INVESTIGATION OF ENHANCER–PROMOTER INTERACTIONS IN HUMAN DISEASE USING CRISPR-BASED FUNCTIONAL APPROACHES

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ABSTRACT
Interactions between Enhancer and promoters are vital in the process of controlling the expression rate of the genes since they allow distal regulatory factors to come in physical contact with target promoters of the genes within the three-dimensional chromatin scaffolding. The maladaptation of these interactions has been more and more associated with numerous human diseases, such as cancer, neurological and complex genetic disorders. Conventional experimental technologies like chromosome conformation capture (3C/Hi-C) and chromatin immunoprecipitation sequencing (ChIP-seq) have also been useful to understand genome organisation, but these techniques are mainly correlation based and do not provide causal relationships. New developments in CRISPR-based functional genomics have completely changed the landscape of gene regulation research, allowing specific and accurate perturbation of enhancer components. CRISPR-Cas9-mediated deletion, CRISPR interference (CRISPRi), and CRISPR activation (CRISPRa) can be used to obtain systematic studies about the role of enhancers and their role in disease-related gene expression. Also, regulatory networks can now be resolved and interpreted more deeply, with the combination of CRISPR screening and multi-omics data, such as RNA sequencing and chromatin accessibility profiling. The current review has given an overview of contemporary information on Enhancer–promoter interaction; emphasised CRISPR based methods in functional validation; and given a discussion on the new challenges and future patients on the understanding of mechanisms of gene regulation in human disease.

KEYWORDS: Enhancer–promoter interactions, CRISPR-Cas9, gene regulation, chromatin architecture, functional genomics, human disease

1. INTRODUCTION

Regulation of genes is a basic mechanism that regulates the cellular identity and development, as well as physiological homeostasis, by regulating the spatial and temporal forms of genes (Andersson and Sandelin, 2020). This control becomes possible via a combination of concerted actions of DNA regulatory processes, transcription factors, chromatin modifiers, and epigenetic interactions (Kagey et al., 2010; Whyte et al., 2013). Among them, the promoters and enhancers are the important elements of the gene regulation scenario. Promoters are found generally near transcription start sites and represent sites of assembling the transcriptional machinery, and the regulators are distal elements that can modulate gene expression regardless of genomic proximity and orientation (Hnisz et al., 2013). These components need to be coordinated accurately with each other to preserve the normalcy in the cellular functioning. The three-dimensional (3D) structure of chromatin mediates Enhancer–promoter interactions in which the distal enhancers are put into physical contact with target promoters through chromatin looping (Rao et al., 2014; Dixon et al., 2012). The structural aspect of organising the chromatin is the use of structural protein CCCTC-binding factor (CTCF) and cohesin complexes that promote or limit such interactions (Nora et al., 2017; Kagey et al., 2010). Furthermore, there are the transcription factors and co-activators that serve in the stabilisation and regulation of enhancer-promoter communication such as Mediator complex (Weintraub et al., 2017). Such interactions are also very dynamic and cell-type-specific, which shows how gene regulation is complicated in various biological situations (Hsieh et al., 2020; Bintu et al., 2018). Interruption of Enhancer–promoter communication has been gaining significant acceptance as a key cause of human disease. Genomic rearrangements in cancer often cause enhancer hijacking which causes aberrant oncogenic activation (Lupiáñez et al., 2015). Super-enhancer development has also been connected with overexpression of tumour as well as metastasis-related genes (Hnisz et al., 2013; Whyte et al., 2013). Likewise, in neurological diseases as well as in other complex genetic conditions, the single nucleotide polymorphisms (SNPs) detected by genome-wide

association studies (GWAS) are frequently found in non-coding enhancers, indicating the role of regulatory factors in the pathogenesis (Nasser et al., 2021; Mumbach et al., 2017). These results further note that there is a need to look beyond the single-gene models to the wider regulatory landscape of the genome. Traditional experimental approaches, which include chromatin immunoprecipitation sequencing (ChIP-seq) and chromosome conformation capture-based measurements (3C, 4C, 5C, and Hi-C), have contributed greatly to increased insights into the chromatin organisation and regulatory interactions (Jung et al., 2019; Mifsud et al., 2015). Nevertheless, these techniques mostly are only correlative, and they lack capabilities to directly identify causal relationships between enhances action and the expression of the genes. They can also be limited to technical and computational limitations which can reduce their resolution and interpretability. The predicate of CRISPR-based genome editing technologies has completely transformed functional genomics because genomic elements can now be manipulated precisely and selectively. CRISPR-Cas9-mediated deletion can be used to remove enhancer regions directly, and CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa) can be used to repress or activate regulatory elements without changing the DNA sequence school of thought (Thakore et al., 2015; Simeonov et al., 2017). These methods offer potent mechanisms of elucidating the function of enhancers and creating a causal relationship between an Enhancer–promoter interaction and altered gene expression (Canver et al., 2015; Guo et al., 2015). Furthermore, the combination of CRISPR-based perturbation and high-throughput sequencing with multi-omics data has provided new opportunities to map regulatory networks with precision never before (Schraivogel et al., 2020). The purpose of this review is to give a critical and in-depth review of Enhancer–promoter interactions, their contribution to human disease, and the recent CRISPR-based functional methods to study them. The rest of the paper is structured as follows: Section 2 is a literature review of studies concerning Enhancer–promoter interaction; Section 3 represents the description of underlying molecular biology mechanisms; Section 4 is a description of CRISPR procedures; Section 5 is an overview of multi-omics integration; and finally, Section 6 concludes with a discussion of future directions and limitations.

2. Review of Enhancer-promoter Interactions in Human Disease.

Historically, the study of how genes are regulated has gone beyond the simple linear models, to complex three-dimensional (3D) genomic deviation models which highlight the importance of distal regulating elements (Andersson and Sandelin, 2020). Initial research on the regulation of genes has determined that enhancers are cis-regulatory DNA elements that can stimulate transcription regardless of their distance and orientation in respect to target promoters (Whyte et al., 2013). Gene expression Researcher Models Promoter activation models noted the recruitment of transcriptional machinery, such as RNA polymerase II and transcription factors, as an important event in gene expression. These building blocks provided the basis to study long-range regulatory interactions in the genome. With the development of high-throughput technologies of chromatin conformation capture, the study of genome organization and interactions between regulators, promoters, and enhancers became possible. Such methods as chromosome conformation capture (3C) and its variants, including Hi-C allowed genome-wide chromatin interactions mapping (Rao et al., 2014; Mifsud et al., 2015). A groundbreaking essay by Dixon et al. (2012) found out topologically associating domains (TADs), structural units through which the genome is composed of specific areas, limiting Enhancer–promoter interactions to certain limits. Equally, chromatin immunoprecipitation sequencing (ChIP-seq) has been used to determine regulatory factors as well as transcription factor binding sites (Hnisz et al., 2013). Hnisz et al. (2013) proposed the use of clusters of enhancers, called super-enhancers, that promote the elevated level of gene transcription and are the factors that are instrumental in preserving cell identity and disease progression. The question has in recent years moved to an interest in the functional significance of Enhancer–promoter interactions, in human pathology. Structural genomic changes brought about by cancer can also cause hacking of the enhancer, causing oncogenes to abnormally activate (Lupiáñez et al., 2015). Super-enhancers are also shown to influence tumor growth and progression of tumors to metastatic ones through controlling growth and survival genes (Whyte et al., 2013). Genome-wide association studies (GWAS) in neurology and related complex diseases have shown that numerous disease-related variants are in non-coding portions and specifically enhancers, which indicate their regulatory role (Nasser et al., 2021; Mumbach et al., 2017). Such results highlight that disease phenotypes can be spurred by regulatory restructuring of cells, and not by a combination of the coding mutations. In spite of these developments, most conventional approaches to studying chromatin interactions are largely correlative. Hi-C and ChIP-seq can be used to have good insight into genome organization, but not necessarily define a causal relationship between enhancer activity and a gene expression (Jung et al., 2019). Moreover, there are also constraints associated with the aspects of resolution, signal interpretation and event specificity of cells, which makes definition of functional interactions difficult. CRISPR-based functional genomics methods have become one of the potent technologies to fill these gaps and perturb regulatory elements to confirm their functions in controlling genes (Thakore et al., 2015; Simeonov et al., 2017). It is noteworthy that Fulco and colleagues (2016) have shown that CRISPR interference (CRISPRi) can be used to conduct a systematic mapping of enhancer-promoter interactions to offer direct functional evidence of regulatory relationship actions. The important representative works, their methodology, their primary findings, and limitations are summarized in Table 1.

Table 1. Summary of Key Studies on Enhancer–Promoter Interactions

Study	Method	Key Finding	Limitation

Dixon et al. (2012)	Hi-C	Identification of topologically associating domains (TADs)	Limited resolution
Hnisz et al. (2013)	ChIP-seq	Discovery of super-enhancers controlling cell identity	Lack of causal validation
Fulco et al. (2016)	CRISPRi	Functional mapping of enhancer–promoter interactions	Limited scalability

This literature raises the need to observe the shift of descriptive mapping of chromatin interactions into functional validation of regulatory elements. Although the previous research gave vital cases regarding how the genome is organised, the recent developments have given prominence to the need to incorporate the use of functional methods that will determine causality. CRISPR-based methodologies that are discussed in the following sections are based on these developments.

3. Enhancer–promoter Interaction Mechanisms.

The interactions of enhancers and promoters are one of the key methods of transcriptional regulation within the eukaryotic genomes, where distant regulatory factors can affect the expression of given target genes. In contrast to promoters, which are found immediately adjacent to transcription start sites as well as platforms on which the basal transcriptional machinery assembles, enhancers can be located many kilobases (or even megabases) away their regulated genes. Nevertheless, in the face of this linear genomic segregation, promoters can be physically engaged by enhancers by three-dimensional folding of the chromatin and, in such a manner, transcriptional outputs can be highly-controlled and context-dependent. The arrangement of chromatin into high-order structures is also a major element of Enhancer–promoter communication. The genome is not assorted in the nucleus in a random fashion but rather it is segmented into coherent spatial areas restricting regulatory interactions. Some of the most significant of those structures are the chromatin loops and topologically associating domains (TADs). Chromatin loops place remote enhancers in close spatial contact with their promoter targets, which enhances transcriptional activator and co-regulators recruitment. TADs have a structural and functional role in vivo where any enhancer-promoter interaction is likely to happen, and the probability of an inappropriate cross boundary interaction with genes within neighbouring domains is restricted. This domain-centered genome organization is fundamental towards regulatory specificity and the disallowance of aberrant gene activation. These chromatin structures rely greatly on the architectural proteins, especially CCCTC-binding factor (CTCF) and the cohesin to be established and maintained. CTCF is an insulator-binding protein which identifies certain DNA sequences and assists in boundary setting of the chromatin domains. Cohesin is a ring-shaped complex protein, which is involved in loop extrusion, encircling chromatin fibres and stabilising the formation of long-range contacts. The combination of CTCF and cohesin supports the organisation of chromatin at the physical level in a way that regulatory elements are localised to advantageous location positions of transcriptional control. The convergent CTCF binding sites are used to stabilize the boundaries of the loop in most instances and the cohesin promotes the integrity of the loop between the two causing a structural framework through which promoters are able to interact with enhancers. Besides chromatin architecture, transcription factor binding is another essential factor which determines enhancer activity. The enhancers are supplemented to contain sequence motifs that are identified by transcription factors that are lineage-specific and signal-responsive. When bound, these factors invoke the co-activation by co-transcription factors including the Mediator complex, chromatin remodelers, and histone-modifying enzymes which facilitate an active state of chromatin. Histone repressions like H3K27ac are commonly found to be linked to active enhancers and are deemed transcriptionally permissive regulatory domains. RNA polymerase II and general transcription factors form a complex at the promoter that is abbreviated as the pre-initiation complex. The interaction between enhancer and promoter is only effective when a product is found to include physically close chromatin as well as the related biochemical recruitment of transcription promoting proteins. Interaction of enhancers and promoters occurs via chromatin looping as illustrated in Figure 1, which entails the introduction of temporal (distal regulatory DNA elements) spatial proximity in the interaction of CTCF and cohesin. The number also depicts the role of the transcription factors, Mediator and the H3K27ac-marked active chromatin as well as the RNA polymerase II in coordinating an effective transcriptional regulatory complex.

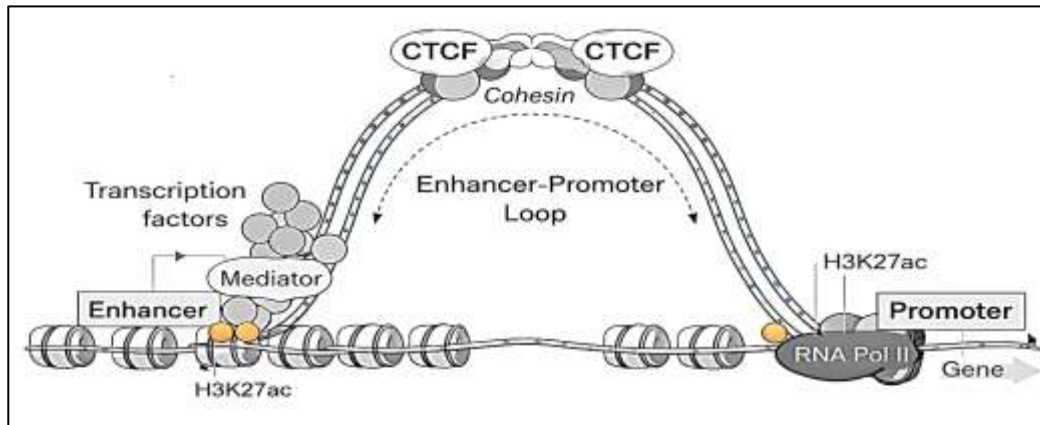

Figure 1. Chromatin looping mediating enhancer–promoter interaction.

In order to characterise the spatial behaviour of chromatin interactions in a quantitative fashion, the genomic distance dependency of the probability of contact between two genomic loci is commonly modelled. One of such relations is written in Equation 1:

$P(s) \propto s^{-\alpha}$, (1)

In this expression, $P(s)$ denotes the probability that two loci separated by a genomic distance s will physically interact, while α is a scaling exponent that reflects the folding properties and compaction state of chromatin. The downward power-law regression shows that overall, loci based on nearness along the linear genome are more likely to be in interaction than loci distant. This equation offers a valuable conceptual framework to help interpret chromatin conformation capture data, in particular, Hi-C maps, in which interaction frequencies are measured on a genome-wide scale. The importance of Equation 1 is that it allows us to consider the effects of not only regulatory specificity but also physical organisation of genome on the enhancement of enhancements by enhancer-promoters. Whereas for regulatory interaction, there is a statistical propensity of nearby enhancers to interact with promoters due to reduced genomic distance, distance is not the sole determinant of regulatory interaction. Simply expecting simple linear relationships, chromatin looping, TAD boundaries, and protein mediated anchoring may permit distal enhancers to interact a specific promoter in a highly selective manner. In this way, the contact probability model is an effective starting point to think about chromatin architecture, whereas biological processes, including, but not limited to, CTCF occupancy, cohesin dynamics, transcription factor recruitment and chromatin accessibility dictate which of these possible contacts are functionally relevant. Combined, enhancer / promoter interactions are the result of a combined mechanism of space genomic organisation, architecture proteins that form loops, and transcription factor-mediated promotion. These long-range contacts are formed and stabilized by chromatin loops and TADs, transcriptionally transduced by transcription factors and co-activators. These mechanisms are fundamental to an understanding of the processes involved in the realisation of regulatory specificity in normal cells and how a breakdown in these processes may lead to human disease.

4. CRISPR-Based Functional Approaches for Enhancer–Promoter Interaction Analysis

4.1 Conventional vs Functional Approaches

As a type of interaction, Enhancer–promoter (E-P) interaction has been studied using chromatin profiling and interaction mapping methods, which include chromatin immunoprecipitation sequencing (ChIP-seq) and chromosome conformation capture-based methods (e.g., Hi-C). Such methods have helped a great deal in enhancing our knowledge on the organisation of genomes and distribution of regulatory elements. CHIP-seq can be used to identify the binding sites of transcription factors and histone modifications of active enhancers and promoters, and hi-c can be used to give a global picture of chromatin interactions through physical interactions between genomic loci. Conventional methods, as they are, are also limited by their failure to build causes and effects. An example of such a method is the Hi-C that shows the frequencies of interaction between genomic regions, but not whether these interactions are functionally significant in the expression of genes. Likewise, ChIP-seq can determine protein-DNA interactions but it cannot directly verify the presence or absence of a particular enhancer to regulate a target promoter. Subsequently, these methods are mainly descriptive and correlational in nature, in most cases, needing further validation to ascertain functional importance. CRISPR-based functional methods have in contrast turned out to be strong instruments of asking genome-wide questions directly dealing with the involvement of regulatory components in gene expression. The CRISPR-Cas9 system allows editing of specific genomic locations that can be eliminated completely to determine the effect of the enhancement areas on transcriptional activities. Moreover, adaptable forms of CRISPR e.g. CRISPR interference(CRISPRi) and CRISPR activation(CRISPRa) can be used to repress or activate regulatory elements in a reversible manner without changing the DNA sequence. These methods offer a straightforward method of assaying further enhancer activity and can be used to draw causal relationships between enhancer-promoter interactions and the results of gene expression.Table 2 represents a comparison between traditional chromatin interaction mapping methods and CRISPR-based methods of test and serve as the main factor of methodological strengths and weaknesses.

Table 2. Comparison of Conventional and CRISPR-Based Methods

Method	Type	Advantage	Limitation
Hi-C	Mapping	Genome-wide interaction profiling	Correlative
CRISPR-Cas9	Functional	Enables causal validation of regulatory elements	Potential off-target effects

This analogy highlights a radical change in the discipline to evade descriptive mapping genomic to functional validation. Although standard techniques give us the necessary information about chromatin structure and possible interaction with regulators, CRISPR-based techniques allow testing enhancer activity directly, thus presenting a more informed picture of the way genes are regulated.

4.2 CRISPR Data Analysis Techniques to Study Enhancer-Promoter Interactions.

CRISPR-based technologies have turned out to be potent platforms of the functional interrogation of Enhancer–promoter (E sequence) associations, which allow in vivo and targeted manipulation of regulatory elements in the genome. In contrast to traditional mapping methods, CRISPR methods permit direct testing of enhancer activity thus enabling the establishment of cause and effect relationships between regulatory elements and gene expression. Originally based on bacterial adaptive immunity, the CRISPR-Cas9 system uses a single-guide RNAs (sgRNA) to target the Cas9 nuclease to a particular genomic locus. Within the framework of enhancer studies, CRISPR-Cas9 may be used to create specific deletions of enhancer regions, and thus disrupt their activity. In this method, enhancer activity is directly evidenced via measurement of the ensuing alterations in expression of the target genes. Nevertheless, nuclease-based editing has a possibility of causing either unintended mutations or off-target effects because of the creation of a break in the two strands. In order to address such constraints, altered CRISPR systems, including CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa) have been created. CRISPRi makes use of transcriptional repressor domains incorporated into dCas9 (dCas9-KRAB) which is catalytically inactive to silence enhancer activity without any changes made to the DNA sequence. CRISPRa, on the other hand, makes use of dCas9 with transcriptional activators fused (e.g., VP64, p300) in order to facilitate stock expression through regulatory element activation. These sensors can be tuned and reversibly switched to different levels of enhancer activity and have thus become especially advantageous in work on dynamically regulating networks of gene expression. These features are also expanded through high-throughput CRISPR screening methods that can be used to interrogate the enhancer elements across the genome. Thousands of regulatory regions can be pooled together in CRISPR libraries which can be introduced to cells to allow systematic identification of functional enhancers and the genes they associate with. Together with a sequencing based readout, these screens offer a platform that scales to map regulatory interactions in a wide range of biological situations. As shown in Figure 2, CRISPR-based enhancer perturbation workflow is characterized by several major steps, including design of sgRNA targeting a particular enhancer region, delivery of the CRISPR components to target cells (e.g. Cas9 and sgRNA), and finally perturbation of the enhancer activity associated with the deletion or silencing of enhancer upon CRISPR action. These perturbations are then evaluated by their functional effects on changes in gene expression which give insights directly on the relationship between enhancers and promoters.

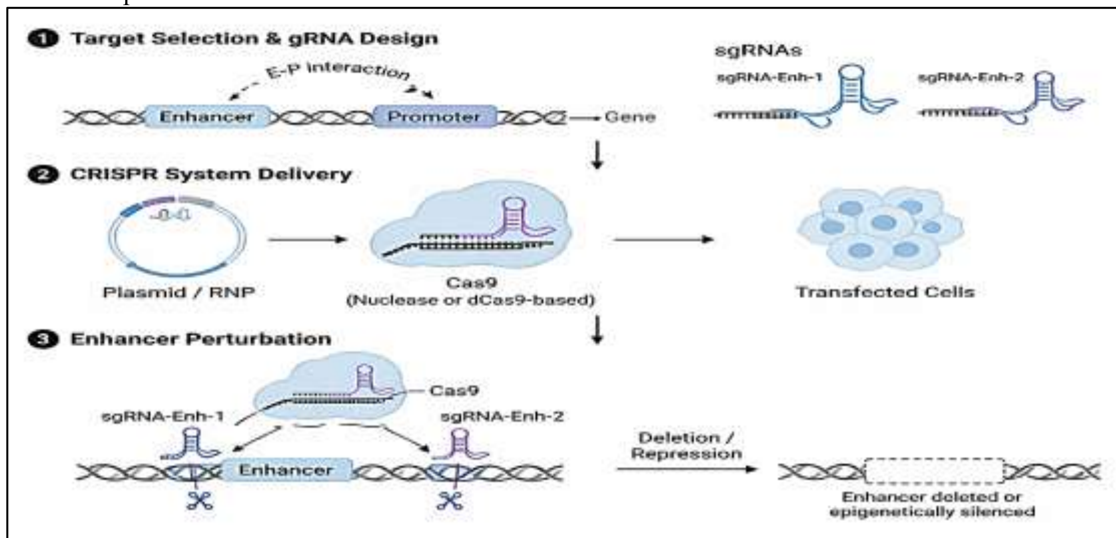

Figure 2. CRISPR-based workflow for enhancer perturbation.

A simple linear equation as demonstrated in Equation 2 can appropriately be used to quantitatively describe the effect of various enhancers on gene expression.

$E = \beta_0 + \sum_{i=1}^{n} \beta_i A_i$,____________________________________(2)

In this model, E represents the expression level of a target gene, while A_i denotes the activity of the i-th enhancer. The coefficient β_i reflects the relative contribution or regulatory strength of each enhancer, and β_0 represents the basal level of gene expression in the absence of enhancer activity. This model describes additive interaction between several promoters who act on a unique promoter. Equation 2 is important in that it provides a framework to understand the regulation of genes as a combinatorial process, whereby a combination of multiple enhancers determines the output of transcription.. In practical applications, CRISPR-based perturbation experiments can be used to selectively disrupt or activate individual enhancers, allowing estimation of their respective contributions β_i to gene expression. Although this linear model is a simplification and does not fully capture complex nonlinear regulatory interactions, it provides a useful framework for interpreting experimental data and understanding enhancer redundancy, synergy, and hierarchy. Altogether, CRISPR methods provide a revolutionary way of studying the Enhancer–promoter relationships through their ability to analyse genes regulation mechanisms in a precise, scalable, and causal fashion. This combination of CRISPR perturbation plan and quantification models with high-throughput screening systems is likely to contribute to the further investigation of genome regulation in health and disease.

5. Multi-Omics Integration and Computation Model.

The intricacy of Enhancer–promoter (E P) interactions requires the synthesis of various layers of genomic and epigenomic data to obtain the knowledge base towards the understanding of gene regulation. The use of single experimental procedures only gives a partial understanding, thus, multi-omics tools, which incorporate transcriptomic, epigenomic, and chromatin interaction data, have taken centre stage in elucidating the process of regulation. RNA sequencing (RNA-seq) has become popular as a technology to measure levels of expression of genes as a direct measure of transcriptional activity. It makes it possible to identify differentially expressed genes after enhancer perturbation and functionally validate regulatory interactions. Simultaneously, Assay of Transposase-Accessible Chromatin with sequencing (ATAC-seq) offers data on the chromatin accessibility, attracting the regions of the open chromatin, which probably can harbour active enhancers and promoters. This method is especially good in determining regulatory factors which are dynamically regulated in varying cell states. A third method to capture chromatin conformation is chromatin conformation capture methods, including Hi-C, that provide insight into the three-dimensional organisation of the genome. Data of hi-c allow determining loops of chromatin, topological associating domains (TADs), and longer range interactions between promoters and enhancers. Combined with RNA-seq and ATAC-seq data, Hi-C offers structural context to functional and accessibility-based data, which help to gain a more comprehensive perspective of regulatory architecture. The most important types of multi-omics data and their role in the study of enhancer-promoter interactions are summarised in Table 3.

Table 3. Multi-Omics Data Integration

Data Type	Role
RNA-seq	Gene expression profiling and functional output
ATAC-seq	Identification of open chromatin and active regulatory elements
Hi-C	Mapping of three-dimensional chromatin structure and interactions

In addition to experimental integration, computational modelling and machine learning has developed a potent method in the analysis of multi-omics datasets. The use of machine learning such as random forests, support vector machine, and deep learning models can be used to train a prediction of enhancer-promoter interactions using their features based on a sequence data, chromatin accessibility, histone modifications, and frequency of interactions. These models allow the determination of regulatory components and their target genes on the genome scale, even when these components have not been experimentally verified. These methods are further extended to predictive regulatory network modeling which are constructed using multi-omics data to build a set of gene regulatory networks, one that defines the connection among enhancers, promoters, transcription factors, and target genes. These networks give a clue to the hierarchy of regulations, redundancy of enhancers and combinatorial control of gene expression. Together with CRISPR-based perturbation data, these computational frames can be used to validate and refine predicted interactions and yield more accurate models of gene regulation. Altogether, combining multi-ounces of data with rigorous computational methods is a multi-dimensional method to cognise Enhancer–promoter interactions. This joint mapping technique not only improves the regulatory mapping course but, as well, facilitates the predictive modeling of gene expression, which may lead to the discovery of the disease-linked regulatory mechanisms and be utilized in therapeutic target identification.

6. CHALLENGES AND FUTURE PERSPECTIVES

Although a great deal has been learned about Enhancer–promoter (E-P) interactions and methods to investigate them with CRISPR, a number of problems have been identified that restrict the precise delineation and interpretation of gene regulatory science. Among the main issues related to the technologies based on CRISPR there is the presence of off-target effects. Despite the sequence-specific targeting properties of CRISPR systems, non-target targeting and cleavage can take place at non-target genomic locations, thus resulting in incorrect inferences about enhancer functionality. This off-target activity can cause changes in gene expression not due to

the desired perturbation, making interpretation of experimental findings more difficult. The development of guide RNA format, more specific Cas9 forms and enhanced computational prediction software is also underway to reduce such effects and increase specificity of targeting. Redundancy of enhancers is yet another serious challenge. Numerous genes are controlled by numerous enhancers able to complement each other, making them strong to express their genes. Deletion or repression of individual enhancers, therefore, may not yield an observable phenotypic or transcriptional effect, and its role can be underestimated. The problem of redundancy makes it difficult to identify important regulatory factors, and requires a combinatorial perturbation approach to completely understand enhancer networks. The study of E-P interactions is complicated further by context-specific regulation. The activity of enhancers is remarkably cell type, developmental-stage dependent, and environment dependent. Elements of regulation that are operational in one cell system may be silent in another, and it is hard to extrapolate findings to other biological systems. This context dependency points out the importance of research that is done in physiologically relevant models and under varied conditions in order to be able to bring the entire regulation spectrum. Single-cell CRISPR screening is also an emerging technology that has the potential to solve these problems. With a combination of CRISPR perturbation and single-cell RNA sequencing (scRNA-seq), they can study the effects of gene regulators on an individual cell-basis scale. The technique allows the detection of cell-to-cell differences in enhancer action and offers more detailed understanding of any heterogeneous biological system, including a tumour and developing tissue. Simultaneously, the use of artificial intelligence (AI) and machine learning in genomics is changing the analysis of regulatory holograms. The models based on AI can be used to predict Enhancer–promoter interactions, infer regulatory networks as well as to identify disease-related regulatory elements with high precision. Such computational methods come in especially handy with large-scale multi-omics data and the discovery of these patterns that would otherwise be difficult to find out using standard methods of analysis. In the future, the integration of high-resolution experimental schemes and single-cell technologies with AI-based computational models will enhance the sphere of gene regulation to a considerable extent. Future research directions will probably include refining the accuracy and usefulness of CRISPR-based technologies, creating scalable procedures of combinatorial perturbed enhancers and merging various datasets to create all-inclusive and forecasting models on gene regulation. These challenges will be important in applying knowledge on enhancer-promoter interactions into clinical practice such as development of specific therapies to treat human diseases.

7. CONCLUSION

Enhancer-promoter interactions are a basic gene regulation mechanism by which genetically correct regulation of transcription occurs without long-range chromatin signalling. The growing body of evidence has shown that distortion of these regulatory interactions is a crucial factor in the development and progression of many human diseases, cancer, disorders of the brain and other complicated genetic diseases. It is then important to understand the dynamics of these interactions in order to unravel the regulatory architecture of the genome. The development of CRISPR-based technologies has dramatically changed how genes are regulated and can now causally and directly interrogate how enhancers work. In contrast to traditional methods yielding more or less correlational information, CRISPR-based perturbation methods, such as CRISPR-Cas9, CRISPR interference, and CRISPR activation, enable studying regulatory elements with great precision and can determine conclusive relationships between the enhancer and its target genes. These devices have offered the chance to systematically deconstruct gene regulatory networks and confirm disease-related regulatory components never before seen before. More so, combination of CRISPR-based functional genomics with multi-omic data and computational modelling has improved the solution and predictive statistical capacity of regulatory analysis. By doing so, a more global view of the coordination of multiple enhancers in regulating gene expression can be achieved, along with how their dysregulation affects disease phenotypes. Finally, Enhancer–promoter interactions are important regulators of gene expression and are central to human disease. The CRISPR-based functional solutions offer a strong framework to discover the causal connection between these interactions, which would facilitate our knowledge of the genome control and provide new opportunities in treatments.

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