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Review

TRIP through the chromatin: A high throughput exploration of enhancer regulatory landscapes

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ABSTRACT

Enhancers are regulatory elements that promote gene expression in a spatio-temporal way and are involved in a wide range of developmental and disease processes. Both the identification and subsequent functional dissection of enhancers are key steps in understanding these processes. Several high-throughput approaches were recently developed for these purposes; however, in almost all cases enhancers are being tested outside their native chromatin context. Until recently, the analysis of enhancer activities at their native genomic locations was low throughput, laborious and time-consuming. Here, we discuss the potential of a powerful approach, TRIP, to study the functioning of enhancers in their native chromatin environments by introducing sensor constructs directly in the genome. TRIP allows for simultaneously analyzing the quantitative readout of numerous sensor constructs integrated at random locations in the genome. The high-throughput and flexible nature of TRIP opens up potential to study different aspects of enhancer biology at an unprecedented level.

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1. Introduction

Transcription regulation in eukaryotes is a complex process with many layers of regulation orchestrating an intricate network of signals and responses. It involves the action of sequence specific transcription factors, chromatin remodelers, epigenetic modifiers and long-distance communication between promoters and enhancers through chromatin looping factors (Fig. 1). Additionally, non-coding RNAs, such as enhancer

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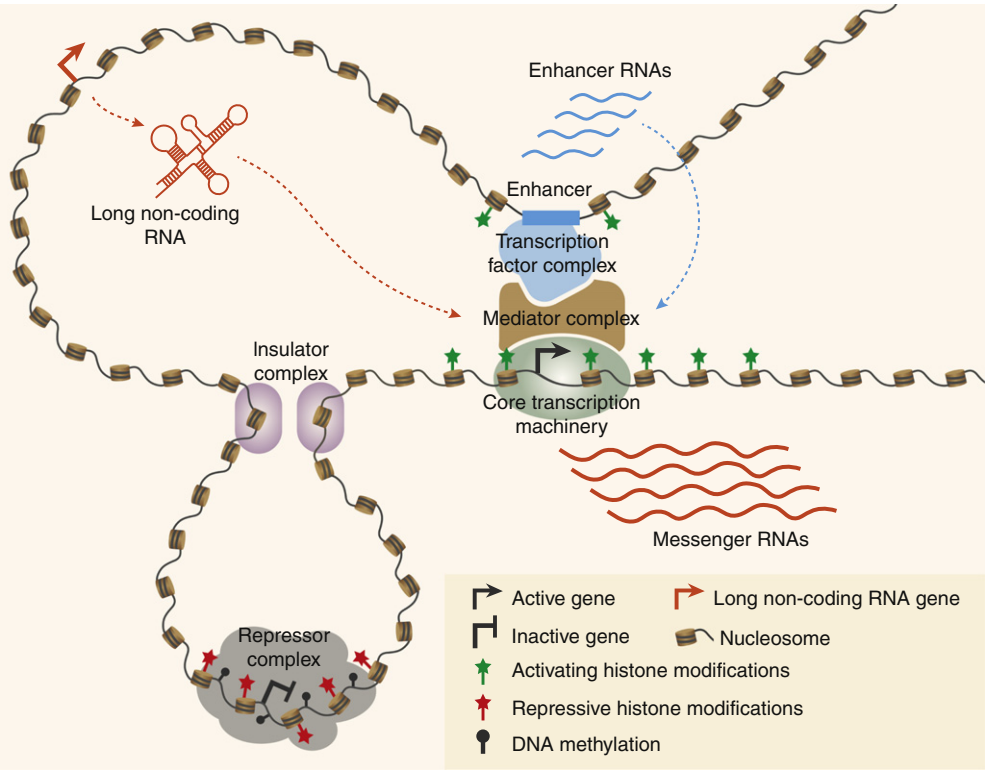

Fig. 1. A simplified model of *cis*- and *trans*-acting factors that shape the regulatory landscape of eukaryotic transcription.

RNAs and long non-coding RNAs, have been implicated in regulation of transcription [1,2]. DNA regulatory elements such as promoters and enhancers are key components of this elaborate machinery. Enhancers are regulatory elements that activate the transcription of their target genes in an orientation-independent manner over long genomic distances, and occasionally across chromosomes [3–7]. Evidence is accumulating that disruptive changes in the DNA sequence of enhancers can cause human disease [8–10].

Identification of all enhancers and their target genes is a prerequisite for complete understanding of spatio-temporal regulation of transcription. Characterization of enhancers has traditionally been a low-throughput and labor-intensive enterprise. In the last decade, however, a number of technologies have emerged, aimed at identifying and characterizing enhancers at high throughput. These technologies can be broadly categorized into three types: (i) profiling of enhancers based on chromatin signatures, (ii) functional testing of genomic fragments for enhancer activity and (iii) enhancer sensors to explore endogenous enhancer landscape. In this review, we briefly describe these technologies and discuss in detail the enhancer sensor assays, with special focus on the TRIP (short for Thousands of Reporter Integrated in Parallel) technology.

2. Identification and characterization of enhancers *en masse*

2.1. Profiling of enhancers based on chromatin signatures

The revolution of genome sequencing at the turn of the century opened the way for computational predictions of enhancers largely based on sequence conservation, and presence and clustering of transcription factor binding sites [11–13]. The development of chromatin immunoprecipitation (ChIP) [14] and DamID [15] approaches, with the parallel revolution in microarray technology and more recently in next generation sequencing, allowed the identification of enhancers based on the occupancy of transcription factors and marking of genomic regions by specific histone modifications, predominantly H3K4me1 and H3K27ac [16–19]. As accessible regions of chromatin often coincide with promoters and enhancers, ChIP- and DamID-based approaches were complemented by technologies that profile chromatin accessibility, such as Formaldehyde-Assisted Isolation of Regulatory Elements sequencing (FAIRE-seq) [20] and DNase I Hypersensitive Site sequencing (DHS-seq) [21]. Many active enhancers also produce balanced bidirectional capped transcripts called eRNAs which have recently been utilized to map their locations in the genome [22,23].

2.2. Functional testing of genomic fragments for enhancer activity

The enhancer profiling methods are mainly descriptive in nature and rely on the presence or absence of certain genomic or epigenomic features. In recent years, medium- to high-throughput technologies have been developed that directly assess the ability of a given segment of DNA to activate transcription from a minimal promoter. These methods rely on screening libraries of genomic fragments, ranging in size from a couple of hundred to a few thousand base pairs, for their ability to activate a nearby minimal promoter-reporter construct. These libraries are either used in an episomal setting [24,25], or are integrated in the genome randomly [26] or at a specific site [27–29]. Although the activity-based methods have brought the throughput of functional enhancer screening to a new level, they have certain limitations in reflecting the character of endogenous enhancers as the candidate sequences are tested out of their natural context, and are often selected in a biased manner [30].

2.3. Approaches based on enhancer sensors

In contrast to the technologies described above, the enhancer trap methods involving integration of sensor constructs in the genome are particularly suited for exploring the enhancer landscape in its native context [31,32]. The expression of the integrated reporter shows the functional output of regulatory activities at and around the integrated