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Review

Chromatin loops in gene regulation

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ABSTRACT

The control of gene expression involves regulatory elements that can be very far from the genes they control. Several recent technological advances have allowed the direct detection of chromatin loops that juxtapose distant genomic sites in the nucleus. Here we review recent studies from various model organisms that have provided new insights into the functions of chromatin loops and the mechanisms that form them. We discuss the widespread impact of chromatin loops on gene activation, repression, genomic imprinting and the function of enhancers and insulators.

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1. Introduction

Control of gene expression can occur locally and over large genomic distances. Regulatory elements are frequently positioned far upstream or downstream of the genes they control and can even influence the expression of genes that lie on separate chromosomes. Most genes exhibit rather unique characteristics with regard to the site and level of expression as well as the timing of transcription throughout development and the cell cycle. This level of specificity is achieved through the combinatorial actions of multiple regulatory elements. In artificial systems to study gene expression, enhancers usually activate linked promoters promiscuously. Hence, in vivo, mechanisms are in place to prevent enhancers from producing unwanted influence on neighboring genes or even genes that are dispersed throughout the genome. This can be achieved in two ways that are not mutually exclusive. In the first, the enhancer may only be able to interact with its target promoter efficiently in the presence of a unique combination of enhancer-binding proteins. Such an enhancer would be expected not to function promiscuously. For example, the locus control region (LCR) of the β-globin locus interacts with embryonic and adult type β-globin promoters at the appropriate time of development. It is thought that the stage-specific transcription factor milieu determines promoter-selective interactions with the LCR. The second way to ensure gene-specific regulation involves enhancer-blocking insulators and barrier elements that reduce the effects of enhancers and block the spreading of repressive chromatin, respectively. Intriguingly, enhancer-blocking insulators resemble enhancers in that they can interact over large distances and between chromosomes. How specific communication among distal regulatory elements is achieved has been the subject of discussion and speculation for many years [1–10]. Substantial recent experimental evidence favors two major models for long-range control of gene expression that might occur alone or in combination, namely looping and tracking/scanning. Specifically, the advent of new techniques has enabled investigators to directly examine long-range interactions between chromosomal sequences in vivo. This has produced strong evidence that genes can be configured into looped structures or chromatin hubs that juxtapose regulatory elements to activate or repress transcription. However, these studies typically provide snapshot images of chromosomal interactions and do not rule out some form of tracking intermediate as a guide to establish gene-specific regulatory chromatin loops. Nevertheless, the detection of loops in vivo at numerous genes has shifted the debate towards the factors that are involved in forming, maintaining and resolving such loops, and how they impact on gene expression. This is the subject of this review.

2. What are chromatin loops?

A chromatin loop occurs when stretches of genomic sequence that lie on the same chromosome (configured in *cis*) are in closer physical proximity to each other than to intervening sequences. This simple definition does not consider the degree of proximity required to be functionally meaningful nor does it speak to the length of the intervening sequence, i.e. the size of the loop. Looped structures have been detected at numerous gene loci in a fashion that juxtaposes important genetic elements. Physical interactions have also been observed between elements residing on separate chromosomes and

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although they do not represent loops in the true sense of the word, these interactions might serve the same functions. Electron micrographs have also impressively demonstrated large chromosomal loops in the so-called lampbrush chromosomes of amphibian and avian oocytes [11], in preparations of metaphase chromosomes [12], and even in interphase chromatin where it appears that large loops are anchored to an insoluble structure often referred to as the nuclear matrix (for review see [13,14]). The term "looping" has also been used to describe chromosomal segments that protrude outwards from their chromosomal territory (for review see [15,16]). However, the impact of these loops on the regulation of gene expression is less well understood. Here we specifically discuss loops that spatially configure defined sequences of known gene loci.

3. Techniques to detect loop formation

Several approaches have been taken to determine whether distal regulatory elements are juxtaposed to form the base of genomic loops (Fig. 1). The most widely used method currently is chromosome conformation capture (3C), in varied form also known as nuclear ligation assay [17,18]. This method relies on the assumption that distal genomic sequences that are brought in close vicinity by protein complexes can be chemically crosslinked. Subsequent restriction digestion produces DNA fragments that can be re-ligated to each other. DNA fragments that are close to each other will be crosslinked and hence ligated with a higher efficiency than those that are not. Quantitative PCR using primers spanning the ligated fragments is used to measure the amounts of specific ligation products. The 3C assay requires numerous essential controls [19] since the results are influenced by crosslinking conditions, digestion efficiency, and have a significant potential for PCR artifacts. Large scale versions of the 3C assay (called 4C and 5C) have been devised using dedicated microarray platforms or high-throughput sequencing [20–23]. Results obtained by 3C do not easily translate into absolute distances that can be described on a micrometer scale. Instead, by comparing the interaction frequencies of multiple fragments, only relative

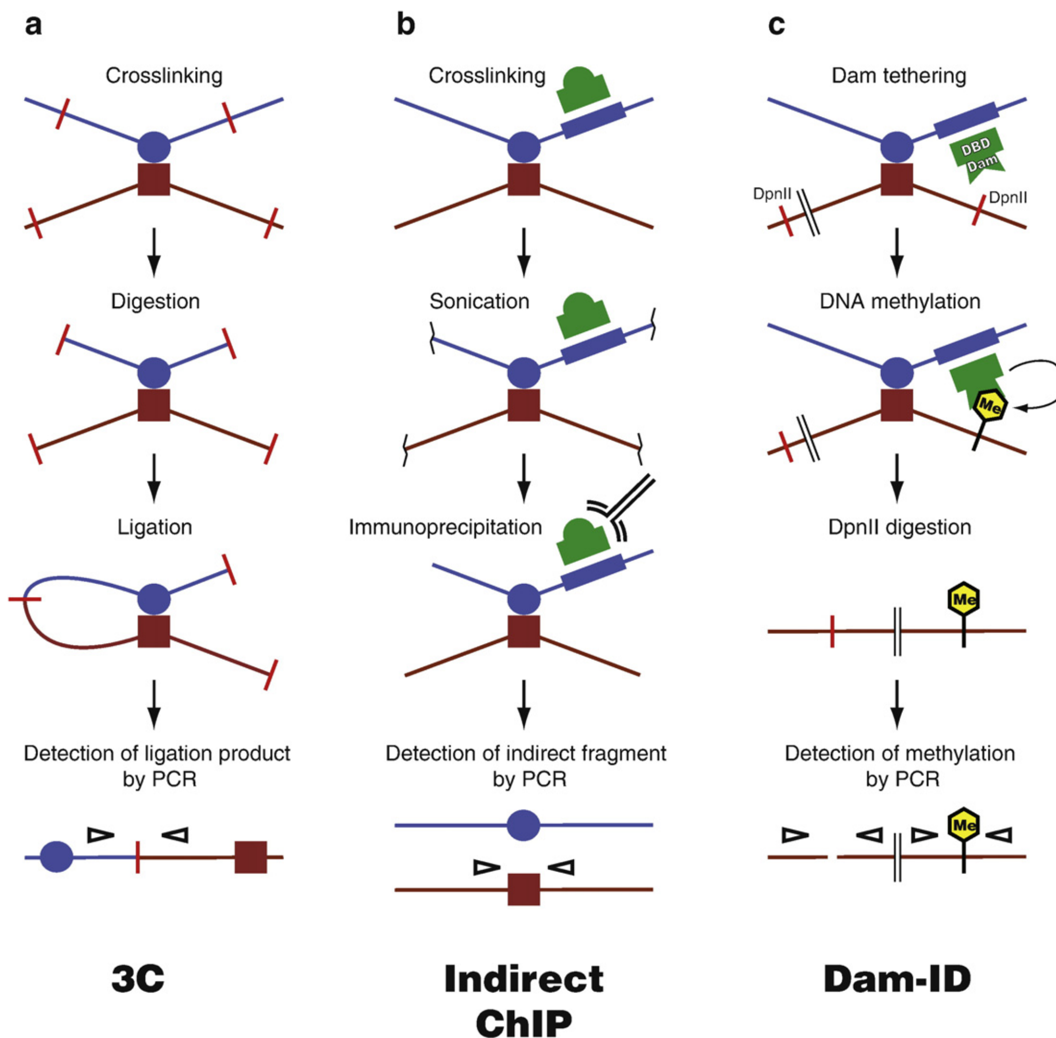

Fig. 1. Methods to detect chromatin loops. Several assays have been developed to detect proximity between two distant genomic sequences, for example an enhancer (blue circle) and a promoter (red square). (a) Chromosome conformation capture (3C). This widely used assay relies on the assumption that sequences which interact with a high frequency can be crosslinked more efficiently than sequences that interact less frequently. Crosslinked chromatin is digested with a restriction enzyme that creates sticky ends (red bars). Ligation generates unique products which do not occur in genomic DNA and which can be detected by PCR (empty arrowheads). The amount of PCR product correlates with the proximity between the genomic fragments in vivo. (b) Indirect chromatin immunoprecipitation (ChIP). This technique exploits sequence-specific DNA-binding proteins (green dome) as anchors to recover indirectly associated sequences. Chromatin is crosslinked and fragmented by sonication. DNA associated with the protein is recovered by immunoprecipitation. Sequences that are indirectly associated with the cognate sequence of the DNA-binding protein are recovered in this process and can be detected by PCR. (c) DNA adenine methyltransferase identification (Dam ID). This method takes advantage of the fact that adenine methylation, which does not naturally occur in eukaryotes, inhibits DNA cleavage by the DpnII restriction enzyme. The bacterial Dam methylase is fused to a DNA-binding domain (DBD) to tether it to a sequence of interest (blue box). Tethered Dam methylates DNA in vivo, after which genomic DNA is digested with DpnII. PCR is used to amplify sequences in a fashion that distinguishes undigested i.e. methylated from unmethylated sites of interest.

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