and enhancer-promoter communication in the presence of epidermal growth factor (EGF) stimulus. The Integrator complex has been shown to recruit the elongation complex SEC to EGF responsive genes to release paused Pol II [74]. Interestingly, a study in neurons suggested that eRNAs could also promote paused Pol II release into productive elongation stage by serving as a decoy factor for the negative elongation factor (NELF) at immediate early genes [62]. A functional link from enhancer to

Figure 1



Model of transcriptional activation by allele specific enhancer. (a) On the active allele, transcription factors bind to enhancer regions through their DNA binding motifs. Enhancer bound transcription factors are able to recruit epigenetic machineries and enhancer binding factors such as ELL3, AFF3 to enhancers. Factors within enhanceosome like the Mediator complex and the architectural protein cohesin facilitate the loop formation between enhancer and promoter. Pre-initiation complex is established at the transcription start site. Enhancer also function in promoting the transition from promoter proximal pausing to productive elongation stage through recruiting the elongation complex SEC. **(b)** On the other allele, transcription factor binding motif is mutated due to SNP. The subsequent transcriptional events do not occur.

elongation control through eRNAs has been proposed here. In support of this scenario, eRNA has been shown to recruit the Mediator complex, which preferentially binds to *cis*-regulatory elements including enhancers and promoters [75]. In a separate study it has been shown that the Mediator complex was able to engage SEC to promote the transition to productive elongation [76].

However, the general biological function of eRNA still remains controversial. The newly developed technology using genomic editing tools, CRISPR-Display, was employed to target eRNAs, TRERNA1, ncRNA-a3 and HOTTIP to a stably integrated reporter gene [77]. Only slight activation of reporter gene expression was observed. While arguing that eRNA might be accidentally produced due to the active interaction of Pol II with the enhancer element, we still need to take into consideration that eRNAs might have their specificity in selecting their functional targets.

Disease/traits linked allele specific enhancers

Disease associated Single Nucleotide Polymorphisms (SNPs) identified by genome wide association studies (GWAS) are predominantly located outside of the protein coding regions of the human genome [78–80]. Many of the non-coding risk SNPs fall within the tissue specific enhancers that are recently annotated by epigenomic profiling [13,79,81–85]. For example, 88% of the SNPs within the known prostate cancer loci lie in the putative enhancer regions identified in human prostatic carcinoma cells [84]. SNPs associated with type 2 diabetes are highly enriched in the clustered pancreatic islet enhancers [85].

SNPs inherited from parents contain allele information. Recent genome wide studies offer evidence in support of SNPs' functions in enhancer activity through demonstrating strong correlation between allele imbalanced histone acetylation at enhancers and allele biased gene expression [86]. Non-coding SNPs or genetic variants could influence diseases and traits through altering the consensus sequences of transcription factor binding sites, reshaping enhancer repertoires, and inducing target gene expression level polymorphisms [76,87,88] (Figure 1). For instance, the inherited 8q24 gene desert SNP rs6983267 is in linkage disequilibrium with the oncogene *MYC*, functioning as an enhancer element recruiting transcription factor 7-like 2 (TCF7L2) to activate *MYC* expression in an allele specific manner and conferring risks for multiple cancers [87,88].

In addition to SNPs, DNA methylation status imbalance could also give rise to allele specific enhancers. During early mouse embryonic development, transcription of the maternally expressed *Meg3* polycistron within the paternally imprinted *Dlk1-Dio3* locus is stimulated by the active *Meg3* upstream enhancer localized on the unmethylated maternal allele [89•,90,91]. The allele specific activity of the Meg3 upstream enhancer is maintained, at least partially, by the allele specific binding of the scaffold protein of the Super Elongation Complex-like 3 (SEC-L3), AFF3 [89•,92]. DNA methylation and its related chromatin modifying machineries can inhibit the binding of AFF3 to the enhancer element and genesis of active enhancer on the paternal allele to control the allele specific gene expression profile of this imprinted locus. Not limited to the imprinted regions, DNA methylation has been shown to affect the function of regulatory regions genome widely [93–96]. Furthermore, the association of diseases/traits associated SNPs with the proximal DNA methylation status changing suggest a functional link between them to drive allele specific gene expression [97,98].

Non-coding variants are the major genetic origins of heterogeneity in inherited phenotypes including drug responsiveness. The anti-diabetic drug rosiglitazone mediates insulin sensitization through activating PPARγ, a master transcription regulator of adipocyte development [99]. However, individuals vary widely in their responses to rosiglitazone. About 20% treated patients experience with poor glycemic control and even adverse effects. Recent study shows that SNPs recast the binding motifs of PPARγ and its cofactors, contributing to the heterogeneity in drug response [100•]. Thus, identification SNP enhancer variants, allele specific enhancers or risk alleles with strong impact on treatment decision making, and delineation the function modes of these enhancers lay the foundation of precision medicine.

Concluding marks

Over the past few years, genome wide mapping of histone modifications has already become a prevailing method in prediction and classification of enhancers *en masse*. Importantly, genetic manipulation of the predicted enhancer element or examination of the impact of the predicted enhancer on reporter gene expression in cell/tissue of interest is necessary to validate the functionality of the putative enhancer captured by epigenomic profiling.

Newly developed genomic sequencing and editing tools enable us to locate traits/disease linked cis-regulatory elements and unravel their functional importance. Coupled with the identification of specific transcription factors functioning on disease driving enhancer, knowledge gained from enhancer studies paves the way for building up a systematic roadmap of disease driving enhancers. We are eager to see the revolution of precision medicine through pushing the development of therapeutic strategies designed to disrupt or enhance the association of transcriptional machinery to disease driving enhancers.

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