Trends in Plant Science

CellPress

Review

Plant Enhancers: A Call for Discovery

Blaise Weber,[1,3] Johan Zicola,[2,3] Rurika Oka,[1,3] and Maike Stam[1,*]

Higher eukaryotes typically contain many different cell types, displaying different cellular functions that are influenced by biotic and abiotic cues. The different functions are characterized by specific gene expression patterns mediated by regulatory sequences such as transcriptional enhancers. Recent genome-wide approaches have identified thousands of enhancers in animals, reviving interest in enhancers in gene regulation. Although the regulatory roles of plant enhancers are as crucial as those in animals, genome-wide approaches have only very recently been applied to plants. Here we review characteristics of enhancers at the DNA and chromatin level in plants and other species, their similarities and differences, and techniques widely used for genome-wide discovery of enhancers in animal systems that can be implemented in plants.

Enhancers in Gene Regulation

The vast majority of eukaryotes consist of numerous different cell types. In a given organism, the different cell types possess the same DNA, and it is fascinating that such diversity of cell types can arise from one and the same set of chromosomes. Cells of all organisms are in addition able to respond to abiotic and biotic environmental cues such as light, temperature, chemicals, and pathogens. The correct temporal and spatial regulation of gene expression is crucial for the successful production of highly specialized cell types and their response to external signals [1]. This is in large part accomplished through the activation and repression of the relevant ***cis*-regulatory elements** (see Glossary), such as **transcriptional enhancers** (hereafter referred to as enhancers) and **silencers**, at the correct moment in time and space [2,3]. Enhancers are non-coding DNA sequences that can be bound by multiple **transcription factors** (TFs) to activate the expression of genes located up to several Mb away (Figure 1A) [4,5]. Silencers are DNA elements that repress gene expression [3]. Both enhancers and silencers can be located up- or downstream of their target genes and function in an orientation-independent manner [6]. Enhancing and silencing functions can also be combined into one and the same DNA element, such as shown for the light-inducible and tissue-specific regulatory elements of *ab80* and *rbcS-3A* in pea (*Pisum sativum*) [7–10]. This review focuses on enhancers.

The general mechanisms by which enhancers are activated and trigger gene expression are well studied [11]. Enhancers are generally activated by the binding of pioneer TFs, followed by the recruitment of coactivators such as histone acetyltransferases and chromatin remodelers that together increase chromatin accessibility [12]. This increased accessibility promotes the binding of other TFs, leading to transcriptional activation of the target genes [12]. To do so, enhancers physically interact with the promoters of their cognate genes (Figure 1B). Ultimately, transcription is initiated by RNA polymerase II at the transcription start-site (TSS) of the gene [13].

In the past decades several examples of enhancers have been identified and characterized in different species, including yeast, fungi, animals, and plants (e.g., [14–21]). These examples have

Trends

Enhancers are one of the key elements in gene regulation in eukaryotes that allow correct temporal as well as tissue- and cell type-specific gene expression.

Thousands of enhancers have been discovered in animals, but only limited numbers are known in plants

Despite the limited number of features known for plant enhancers, they appear to share a number of common properties with the well-characterized animal enhancers. Plant-specific enhancer features are yet to be discovered.

The use of high-throughput sequencing-based methods enables the genome-wide discovery and characterization of plant enhancers.

Adaptation of powerful techniques such as STARR-seq, developed in the animal field, would greatly contribute to the identification and characterization of plant enhancers.

[1]Swammerdam Institute for Life Sciences, Universiteit van Amsterdam, Science Park 904, 1098 XH Amsterdam, The Netherlands
[2]Max Planck Institute for Plant Breeding Research, Department Plant Developmental Biology, Carl-von-Linné-Weg 10, 50829 Köln, Germany
[3]These authors contributed equally to this work.

*Correspondence: m.e.stam@uva.nl (M. Stam).

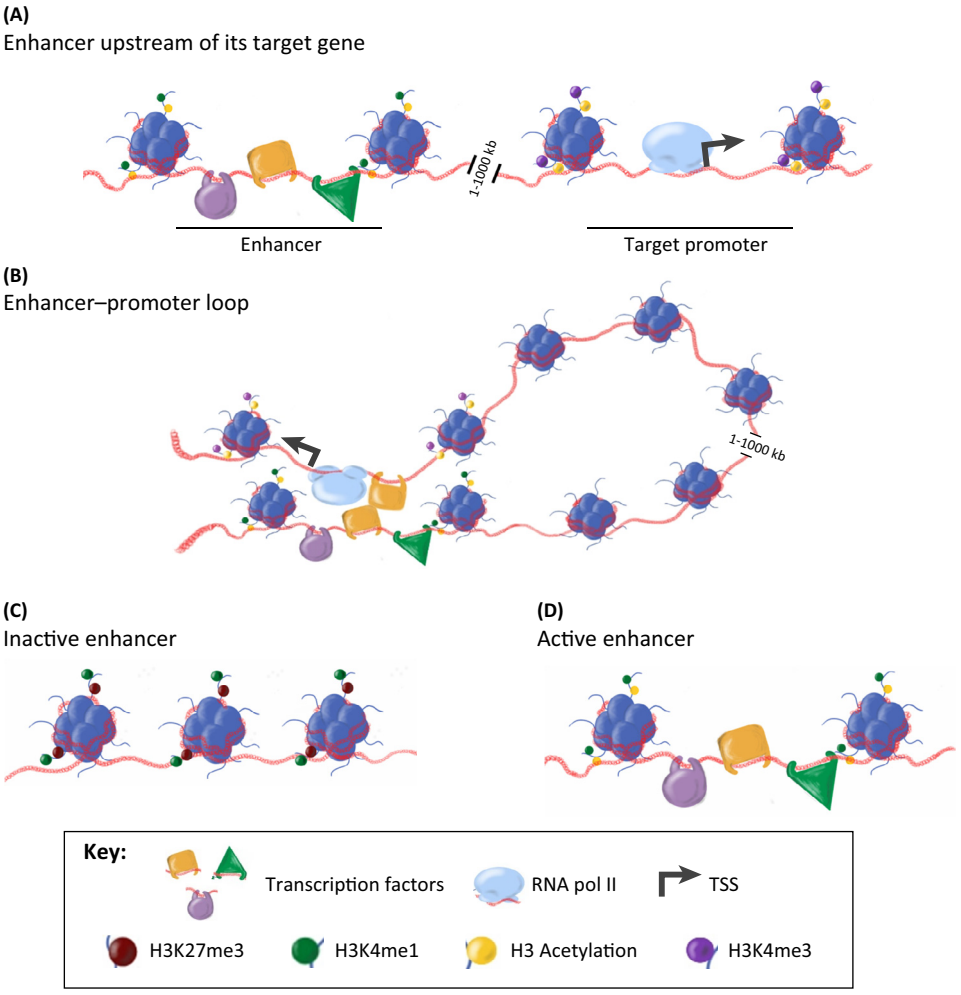

Figure 1. Schematic Illustrations of Chromatin Features and Associated Proteins Observed at Enhancer Regions and Target Genes in Animals.

For a Figure360 author presentation of Figure 1, see the figure online at http://dx.doi.org/10.1016/j.tplants.2016.07.013#mmc1.

(A) Enhancer located at a distance from its target gene. The presence of H3K4me1 and absence of H3K4me3 distinguishes enhancers from promoters. (B) Active enhancers physically interact with the promoter of their target gene through protein complexes. (C) Inactive enhancers are associated with H3K27me3 and H3K4me1. (D) Active enhancers are associated with nucleosome-depleted regions as well as H3K4me1, H3K9ac, and H3K27ac (annotated as H3 acetylation). Abbreviation: TSS, transcription start-site.

mainly been identified using low-throughput methods such as enhancer trapping, promoter deletion analysis, recombinant analysis, and quantitative trait locus mapping. The recent development of affordable next-generation sequencing technologies, in combination with the identification of general enhancer features, especially DNA and chromatin features, has allowed the genome-wide identification of enhancers in a high-throughput manner. This led to the discovery of over 43 000 enhancer candidates in the human genome [22] and up to 100 000 predicted enhancers in drosophila (*Drosophila melanogaster*) [23]. Remarkably, genes are often shown to be regulated by more than one enhancer [23–25]. The crucial roles of enhancers in gene regulation have been emphasized in studies linking enhancers not only with proper embryonic development and the specialization of cell types, but also with a large set of diseases

Glossary

Active enhancers: enhancers that are upregulating the expression of their target genes. They are located within accessible chromatin regions, are associated with activating histone marks and low levels of DNA methylation, and are bound by TFs.

Chromosome conformation capture (3C): 3C reveals the relative frequency of physical interactions between a given chromosomal fragment, called the bait or viewpoint, with other known fragments (one-to-one). Derivative techniques increase the number of detected interactions: (i) 4C (circular 3C) reveals all interactions of a given bait (one-to-all); (ii) 5C reveals all interactions for many baits (many-to-many); (iii) Hi-C reveals all interactions genome-wide (all-to-all).

***Cis*-regulatory elements:** non-coding DNA sequences that regulate gene expression by recruiting TFs. The elements can be located nearby or at a distance from their target genes.

DNA footprinting: method allowing the identification of protein binding sites using techniques such as DNAse I footprinting, DNase-seq, ChIP-seq or ATAC-seq. In combination with next-generation sequencing, footprinting allows to elucidate TF binding motifs. For example, when using DNase-seq for DNA footprinting, TF binding to DNA protects binding sites from DNase I cleavage. TF binding motifs can then be determined by sequence analysis of the protected fragments.

Inactive enhancers: enhancers that are silenced. They can be stably silenced or ready to be activated (also known as poised enhancers). Stably silenced enhancers are located in inaccessible chromatin regions and carry repressive histone marks (e.g., H3K9me2) and high DNA methylation. Poised enhancers are associated with both repressive (H3K27me3) and activating histone marks (e.g., H3ac), and display an increased level of accessibility compared to stably silenced enhancers.

Insulators: *cis*-regulatory elements that block the interaction between enhancers or silencers and non-target genes.

Position effect: the effect of the genomic location of an endogenous