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REVIEW ARTICLE

Enhancer and super-enhancer: Positive regulators in gene transcription

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Abstract

Enhancer is a positive regulator for spatiotemporal development in eukaryotes. As a cluster, super-enhancer is closely related to cell identity- and fate-determined processes. Both of them function tightly depending on their targeted transcription factors, cofactors, and genes through distal genomic interactions. They have been recognized as critical components and played positive roles in transcriptional regulatory network or factory. Recent advances of next-generation sequencing have dramatically expanded our ability and knowledge to interrogate the molecular mechanism of enhancer and super-enhancer for transcription. Here, we review the history, importance, advances and challenges on enhancer and super-enhancer field. This will benefit our understanding of their function mechanism for transcription underlying precise gene expression.

KEYWORDS
enhancer, next-generation sequencing, super-enhancer, transcription regulation

1 | TRANSCRIPTION REGULATION IN EUKARYOTES

DNA is the genetic information storage in cell/organisms. Transcription is an intermediate process that synthesizes RNA and then RNA translates the message into protein to perform a specific biological function. As the first step, transcription switches on and regulates gene expression. Therefore, scientists put lots of effort and attention to the field in the long run. In 1860s, scientists proposed genetic factor to explain "one gene-one trait" which was based on Mendel's pea experiments.[1] In 1941, Beadle and Eatum proposed "one gene-one enzyme" to explain inborn errors of metabolism.[2] In 1957, "one gene-one polypeptide" was introduced due to the progress of biochemical genetics.[3] In 1958, Crick proposed central dogma which is often stated as "DNA makes RNA and RNA makes protein" (Figure 1).[4] Central dogma defines the genetic information flow of DNA, RNA, and protein. It has clarified the role of these three macromolecules in transcription. Since then, transcription has become the central field of biologists. In 1970s, "one gene-multiple RNAs" hypothesis was proposed due to splicing and other progresses on molecular biology.[5] Meanwhile, transcription has been recognized as a dynamic process. Scientists divide it into multiple sub-processes, mainly including initiation, elongation, and termination (Figure 2).[6] RNA polymerase II (RNAPII) is identified as the core factor to initiate and regulate gene expression by coordinating with lots of other factors, including general transcription factors, enhancers, mediators, cohesions, insulators, and silencers accompanying with other epigenetic mechanisms.[7] In the past decades, next generation sequencing (NGS) has been innovated into transcription research.[8-10] Genome architecture, methylation, acetylation, and other histone modifications have also been brought into the field, which dramatically extended the view of transcription regulation.[10] Among them, as the vigorous positive factor, enhancer attracts special interests of scientists.[11-13]

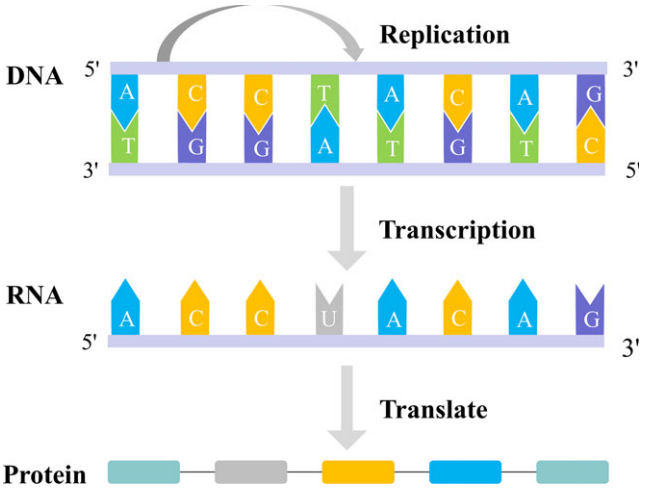

FIGURE 1 Diagram of central dogma

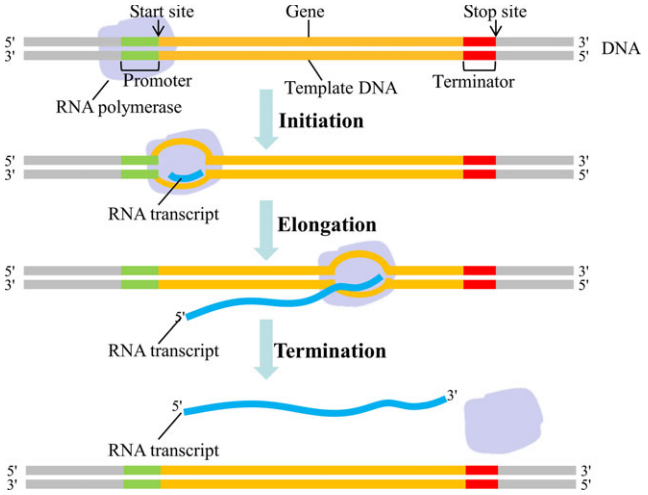

FIGURE 2 Diagram of transcription sub-processes, including initiation, elongation and termination

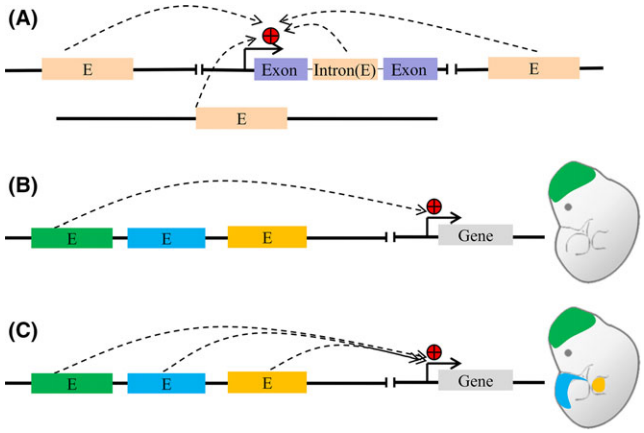

FIGURE 3 A, Enhancers are *cis*-regulatory elements that can increase expression of target genes in *cis* and *trans*-acting manner; (B and C) Enhancer regulate spatiotemporal gene expression

2 | ENHANCER IS A POSITIVE REGULATOR IN TRANSCRIPTION

2.1 | Enhancer is a positive regulator

Enhancer is a short region of DNA that can be bound by proteins (activators) to activate transcription of a gene.[14] It can positively regulate spatiotemporal gene expression during development through either *cis*- or *trans*- interaction manner (Figure 3).[13,15-17] In 1981, enhancer was first described as a 72-bp repeated sequence in simian virus 40 (SV40) genome, which could increase the ectopic expression of a reporter gene by ~200-fold.[18,19] In 1983, enhancer was discovered within a mouse immunoglobulin heavy chain gene in mammals.[20] Subsequently, different enhancers in various cells and tissues have been reported.[14-17]

2.2 | Properties of enhancer chromatin

Enhancers activity are usually linked with certain properties of chromatin (Figure 4). Active enhancers are typically bound with transcription factors (TFs).[21] The flanking of enhancers are commonly marked by histone modifications such as histone H3 lysine 4 monomethylation (H3K4me1) and H3K27 acetylation (H3K27ac).[22-24] Active enhancers are marked by both H3K4me1 and H3K27ac, with depletion of histone H3 lysine 4 trimethylation (H3K4me3);[22] inactive, poised enhancers are marked only with H3K4me1.[24] In addition, enhancers are typically depleted of nucleosomes and sensitive to DNase I digestion.[25] Distal enhancers are brought into close proximity with their target promoters through chromatin looping,[14] which is facilitated by mediators and cofactors.[11,21] Moreover, active enhancer can recruit RNAPII and produce RNAs that contributes to its function and gene regulation.[26,27]

2.3 | Enhancer identification

Traditionally, enhancers have been identified based on their ability to increase transcription by using reporter gene assays.[14,18] Transgenic reporters are widely used for enhancer identification in animal models such as nematode, fruit fly, and mouse.[14] Traditional transgenic reporter assays, for example, those based on luciferase, are usually low throughput as they could only validate individual enhancer in a relative simple mode.[14,18] In the recent years, with the advent of NGS, high-throughput computational and experimental methods have been adapted to predict enhancers.[14,28] These are mainly included in several categories: (a) Computational analysis of conserved noncoding sequences and TF binding motif[29-31]; (b) Chromatin immunoprecipitation and sequencing (ChIP-seq)[28] for transcription factors,[32,33] mediators and cofactors such as P300,[34,35] and histone modifications such as H3K4me1 and H3K27ac[23,24]; (c) Chromatin accessibility assays, including DNase I digestion coupled to sequencing (DNase-seq),[25,36] formaldehyde-assisted isolation and sequencing (FAIRE-seq),[37] and transposase-accessible chromatin followed by sequencing (ATAC-seq)[38]; (d) Multiple methods depending on the detection of enhancer RNAs,[28] including global run-on sequencing (GRO-seq),[39] precision nuclear run on and sequencing (PRO-seq),[40] native elongating transcript sequencing (NET-seq),[41] cap-analysis gene expression (CAGE)[42]; (e) Methods based on

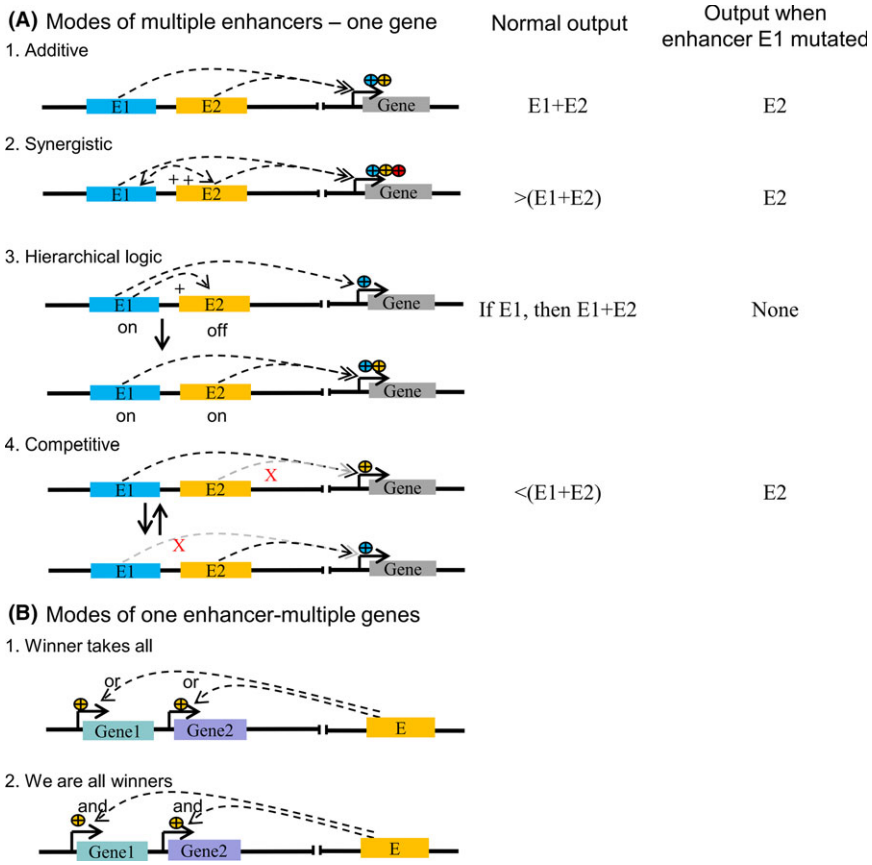

FIGURE 4 Modes of enhancer action

enhancer-promoter interactions, including chromosome conformation capture (3C),[43] 4C,[44] 5C,[45] Hi-C,[46] and chromatin interaction analysis by paired-end tag sequencing (ChIA-PET)[47]; (f) Methods of testing enhancer activity,[28] such as massively parallel reporter assays (MPRAs),[48] self-transcribing active regulatory region sequencing (STARR-seq),[49] and functional identification of regulatory elements within accessible chromatin (FIREWACh).[50] Currently, enhancers can be defined by using one or combinations of these methods.

Accordingly, thousands of enhancers in different model animals such as fruit fly, nematode and mouse, as well as human have been annotated by different international genome annotation consortia, such as ENCODE,[51] NIH Epigenome Roadmap,[36] FANTOM5,[42,52] and Blueprint/IHEC.[53] At the same time, enhancer related databases such as VISTA Enhancer Browser,[54] Enhancer Atlas,[55] and HEDD[56] have been developed for visualizing and sharing information of enhancers annotations across mammalians. These useful resources provide new insight into their roles and mechanism of enhancers-mediated gene regulation.

3 | ROLE AND ADVANCE ON TRANSCRIPTION RESERCHES

3.1 | H3K4me1 and H3K27ac

H3K4me1 and H3K27ac are commonly used hallmarks to identify putative genome-wide enhancers.[11,57] H3K4me1 and H3K27ac are conferred by the mixed lineage leukemia (MLL) family of methyltransferase (MLL2/3/4) and the CREB-binding protein (CBP)/P300 acetyltransfereases, respectively.[11,57] Knocking out H3K4 methytransferases MLL3 and MLL4 have resulted in a global loss of H3K4me1 binding, and subduction of H3K27ac, mediators and RNA-PII bindings as well.[58,59] It has been found that H3K4me1 can facilitate recruitment of the cohesion complex to chromatin, which provides a potential mechanism for MLL3/4 to promote chromatin interactions between enhancers and promoters.[60] In addition, a recent study has suggested that H3K4me1 might play a fine-tune role in enhancer activity by facilitating binding of the BAF complex and possibly other chromatin regulators.[61] Meanwhile, active enhancers in both flies[62] and mice[63] are not necessarily marked by H3K27ac, but H3K27ac has been supposed to affect enhancer activity through destabilizing nucleosomes or recruiting H3K27ac-binding proteins.[64] All these evidences imply that H3K4me1 and H3K27ac themselves are not required for enhancer activity.

3.2 | Diverse modes of enhancer action

As time goes by, enhancer has been recognized that it could regulate gene expression in quite diverse manners, which are summarized as "multiple enhancers—one target gene" (Figure 4A) and "one enhancer—multiple target genes" patterns (Figure 4B).[65,66] The former pattern includes addictive, synergistic, hierarchical, and redundant mode. (a) An additive mode represents that gene transcription is determined by the superimposed effect of multiple enhancers (Figure 4A-1). For

example, the *even skipped* (*eve*) gene is expressed in seven pair-rule stripes along the length of *Drosophila* embryo due to five separate enhancers,[16] so as enhancers of α- and β-*globin* genes in mouse erythroid cells,[67,68] within the developing limb[69]; (b) A synergistic mode proposes that multiple enhancers produce an effect greater than the sum of their individuals (Figure 4A-2),[70] for example, enhancers near *hunchback* and *knirps* in *Drosophila*,[71] and murine *Fgf8* locus[72]; (c) A hierarchical logic mode supposes that one or some enhancers can first activate one gene transcription to a basal level, while these enhancers could initiate the activity of their nearby enhancers to amplify its expression (Figure 4A-3). As an example, a conditional relationship between two enhancers near the *PU.1* locus in mouse myeloid cells[70]; (d) A redundancy mode describes that lossing one of gene-associated enhancers would not greatly affect its expression pattern due to their functional redundancy.[73] A potential mechanism of this might be a competition model that two enhancers compete for one target gene, which could ensure a relative constant gene expression in the case of one enhancer loss (Figure 4A-4).[73] Enhancer redundancy is a remarkably widespread feature in mammalian genome.[66,74,75]

On the other hand, the solo enhancer is able to regulate multiple genes (Figure 4B). Two types of competition modes, "winner takes all" and "we are all winners," have been proposed to explain this.[65] For the first one, only one target gene is activated and expressed in each cell (Figure 4B-1). As an example, to ensure unique identity of neurons, only one olfactory receptor gene or protocadherin gene is expressed in each cell of its sensory system and brain.[76,77] For the second one, multiple genes are activated and expressed in all cells, but they are not necessarily expressed at maximum levels (Figure 4B-2). This mode can be detectable when the deletion of one such gene would increase other gene expression,[78] or the introduction of an extra gene copy would decrease other gene expression.[79,80] The interaction of multiple genes expression are switched by a shared enhancer in one cell is thought to belong this mode.[81]

3.3 | Enhancer-promoter interactions

3.3.1 | DNA-Looping

Enhancer-promoter interactions can be commonly found to determine spatiotemporal gene expression pattern in eukaryotes.[82,83] This has been well presented by studies of the globin locus control region (LCR) and its target gene.[84,85] During erythroid development, LCR activates distinct globin genes in a stage specific manner through the formation of DNA looping.[86] LCR-β-*globin* interactions are established dependent on gene-specific transcription factors, including the hematopoietic-specific factors GATA1 and FOG1,[87] KLF,[88] and the widely expressed factor LDB1.[89] The depletion of LDB1 has been previously reported to disrupt long-range LCR loop formation, and thus affect gene transcription.[89] There are other examples of specific gene regulation involving in enhancer-promoter looping. The *Satb1* gene is silent when its promoter does not contact with enhancers in the brain, whereas it is highly expressed when enhancer-promoter looping has been de novo formed in the thymus.[90] In the latest study, a distal enhancer of *Sox9* can reverse sex in mouse,[91] which suggests DNA-Looping could also determine specific traits.

The protein yin and yang (YY1) has been recognized as a structural mediator of DNA looping in recent study.[92] YY1 could globally mediate enhancer-promoter interactions by binding to DNA and facilitate the formation of chromatin loops, probably through its dimerization.[92] In addition, YY1 has been further indicated to positively regulate transcription by targeting promoters and enhancers to through the BAF complexes in embryonic stem cells.[93]

3.3.2 | TADs

Along with the 3D genome architecture, topologically associating domain (TAD) has been realized as a popular pattern for enhancer function. TAD is a proposed selfing-interaction genomic territory, meaning that DNA sequences physically interact with each other more frequently within than outside.[90] Recent studies have indicated that TADs might ensure proper physical interactions between promoters and distal enhancers.[94] For example, *Shh* expression is not affected by changing the distance between *Shh* gene and its associated enhancer (ZRS) within TAD.[94] Conversely, it has been altered by inversions disrupting the TAD between them.[94]

The mechanism leading to the TAD boundary formation have attracted the study interest of many biologists. TADs are suspected to be bordered by dimerization of the zinc finger protein CTCF bound to chromatin.[95] Disruption of a conserved CTCF-cohesion boundary extends the sub-TAD of the mouse α-*globin* gene cluster to adjacent CTCF-cohesin-binding sites.[96] This in turn allows α-*globin* enhancers to interact with more additional promoters located within extended sub-TAD. In addition, a study of the *Sox9* locus has showed that duplication of boundary-containing regions results in the formation of a new TAD that is insulated from its neighbors by the duplicated boundary.[97] However, the research field of TAD remains controversial, more efforts and data will be eager for further interpreting its mechanism.

3.4 | Enhancer RNAs

Enhancer RNAs (eRNAs) are a new class of long noncoding RNAs synthesized at enhancers,[98] which are correlated with enhancer activity and contribute to gene regulation.[98,99] The transcription of enhancer was first reported in the locus control region (LCR) of the β-*globin* gene.[100] Subsequently, enhancers have been found to be broadly transcribed.[26,101-103] Unlike messenger RNAs (mRNAs), eRNAs are generally short, non-coding, bidirectionally transcribed, and their 3′-end are not polyadenylated.[42,102,104] Meanwhile, they are susceptible to exosome-mediated degradation and express at very low levels.[104,105] Recent studies have revealed that eRNAs can be generated through unidirectional transcription, that are longer and contain a poly A tail.[106] eRNAs could promote transcription by facilitating nucleosomes depletion and establishing DNA accessibility.[107,108] Moreover, nascent eRNAs have been found to contribute to the stabilization of TF binding,[109] the recruitment and activation of cofactors,[110-113] the release

of negative elongation factor (NELF) from promoters.[114] In addition, eRNAs have been indicated to play a role in gene regulatory networks by controlling promoter and enhancer interactions and topology of higher order chromatin structure.[115]

4 | SUPER-ENHANCER DETERMINES CELL INDENTITY AND FATE

4.1 | Super-enhancer is a cluster of enhancers

Super-enhancer is emerging as cluster of enhancers that is densely occupied by the master regulators and mediators, which is speculated to act as switches to determine cell identity and fate.[116,117] This notion was first described as genomic regions with high levels of five master transcription factors (Oct4, Sox2, Nanog, Klf4, and Esrrb) and the Mediators in mESCs.[117] Subsequent studies have extended the concept of super-enhancers as genomic regions densely occupied by high levels of H3K4me1, H3K27ac, p300 or master transcription factors in multiple cell types and tissues.[116,118] The main identification procedure has been summarized as five steps (taking H3K27ac as example, Figure 5): (A) performing H3K27ac ChIP-seq experiment in the interested cell types or tissues; (B) mapping H3K27ac ChIP-seq data to reference genome; (C) calling peaks using peak calling algorithm, for example, MACS2[119]; (D) stitching enhancers within 12.5 kb of each other (performing in ROSE); (E) plotting the ranked stitched enhancers and the remaining individual enhancers by the total background-normalized levels of H3K27ac within the genomic region; a line with a slope of one tangent to the curve is used as a cutoff to distinguish super-enhancers above the point and typical enhancers below the point of tangency (performing in ROSE).

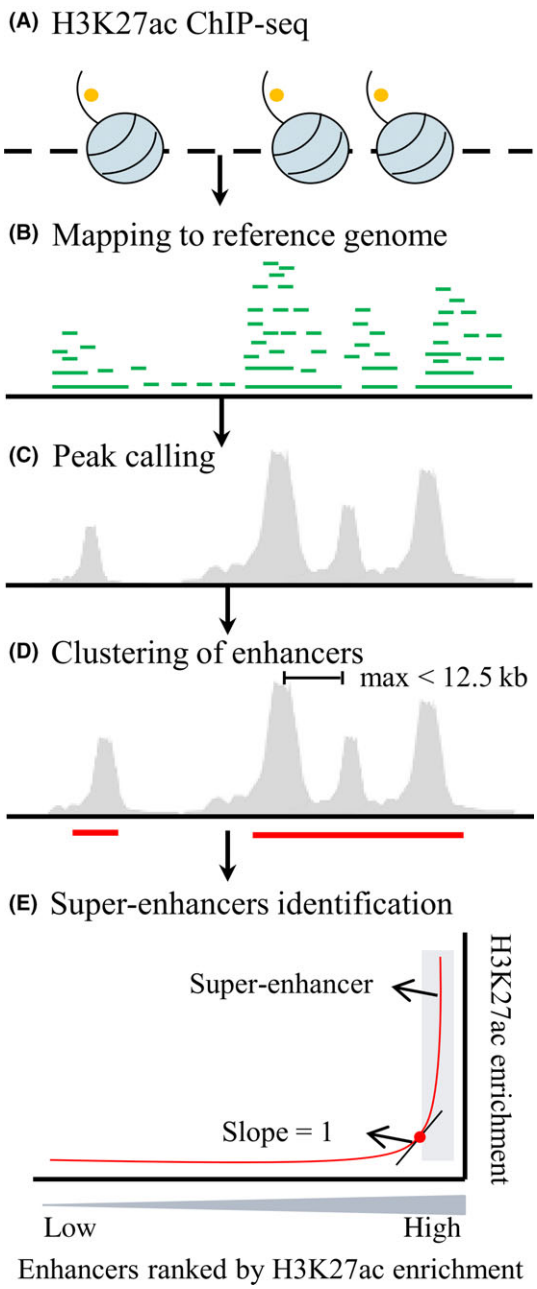

FIGURE 5 Identification procedure of super-enhancers

4.2 | Properties of super-enhancers

Super-enhancers differ from typical enhancers in size, transcription factor density and content, and ability to activate transcription (Figure 6). In addition, super-enhancers produce higher level of eRNAs than typical enhancers,[116,117] for example, about 93% of super-enhancers and about 30% of intergenic typical enhancers are associated with eRNAs during toll-like receptor 4 (TLR4) signaling in macrophages, respectively.[120] Super-enhancer and its associated genes are frequently located within a loop connected by two CTCF sites co-occupied by cohesion within TADs, as an example, 84% of super-enhancers and 48% of typical enhancers are located within such structures in mESCs, respectively.[121] Remarkably, super-enhancers are capable of maintaining cell identity, determining cell fate, driving oncogene transcription in cancer cells.[118,122]

4.2.1 | Maintaining cell identity

A series of studies have indicated that super-enhancers are capable of maintaining cell identity. In mESCs, both super-enhancers and typical enhancers are co-occupied by master TFs Oct4, Sox2, and Nanog, which are important for pluripotency; but only super-enhancers are densely occupied by TFs KLF and ESrrb, which play important role in cell identity.[117] In the same study, the crucial role of super-enhancers in cell identity has been further revealed by that reduced levels of Oct4 or Mediators cause preferential loss of expression of super-enhancer-associated genes relative to other genes in mESCs.[117] Likewise, key TFs that control cell identity have been found to bind at super-enhancer in other differentiated cell types, such as myotubes (MyoD), T helper (Th) cells (T-Bet) and macrophages (C/EBPα).[117] Subsequently, super-enhancers co-occupied by lineage-specific factors have been identified in diverse cell types such as adipocytes, hair follicle stem cells, and mammary epithelial cells.[12,123-126] For example, in the mammary epithelium, mammary-specific super-enhancers have been identified

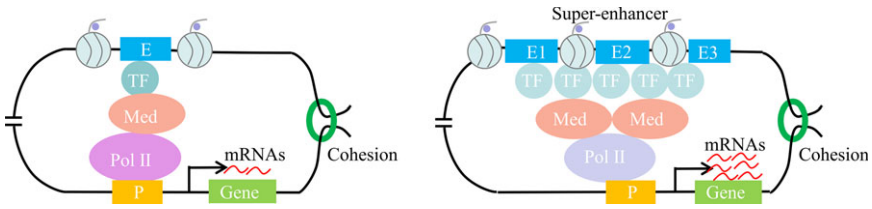

FIGURE 6 Differences between organization and function of typical enhancers and super-enhancers

with mammary-enriched transcription factors, such as signal transducer and activator of transcription 5 (STAT5), glucocorticoid receptor (GR), E74 like ETS transcription factors 5 (ELF5), and nuclear factor I B (NFIB).[124]

In addition, super-enhancers are correlated with lineage-specific transcriptional factors and oncogenes in a broad spectrum of cancers, such as neuroblastoma,[127] small-cell lung cancer (SCLC),[128] medulloblastoma,[129] breast,[57] esophageal,[130] gastric cancers,[131] and melanoma.[132] Moreover, in medulloblastoma, super-enhancers are able to distinguish its four main subtypes based on underlying biochemical and genetic signatures, suggesting that super-enhancers are correlated with tumor heterogeneity and define cell identity.[129] In addition, studies have revealed that different super-enhancers have same target oncogenes in various tumor types, for example, tumor-specific super-enhancer profiles have been found at the *MYC* and *MYCN* loci,[127,128] which further indicate the importance of super-enhancers on maintaining cell identity.

4.2.2 | Determining cell fate

Analyses of the super-enhancer dynamics during lineage commitment of specific cell types have shown that super-enhancers are remodeled during differentiation, having crucial roles in cell fate determination.[123,133,134] Deletion of super-enhancer constituents at the *Nanog*[135] or *Sox2*[136] locus in mouse embryonic stem cells reduces the corresponding target gene expression and impaired some other key pluripotency genes, resulting in cellular differentiation. In another example, distinct super-enhancer landscape and super-enhancer-associated TF network have been identified for mesenchymal and adrenergic cells, and the state of adrenergic cells towards mesenchymal is associated with the changes of super-enhancers landscape.[137]

4.2.3 | Driving oncogene transcription in cancer cells

Super-enhancers have been found to drive the expression of a few critical oncogenes in several types of tumor cells.[122] In *Nasopharyngeal carcinoma*, super-enhancers are linked to genes important for oncogenesis including *ETV6*.[138] In *Oesophageal squamous cell carcinoma* (OSCC), super-enhancers are associated with oncogenes including *PAK4*, *RUNX1*, *DNAJB1*, *SREBF2*, and *YAP1*.[130] Deletion of a super-enhancer reduces the expression of cancer-related genes and impairs some oncogenic properties.[139] In contrast, duplication of super-enhancers leads to overexpression of a key oncogenic transcription factor, which then activates other cancer-related genes in squamous cell carcinomas.[140] Super-enhancers can be targeted through inhibition of chromatin and transcriptional regulators that disproportionately bound to these regulatory elements super-enhancers.[122] Recent studies have demonstrated that JQ1 (a competitive inhibitor of BRD4, and a covalent inhibitor of CDKs), selectively kill cancer cells by inhibiting SE-driven oncogenic transcription, with both agents lacking systemic toxic effects in vivo.[127,128,141,142]

5 | CHALLENGES AND ONGOING STUDIES

As positive transcription regulators, scientists have put lots of efforts and made significant progress on enhancer and super-enhancer related studies. So far, there are still a few challenges remained for understanding their role and mechanism in gene transcription: (a) precisely identifying enhancer motif across the genome; (b) validating vast enhancer candidates identified by ChIP-seq and other methods; (c) precisely annotating enhancers to their target genes in genome; (d) the ambiguous definition and unclear composition of super-enhancer.

5.1 | Precision identification of motif

Precisely, identification of motifs is essential for understanding the enhancer function mechanism and genome constitution. Motif is a degenerate short (6-10 bp) DNA sequence pattern that summarizes the DNA sequence binding preference of a transcription factor.[14] Enhancer motifs recruit transcription factors, which in turn enroll cofactors, and thus activate transcription.[14] They are highly linked to enhancer activities and gene expression.[66] The space between motifs is one of factors contributing to enhancer activities. For example, the neural plate-specific *Otx-a* enhancer in *Ciona* controls *Otx-a* expression in a moderate and proper manner.[143] This enhancer contains GATA and ETS DNA sequence motifs.[143] A 3 bp insertion between one set of them has been found to result in a threefold increase of *Otx* expression.[143] Thus, precisely motifs are important for understanding enhancers function. However, up to now, the identified potential enhancer candidates, by various methods such as ChIP-seq, bestrow hundreds of base pairs along the genome (Figure 7).[28,34] The conflict size differences between motif and potential enhancer candidates would result in the difficulty for dissecting enhancer, its function and genome annotation.

Scientists have started to put their effort to position motif precisely. There are several methods developed to identify enhancers at high resolution and low background. For example, ChIP-exo, a derivation of ChIP-seq, has been adapted.[144] Compared to ChIP-seq, ChIP-exo includes an additional step of exonuclease digestion that trims DNA fragments.[144] This step allows identifying putative enhancer candidates at high resolution and low background noise, and in turn positioning motifs more precisely.[144] However, the current ChIP-exo technique has been applied to limited cell types. Thus, more efforts are required for developing new experimental methods and algorithms of enhancer identification and motif position in the future.

5.2 | The validation of enhancer activity

Identifying functional enhancers is an important step for understanding their mechanism in gene transcription. Up to now, hundred thousands of putative enhancer candidates have been identified across human and multiple model animals,[23,24] but not all of them are representative of functional ones. Indeed, with the data generated by the ENCODE Project, only a fraction (26%) of enhancer candidates display enhancer activity with reporter assays.[51,145] In addition, the data in VISTA Enhancer Browser reveals that only 50% of putative candidate elements exhibit enhancer activity in transgenic mouse (up to date 23 June 2018). With the development of NGS, several high-throughput screening methods, such as MPRA, STARR-seq and FIRE-WACh, have been adapted to validate enhancers activity.[48-50] These related methods have greatly improved our ability to validate enhancers activity, but there are still a lot of putative enhancer candidates have not been functionally tested.[23,24] Therefore, enhancers activity validating remains as a challenge for biologists.

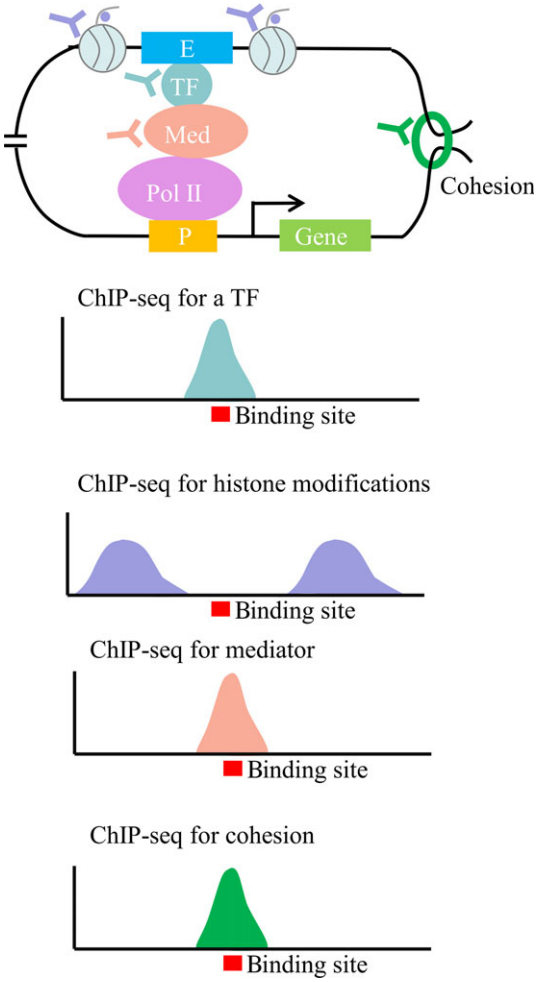

FIGURE 7 Scheme of binding site for one TF

5.3 | The assignment of enhancers to their target promoters

Enhancer-promoter interaction is important for gene transcription and has commonly occurred in eukaryotes. However, their related information or data in multiple cell types/tissues is still lacking. A few years ago, enhancers have been typically assigned to their neighboring promoters based on linear proximity or shared chromatin states.[116] However, enhancers do not always regulate their neighboring genes. A well-characterized example is that the ZRS enhancer, which resides in an intron of *Lmbr1* (encoding limb region 1 protein), contributes to the limb bud activation of the *Shh* gene, which locates in nearly 1 Mb away.[146,147] Sanyal et al[148] have found that only 7% of distal regulatory elements control their closed promoters in human cell lines. Moreover, Zhang et al[149] have found 76% of the putative enhancers do not interact with their neighboring promoters in mESCs. Thus, direct approaches for detecting enhancer-promoter interactions are required. Several three-dimensional technologies, such as 3C,[43] 4C,[44] and 5C,[45] Hi-C[46] and ChIA-PET[47] have been adapted to directly identify physical contacts. However, the available data of these associations is still far more insufficient. Data accumulation might be an option to solve this, which might need global efforts to achieve.

5.4 | Definition and composition of super-enhancers

Despite of biological effects of super-enhancers, its definition is ambiguous and molecular composition is unclear.[118] Super-enhancer can be termed as enhancer cluster. However, according to its identification procedure (Figure 5), a few defined super-enhancers are single enhancers, for example, 15% (35 of 231) are proposed as single in mESCs.[117] Most defined super-enhancers contain several ones, which are difficult to distinguish their boundaries. Accordingly, these would be an obstacle for understanding their functional mechanism in gene transcription. The ambiguous definition and unclear composition of super-enhancers would be caused by the current low resolution methods. The concept indeed fits researcher's need to shrink the list of regulatory candidates. Therefore, the related studies have been explosively increased during the last a few years. However, its ambiguous definition and molecular composition remind us that it is a long way to uncover their mechanism.

6 | CONCLUDING REMARKS

Enhancer and super-enhancer are positive regulators for gene transcription. Scientists have made great processes on their effect and mechanism research. Their function is tightly dependent of the recruitment of transcriptional factors, cofactors, and mediators, as well as the formation of enhancer-promoter interactions. Recent advent of NGS has greatly expanded our knowledge and skill to explore genome-wide composition. We review their history, definition, importance advance and challenge with different aspects. Currently, precision motif, activity validation, targeted gene, and molecular mechanism are the central of the field. To achieve these goals, more efforts on developing new methods and accumulating data across different cell types/tissues are required. We hope this essay would be beneficial for further understanding the role and mechanism of enhancers and super-enhancers in transcription, as well as for providing future clues in the field.

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CONFLICT OF INTEREST

None.

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