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Opinion

Genome-wide Mapping of the Nucleosome Landscape by Micrococcal Nuclease and Chemical Mapping

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Nucleosomes regulate the transcription output of the genome by occluding the underlying DNA sequences from DNA-binding proteins that must act on it. Knowledge of the precise locations of nucleosomes in the genome is thus essential towards understanding how transcription is regulated. Current nucleosome-mapping strategies involve digesting chromatin with nucleases or chemical cleavage followed by high-throughput sequencing. In this review, we compare the traditional micrococcal nuclease (MNase)-based approach with a chemical cleavage strategy, with discussion on the important insights each has uncovered about the role of nucleosomes in shaping transcriptional processes.

Trends

Genome-wide nucleosome positioning maps provide deep insight into the regulatory role of nucleosomes in transcriptional processes.

Micrococcal nuclease (MNase) digests linker DNA in between nucleosomes while nucleosome-protected DNA remains intact. Sequencing the protected DNA allows for the determination of nucleosome positions genome-wide.

The chemical mapping method relies on site-directed hydroxyl radical cleavage of nucleosomes carrying modified histones to determine the positions of nucleosomes in the genome.

MNase-defined NDRs of cis regulatory elements are nucleosome enriched in the chemical map of mouse ES cells. Emerging evidence shows that such regions are occupied by ‘fragile nucleosomes’, which are lost due to overdigestion by MNase. Results in mouse ES cells illustrate that fragile nucleosomes exist in the mouse genome and chemical mapping is capable of detecting them.

Nucleosome Positioning Plays a Critical Role in the Regulation of Transcription

Eukaryotic genomes are packaged hierarchically into a nucleoprotein complex called chromatin. The basic unit of chromatin is the nucleosome, which comprises 147 base pairs (bp) of DNA wrapped 1.65 superhelical turns around a small disk-shaped octamer of histone proteins [1,2]. Nucleosomes are repeated throughout the genome, each separated by unwrapped linker DNA of lengths varying from a few bp to over 100 bp. Approximately 20 bp of linker DNA may associate with a fifth histone, such as H1, thus forming a stable complex of the histone octamer core, H1, and ~165 bp of DNA known as the chromatosome [3].

Nucleosomes sterically occlude their wrapped DNA from proteins that must bind to it for DNA-related processes, including gene regulation and transcription. Access to occluded DNA often requires ATP-dependent nucleosome remodeling factors to unwrap nucleosomal DNA or slide nucleosomes to new locations along DNA [4]. However, remodeling factors are not always required as proteins can still bind nucleosomal targets through ‘DNA breathing’, in which a stretch of DNA lifts off the histone core and allows transient access to occluded DNA regions [5–7]. *In vitro* experiments demonstrate that breathing occurs every ~250 milliseconds on the outer stretches of the nucleosome (~20 bp), making these sites relatively accessible [5–7]. At ~40 bp into the nucleosome, however, the breathing frequency decreases to every 10 minutes and even slower at sites close to the nucleosome dyad [8]. It is evident then that the precise location of a nucleosome relative to a target site can influence factor binding by orders of magnitude. In the context of transcriptional regulation, a consequence of this competition over binding sites is that the outcome can have a dramatic effect on gene expression. As an

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illustration, *in vivo* work has shown that repositioning nucleosomes by as little as a few base pairs can alter the accessibility of once-hidden TATA boxes, leading to changes in transcription activation [9,10]. Another example comes from recent studies in yeast: insertion of nucleosome-disfavoring poly(dA) tracts near transcription factor (TF) binding sites influences gene expression in a manner that is tunable by the location, length, and composition of the poly(dA) tract [11,12]. In addition to competing with TFs for DNA accessibility, nucleosomes can function as physical barriers to an elongating **RNA polymerase II** (RNAPII) (see Glossary) [13–19]. Transcription studies on chromatin templates *in vitro* showed that nucleosomes decrease the rate of transcription elongation by RNAPII [20]. Both *in vitro* and *in vivo* studies suggest that nucleosomes contribute to RNAPII pausing. Significantly, *in vitro* data shows that RNAPII often pauses at the **nucleosome dyad**, where the histone–DNA contacts are the strongest [15,16], although *in vivo* experiments suggest that RNAPII pausing occurs predominantly near the nucleosome entry site [17–19]. Thus, **nucleosome positioning** along DNA plays a critical role in regulating the transcription output of the genome.

Recent technological advances have enabled nucleosome landscapes to be determined for many different organisms and cell types. The most widely used nucleosome mapping method is micrococcal nuclease (MNase) digestion of chromatin followed by high-throughput sequencing of the resulting DNA fragments (MNase-seq) [21,22]. Additional enzymatic methods have been put forward for mapping nucleosomes, including DNase-seq [23], NOME-seq [24], and ATAC-seq [25,26]. Another approach to determining nucleosome positioning maps is with targeted hydroxyl radical cleavage directed by genetically modified histones (chemical mapping) [27], methidiumpropyl-EDTA-Fe(II) (MPE-seq) [28], or ionizing radiation (RICC-seq) [29]. All existing mapping methods take advantage of the biophysical properties of nucleosomes and involve either enzymatic digestion or chemical cleavage of chromatin. These mapping techniques have collectively contributed to our understanding of nucleosome organization throughout eukaryotic genomes. Due to space constraints, we focus the present article on a comparison between the traditional use of MNase and the chemical mapping approach and discuss the insights they have revealed about the role of nucleosomes in transcriptional regulation.

MNase-seq: A Nuclease Protection Assay to Generate Global Nucleosome Maps

MNase Digestion Yields Nucleosome-Protected DNA

Most nucleosome mapping experiments are based on the protection of nucleosomal DNA against micrococcal nuclease (MNase) digestion. MNase is an endo-exonuclease that preferentially degrades the accessible linker DNA between nucleosomes, while most of the nucleosome-bound DNA is left intact (Figure 1A). Brief digestion of chromatin with MNase yields a 'ladder' of discrete DNA fragments by agarose gel electrophoresis, where each rung of the ladder corresponds to DNA protected by integer units of nucleosomes (Figure 1B). Thus, the fastest migrating fragment (~147–200 bp) represents DNA wrapped by a single nucleosome (or 'mononucleosome'), with fragment sizes increasing by multiples of this length. This characteristic ladder pattern provided early evidence that the regular repeating unit of chromatin consisted of histones wrapped by about 200 bp of DNA [30–33]. As MNase digestion proceeds, the fragment sizes decrease (as indicated by the gradual shortening of the ladder) and, accordingly, the proportion of mononucleosomes is increased. This process can be divided into three major stages [34]. First, monomers produced by initial MNase cleavage are nucleosomes still attached to linker DNA. They tend to be ~200 bp for the most part, but sizes can vary from as little as 154 bp (in *Aspergillus* fungus [35]) to 260 bp (in sea urchin [36]) due to the wide range of linker lengths. Second, MNase processively trims residual linkers until the fragment lengths are reduced to ~165 bp, as it reaches the boundary of the chromatosome. Third, digestion of the chromatosome eventually results in the concomitant loss of histone H1

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