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REVIEW

OPERating ON Chromatin, a Colorful Language where Context Matters

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Histones, the fundamental packaging elements of eukaryotic DNA, are highly decorated with a diverse set of post-translational modifications (PTMs) that are recognized to govern the structure and function of chromatin. Ten years ago, we put forward the histone code hypothesis, which provided a model to explain how single and/or combinatorial PTMs on histones regulate the diverse activities associated with chromatin (e.g., gene transcription). At that time, there was a limited understanding of both the number of PTMs that occur on histones and the proteins that place, remove, and interpret them. Since the conception of this hypothesis, the field has witnessed an unprecedented advance in our understanding of the enzymes that contribute to the establishment of histone PTMs, as well as the diverse effector proteins that bind them. While debate continues as to whether histone PTMs truly constitute a strict "code," it is becoming clear that PTMs on histone proteins function in elaborate combinations to regulate the many activities associated with chromatin. In this special issue, we celebrate the 50th anniversary of the landmark publication of the *lac* operon with a review that provides a current view of the histone code hypothesis, the lessons we have learned over the last decade, and the technologies that will drive our understanding of histone PTMs forward in the future.

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Abbreviations used: PTM, post-translational modification; TAF, TBP-associated factor; MS, mass spectrometry; ChIP, chromatin immunoprecipitation; bp, base pairs; SILAC, stable isotope labeling with amino acids in cell culture; modENCODE, model organism ENCyclopedia Of DNA Elements; ChIP-chip, ChIP combined with DNA microarray analysis; ChIP-seq, ChIP coupled with next-generation sequencing technology; SNAP, SILAC nucleosome affinity purification.

> "Small changes modifying the distribution in time and space of the same structures are sufficient to affect deeply the form, the functioning, and the behavior of the final product…. It is always a matter of using the same elements, of adjusting them, of altering here or there, of arranging various combinations to produce new objects of increasing complexity. It is always a matter of tinkering."
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> – François Jacob, "Evolution and Tinkering" (*Science* 1977)

The adult animal was in actuality the final product that François Jacob was referring to in this eloquent

statement taken from his article "Evolution and Tinkering."[1] Yet, as chromatin biologists, we delight in the applicability of Jacob's quote regarding the plasticity of a single template to the chromatin landscape. However, François Jacob is not best known for his theories on how patterns of gene expression affect evolution, but rather for his seminal work with Jacques Monod establishing the basis of the *lac* operon. In celebration of the 50th anniversary of François Jacob and Jacques Monod's landmark publication on the *lac* operon,[2] we are honored to contribute this piece in which we reflect on how several of the scientific themes put forward by Jacob and Monod in their historic work are widely applicable to topics as diverse as chromatin biology and the histone code hypothesis.

In simplistic terms, an operon is a functional genomic unit composed of a cluster of genes that are controlled by a single regulatory element or promoter.[3] Complementary genetic and biochemical studies revealed that the basic principle underlying the *lac* operon is that the coordinated expression of the genes necessary to metabolize lactose is under the control of the *lac* repressor protein and activator protein CAP, which negatively and positively control transcription of the *lac* operon, respectively.[2] From the pioneering studies on the *lac* operon completed by Jacob and Monod, we now know that there are three major types of regulatory DNA sequences that function in the control of gene expression in prokaryotes: (1) promoter sequences to which RNA polymerase binds; (2) operator sequences to which transcriptional repressors bind; and (3) positive control elements to which transcriptional activator proteins bind.[4] While the *lac* operon provides a simple yet elegant mechanism by which gene expression is controlled in prokaryotes, it is unreasonable to think that such a system would adequately provide a means by which efficient regulation of gene expression could occur in eukaryotes, where DNA must be highly compacted to fit within the confines of the nuclear space. The need for differential patterns of gene expression to specify diverse types of tissues from a single genome in multicellular organisms also calls for the existence of additional regulatory mechanisms. For example, cellular identity must be faithfully maintained through cell divisions for a lifetime, despite differentiation occurring earlier during embryonic development. The plasticity of cellular differentiation and the stability of cellular memory are thought to represent *epigenetic* phenomena wherein inherited changes in phenotype occur independently of changes in the underlying DNA sequence and without the need for *trans*-factors that establish the initial programs of coordinated gene regulation. Hence, while the historic work of Jacob and Monod reveals an elegant mechanism for prokaryotic gene regulation, it is clear that more sophisticated means of gene regulation involving components that do more than engage the DNA template alone are necessary for processes such as cellular memory in multicellular eukaryotes.

On the basis of many insightful studies on chromosome structure, we know that in eukaryotes, DNA is assembled on a histone scaffold to form chromatin.[5] The nucleosome core particle, or fundamental repeating unit of chromatin, consists of approximately 147 bp of DNA wrapped around an octamer containing one tetramer of histones H3 and H4 (two copies each) and two histone H2A–H2B dimers.[5–8] Nucleosomes are packaged into progressively higher-order structures to ultimately form chromosomes. Chromatin structure largely affects DNA-templated processes such as transcription, thus necessitating that access to DNA be tightly controlled to allow factors that function in such processes to make appropriate contacts with the DNA template itself.[5,9] Post-translational modifications (PTMs) to the histone proteins themselves can significantly affect the levels of chromatin compaction by creating generally condensed "heterochromatic" or more open "euchromatic" regions, and therefore provide a means by which rapid and localized access to DNA can be accomplished.[10,11] Additionally, other well-studied mechanisms, such as ATP-dependent chromatin remodeling and the exchange of primary sequence histone variants, introduce meaningful variation into the chromatin polymer, "tinkering" in such a way that one relatively stable genome can give rise to the demands of multicellular development.[12–14]

The "histone code hypothesis": the first 10 years

In 2000, we proposed what has come to be commonly referred to as the "histone code hypothesis," which, in its original form, posits that "multiple histone modifications, acting in a combinatorial or sequential fashion on one or multiple histone tails, specify unique downstream functions."[15] Parallels to François Jacob's quote from "Evolution and Tinkering" are readily apparent. The same fixed set of amino acids that make up the histone proteins have the potential of being post-translationally modified within the chromatin template, where distinct spatiotemporal patterns of modifications ultimately shape functional outcome. One of the more striking phenomena predicted by such a code is that subtle variations to the same template can result in vastly different outcomes, especially in the context of regulation of gene expression.

At the time that we proposed the histone code hypothesis, we had a limited understanding of the true breadth of the number and type of PTMs that exist on histone residues either on the unstructured N-terminal tails that protrude from the nucleosomal surface or within the structured globular domains.