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Review

Identification of the gene-regulatory landscape in skeletal development and potential links to skeletal regeneration

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ABSTRACT

A class of gene-regulatory elements called enhancers are the main mediators controlling quantitative, temporal and spatial gene expressions. In the course of evolution, the enhancer landscape of higher organisms such as mammals has become quite complex, exerting biological functions precisely and coordinately. In mammalian skeletal development, the master transcription factors Sox9, Runx2 and Sp7/Osterix function primarily through enhancers on the genome to achieve specification and differentiation of skeletal cells. Recently developed genome-scale analyses have shed light on multiple layers of gene regulations, uncovering not only the primary mode of actions of these transcription factors on skeletal enhancers, but also the relation of the epigenetic landscape to three-dimensional chromatin architecture. Here, we review findings on the emerging framework of gene-regulatory networks involved in skeletal development. We further discuss the power of genome-scale analyses to provide new insights into genetic diseases and regenerative medicine in skeletal tissues.

Contents

1. Framework of gene regulation

The sequence from gene expression to protein translation (DNA to RNA to protein) comprises the central dogma of biology, as these phenomena collectively mediate the biological actions of cells. Gene expression is initiated through activation of the basal promoter, generally a DNA sequence less than 100 bp, in conjunction with the transcription start site (TSS). The basal promoters recruit RNA polymerases and basal transcription factors [1]. This action is necessary to initiate the process of transcription; however, it is not sufficient for the proper expression of the genes. Additional regulatory elements, called enhancers, are the main mediators specifying quantitative, spatial, and temporal regulation of the gene expression.

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The first enhancer described was a 72-bp tandem repeat of SV40 DNA, which was shown to function as a cis-regulatory element activating the transcription of a cloned β-globin gene. This activation was observed even after changing the orientation or location of the sequence among several positions [2]. Currently, an enhancer is defined as a noncoding DNA sequence that can drive the target gene expression, regardless of the distance, location, or orientation of the sequence from the target basal promoter [3]. The size of enhancers ranges from 50 bp to 2 kb, and transcription factors bind to enhancers through specific DNA sequences called motifs, resulting in activation of the enhancers.

Although the basic machinery of the enhancer action is well conserved, the enhancer landscapes differ widely among species (Fig. 1; see the detail in Ref. [4]). In organisms with small genomes, such as bacteria and yeast, local regulatory controls are predominant, primarily through activation of the basal promoter and/or very few enhancers located mainly within 1 kb of the respective genes. Invertebrate species, such as *Drosophila*, have relatively complex machinery. Multiple enhancers targeting individual genes are often observed, although the distance of enhancer–promoter interactions is usually less than 10 kb. In contrast to these species, vertebrae have much more complex regulation for transcription. Enhancers can interact with target promoters over a range of 100 or even 1000 kb. Multiple enhancers can target genes even beyond the nearest genes. This complexity is probably due to the two rounds of whole genome duplication, which generate paralogous genes and additional regulators. Thus, *in vitro* studies such as reporter assays using several-kilobase-long genomic fragments around the basal promoter could cover gene-regulatory elements in invertebrates; however, capturing the vertebrate gene-regulatory landscape was difficult until next-generation sequencers became available.

Genome-scale studies using next-generation sequencers have provided novel insights into gene regulations, shifting interest from local gene regulations to multiple dimensions of the gene-regulatory landscape that integrates primary actions of the transcriptional regulators, the enhancer landscape, and the three-dimensional chromatin architecture (Fig. 2). Uncovering this landscape will provide new insights into the development,

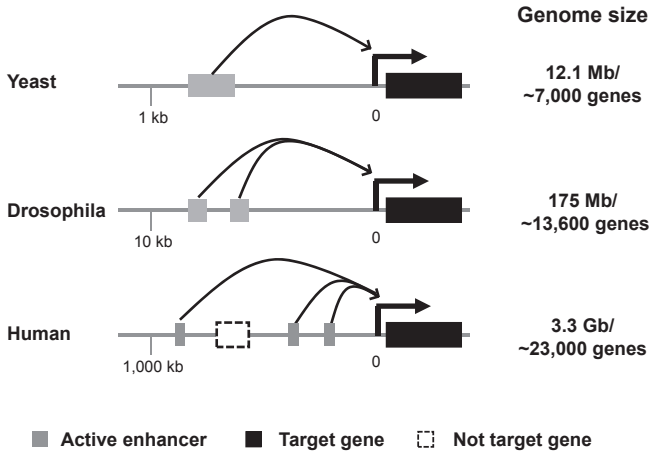

Fig. 1. Enhancer landscape in various species. A schematic model of the gene-regulatory landscape and genome size information of yeast, *Drosophila*, and humans. In yeast, many genes are regulated only by activation of the basal promoter. A few enhancers are reported to control the target gene, but most of the enhancers are located within a 1-kb range of the basal promoter. In *Drosophila*, multiple enhancers work together to control a target gene, although the distance of enhancer–promoter interactions is usually less than 10 kb. In mammals, including human, enhancers can interact with target promoters over a range of 100 or even 1000 kb. The interactions can occur beyond several of the nearest genes. Multiple enhancers likely control quantitative, temporal, and spatial aspects of gene expression.

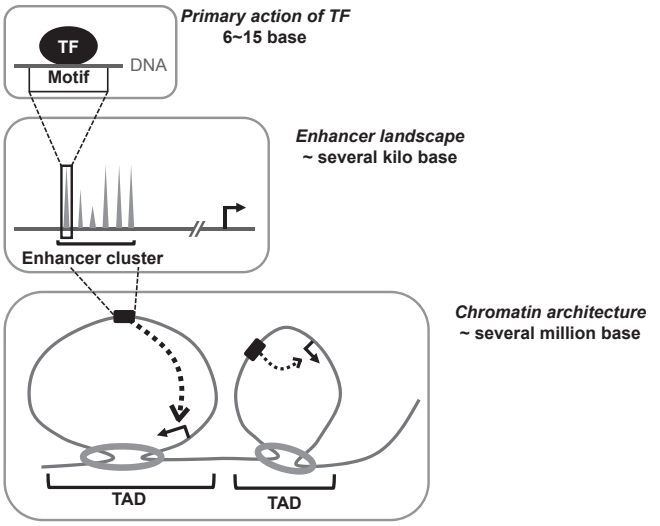

Fig. 2. Emerging framework of gene regulations. Transcription factors (TFs) prefer to bind to specific DNA sequences called motifs. Multiple associations of TFs to DNA occur at enhancers. On the genome, multiple enhancers form clusters; their distributions differ among cell types, representing cell type-distinct signatures. Topologically associating domains (TADs), which are defined as a three-dimensional chromatin landscape, have emerged as a new concept. Within a TAD, physical interactions of enhancers and promoters frequently occur, whereas these interactions are generally not observed across the boundary of TADs. In the boundary regions, CTCF and cohesin are thought to be involved in the looping of the chromatin structure.

evolution and pathologies of organisms. In this review, we summarize the emerging gene-regulatory networks involved in the development of skeletal tissues, which both create a supportive framework and systematically control the supply of minerals for the body.

2. Primary mode of action of key transcription factors in skeletal development

The mammalian skeleton is derived from cells of three origins: the neural crest, paraxial mesoderm, and lateral plate mesoderm. It is formed through two distinct modes of ossification: intermembrane ossification and endochondral ossification (see review [5]). Regardless of the origin or the mode of ossification, key transcription regulators are crucial for the specification and differentiation of the skeletal cells. So far, three transcription factors, Sox9, Runx2 and Sp7/Osterix, have been identified as master regulators in skeletal development [6–10], whereas others were identified mainly as co-regulators of these master regulators that modify their functions or expressions at either the transcriptional or protein level [11–13]. In this section, we briefly summarize the biological roles of these master regulators in skeletal development and discuss the gene-regulatory networks that have emerged from recent genome-scale studies, particularly focusing on how the master regulators program the regulatory networks cooperatively with co-regulators.

2.1. Sox9-mediated chondrocyte regulatory program

Sox9 is a high-mobility group (HMG) domain-containing transcription factor, closely related to the Y chromosome-encoded testis-determining factor SRY. Mutations in *SOX9* are associated with campomelic dysplasia, in which both sex and skeletal development are affected [14]. During skeletal development, Sox9 is expressed in mesenchymal condensation; the Sox9-positive cells in the condensation give rise to osteoblasts, chondrocytes, tendon