Starting from a single fertilized cell, a wide variety of cell types are formed as a consequence of development. It is widely known that a subset of pluripotent cells is committed to a specific lineage during development. For example, the cartilage tissue is developed through the process termed chondrogenesis, where an initial population of mesenchymal stem cells ultimately develops into differentiated cells (chondrocytes) characterized by abundant extracellular matrix such as type II collagen. This process consists of several stages: mesenchymal condensation, chondrocyte proliferation, matrix, and ossification.1

DNA Methylation

The only modification of mammalian genomic DNA is the methylation at the 5-position of the cytosine residue within cytosine–guanine dinucleotides (CpG), resulting in the formation of 5-methylcytosine, which is inherited over cell divisions.4 Regions of genome that have many CpG sequences are commonly referred to as ‘CpG islands.’ CpG islands often exist in the promoter region of the genome, and methylation of the CpG island leads to transcriptional repression.1,14 At least two major mechanisms of this transcriptional repression have been proposed. One is the inaccessibility of transcription factors to the binding motif sequence due to the altered DNA structure by methylation; the other is the formation of heterochromatin, which is caused by chromatin remodeling proteins bound to methylated DNA. The methylated CpGs recruit methyl-CpG-binding domain proteins (MBDs), which then recruit more proteins, such as histone deacetylases (HDACs) and heterochromatin protein 1 (HP1), resulting in the formation of compact inactive chromatin.10

In mammals, while the methylation patterns of the genome are constantly inherited after tissue differentiation, dynamic changes in genome-wide de novo methylation are observed during at least two developmental E: asahara@scripps.edu

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Epigenetic Control in Cartilage Formation

The significance of epigenetics in cartilage development has begun to be realized, and several experimental studies have been published accordingly (see Figure 2). In chondrogenesis, the fundamental transcription factor is Sox9, which recognizes and binds to the specific DNA sequence of six to seven nucleotides referred as Sox-binding motif, and also activates the transcription of cartilage-specific genes. Sox5 and Sox6 are members of the Sox gene family with a DNA-binding HMG domain, and co-operatively activate transcription with Sox9 in chondrogenic differentiation. Sox9 is believed to have a lot of downstream target genes in chondrogenic-lined periods: germ cells and pre-implantation embryos.11 Germ cells with paternal and maternal genomes have different epigenetic marks due to genomic imprinting. Thus, the original methylation patterns in primordial germ cells are deleted and reprogrammed during gametogenesis. Similarly, the genome undergoes de novo methylation following demethylation in the pre-implantation embryo. This process is presumed to be necessary for the embryo to obtain totipotency.11 However, the mechanism of demethylation has remained controversial, whereas DNA methyltransferase-mediated methylation is well understood.12,13 All of the aforementioned processes of methylation are catalyzed by members of the DNA methyltransferase (Dnmt) family: Dnmt1, 3a, and 3b in mammals. It has been proposed that Dnmt1 is responsible for the maintenance of DNA methylation patterns in daughter strands over DNA replications, while Dnmt3a and 3b catalyze de novo methylation.14,15 Interestingly, it has been reported that in particular genes, such as trefoil factor 1 or oestrogen receptor alpha, the CpG sequences in the promoter region are controlled by cyclical changes of DNA methylation and demethylation with a periodicity of around 100 minutes.14

**Figure 2: Initiation of Transcription on Col2a1 Promoter**

The Sox9–CBP/p300 complex initiates the transcription of cartilage-specific genes such as the type II collagen alpha 1 (Col2a1) gene. Sox9-CBP/p300 complex looses the nucleosome by histone acetylation and uncovers the underlying genetic code. RNA polymerase II (Pol II) complex mechanism of demethylation has remained controversial, whereas DNA methylation following demethylation in the pre-implantation embryo. This process is presumed to be necessary for the embryo to obtain totipotency. However, the mechanism of demethylation has remained controversial, whereas DNA methyltransferase-mediated methylation is well understood. All of the aforementioned processes of methylation are catalyzed by members of the DNA methyltransferase (Dnmt) family: Dnmt1, 3a, and 3b in mammals. It has been proposed that Dnmt1 is responsible for the maintenance of DNA methylation patterns in daughter strands over DNA replications, while Dnmt3a and 3b catalyze de novo methylation. Interestingly, it has been reported that in particular genes, such as trefoil factor 1 or oestrogen receptor alpha, the CpG sequences in the promoter region are controlled by cyclical changes of DNA methylation and demethylation with a periodicity of around 100 minutes.

**3D Positioning of Chromosomes**

After cell division, highly compacted chromosomes are relaxed and begin to express particular genes. Numerous factors generate and modulate the spatially high-ordered chromatin structure. The chromatin structure allows for intimate communications between distantly spaced genomic regions, and co-ordinates the expression of a gene cluster according to the spatial field. Recent studies have revealed the importance of spatial regulation.

**Nucleosomal Modification**

The nucleosome is an efficient packaging unit of DNA in all eukaryotic chromatin, and consists of two sets of the four core histones (H2A, H2B, H3, and H4). They exist as the histone octamer, in which 147bp of DNA are tightly wrapped. Hundreds of thousands of nucleosomes are further packaged into multiple hierarchical levels. Histone N-termini are referred to as ‘histone tails,’ which undergo diverse post-translational modifications, including acetylation, phosphorylation, methylation, ubiquitination, and adenosine diphosphate (ADP) ribosylation. Many modification enzymes participate in the modification process. Acetylation and phosphorylation mainly participate in the dynamic chromosomal changes. The acetylation reaction produces a more relaxed chromatin structure, resulting in the upregulation of gene transcription; this is because the acetyl group offsets the positive charge on histone proteins, thereby attenuating the interactions between histones and negatively charged DNA. On the other hand, histone methylation is a stable covalent modification, which usually contributes to long-term transcriptional regulation.

These histone tail modifications are suggested to provide information that serves as a transcriptional controller called the histone code. The histone code is hypothesized to function as a ‘barcode’; it determines the specific status of histone–DNA interactions by combination of histone tail modifications (see Figure 1). The precise mechanisms by which the barcode determines the status of histone–DNA interactions remain to be elucidated. However, recent studies have deciphered some specific barcodes. For instance, methylation of histone H3 lysine 4 (H3K4me) represents transcriptionally activated status, and methylation of H3 lysine 9 (H3K9me) usually represent inactive status. Each modification often accompanies specific effectors: trithorax group (TrxG) proteins related to H3K4, HP1 to H3K9, and polycomb group (PcG) proteins to H3K27. The covalent histone tail methylation is a stable modification, and has been believed to be almost permanent. Recently, however, several enzymes with the capability of active histone demethylation have been reported, and this process appears to be essential for normal development.

In addition to histone core proteins, non-histone proteins such as high-mobility-group (HMG) proteins are involved in epigenetic controls. Non-coding RNA also has the power to modulate epigenetic status.

Figure 1: Encoding of Transcriptional Control Signals

Transcriptional control signals are encoded in various histone tail modifications, referred to as ‘histone codes,’ like barcodes.

**Figure 2: Initiation of Transcription on Col2a1 Promoter**

The Sox9-CBP/p300 complex initiates the transcription of cartilage-specific genes such as the type II collagen alpha 1 (Col2a1) gene. Sox9-CBP/p300 complex looses the nucleosome by histone acetylation and uncovers the underlying genetic code. RNA polymerase II (Pol II) complex is recruited by the Sox9 complex mediated by thyroid hormone receptor-associated protein complex (TRAP230/Med12) and starts the transcription. The extracellular cytokine signal of transforming growth factor 1 or oestrogen receptor alpha, the CpG sequences in the promoter region are controlled by cyclical changes of DNA methylation and demethylation with a periodicity of around 100 minutes.
cells. Among these target genes, transcription of type II collagen alpha 1 (Col2a1), a gene of the dominant cartilage component protein, has been intensively analyzed as a typical model. The regulatory enhancer sequence exists in the first intron of the Col2a1 gene, which contains four Sox-binding motifs. In undifferentiated mesenchymal stem cells, the enhancer area is believed to hold the inactive state tightly folded round with deacetylated histones. Although Sox9 is essential for initiating the transcription of Col2a1, alone it can introduce a transcriptional active state using underlying genetic information wrapped on the nucleosomes.19 It is necessary for other factors to convert the epigenetic inactive chromatin into an active form, although these factors remain unknown.

Tsuda et al. and Furumatsu et al. demonstrated that a cyclic adenosine monophosphate (cAMP)-response element-binding protein (CREB)-p300 possesses chondrocyte-specific histone acetyltransferase (HAT) activity and co-operatively unfolds the tight nucleosomes and initiates the transcription.28,29 Interestingly, this epigenetic chromosomal conversion is accomplished when both Sox9 and CBP/p300 co-operatively exist.28 Additionally, although TGF-β signals have an important role in chondrogenesis from mesenchymal stem cell to chondrocyte, the precise mechanism of chondrogenic commitment has been unknown. Furumatsu et al. showed that phosphorylated Smad3/2 complex enters the nucleus after stimulation of mesenchymal stem cells by Col2a1 enhancer region and stabilizes the complex, resulting in the activation of transcription.28 In addition, peroxisome proliferation-activated receptor-gamma co-activator 1-α (PGC1-α) has been found by screening the spatially and timely specific genes for chondrogenesis using whole-mount in situ hybridization. PGC1-α showed parallel expression to that of Sox9. Kawakami et al. have showed that PGC1-α directly binds to Sox9 and acts as co-activator on the transcriptional chromatin of Col2a1.30

By yeast two-hybrid assay, Zhou et al. have showed that Sox9 interacts with a component of the thyroid hormone receptor-associated protein complex (TRAP230/Med12).31 Using genetic screening for detecting the chondrogenic phenotype in zebrafish, TRAP230/Med12 has also been detected. TRAP230/Med12 mutant zebrafish showed a cartilaginous deformity very similar to that of the Sox9 (Sox9a and Sox9b) mutant.32 The mediator complex is a key co-activator acting as a bridge between transcription factors and RNA polymerase II (pol II), thus conveying regulatory information to enhancer elements to the basal transcription machinery32,33 TRAP230/Med12 has been shown to physically bridge the Sox9 complex on the enhancer region of Col2a1 and pol II on the promoter region. Barx2 and c-Maf transcription factors have the ability to bind to the enhancer region of Col2a1, and co-operatively act with Sox9.34,35

As mentioned above, detailed information about the epigenetic activation of chondrogenic genes has been revealed, especially for histone acetylation by the Sox9 complex on Col2a1. However, other mechanisms of epigenetic control in cartilage remain unclear. Very few reports have been published focusing on methylation and demethylation of DNA or histone tails in chondrocytes, partly because knockout mice of important enzymes such as Dnmts or histone methyltransferases showed embryonic fatality before chondrogenesis. However, almost all of these mutant mice show growth retardation, which may indicate the important role of epigenetic activation of chondrogenic genes on cartilage-specific development. In the near future, precise mechanisms of epigenetic control in cartilage will be revealed.

Applications for Epigenetic Treatment in Musculoskeletal Diseases

A lot of attention has been paid to epigenetic research for novel aspects of potential treatments for musculoskeletal diseases. Indeed, several insights into epigenetic targets for musculoskeletal diseases have been reported. Systemic administration of the HDAC inhibitor FK228 suppresses rheumatoid arthritis-like synovitis in a mouse model.36 HDAC inhibitors, suberoyl anilide hydroxamic acid, sodium butyrate, and MS-275 have effectively induced apoptosis in cell lines from Ewing’s sarcoma.37 From a broader perspective, however, one of the biggest innovations is the production of human induced pluripotent stem (iPS) cells, which are epigenetically reprogrammed into the undifferentiated status from adult human skin fibroblasts by inducing only a few genes.38,39 Application of iPS cells for treatment of disease has already been reported in a humanized sickle cell anemia mouse model,40 and further research will contribute to the development of effective treatments for musculoskeletal diseases as well. Epigenetic treatment will become one of the central methods in this area in the future.

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