Chromosomes and Expression Mechanisms

Editorial Overview

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Toshio Tsukiyama's lab is interested in the mechanism of chromatin regulation. His lab currently focuses primarily on the functions and the mechanisms of ISWI-dependent chromatin remodeling *in vivo* using the budding yeast as a model system.

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Susan Parkhurst's lab is interested in the different regulatory mechanisms and pathways required for proper *Drosophila* embryonic development. Their current efforts are divided between three areas of study: molecular mechanism(s) of transcriptional repression and cofactor recruitment, molecular mechanisms of Rho GTPase function, and molecular genetic analysis of wound healing.

Introduction

Whether one considers a single cell or a multicellular organism, a complex and precisely coordinated series of regulatory events and communications is required to ensure its proper configuration and function. One of the major goals in biology is to understand how cells differentiate into specific types to perform their roles *in vivo*. Genome sequencing projects have produced enormous amounts of data that are beginning to reveal the blue print of body plans for various organisms. Despite this wealth of new information, we are still far from understanding how cells differentiate. This is, in part, because we are not yet able to fully appreciate how this genetic information is being read by the transcription machineries. It is widely accepted that specific gene expression patterns are responsible for differentiation and maintenance of specific cell types, with mistakes in these regulatory steps often leading to developmental defects and the onset of cancers. Therefore, understanding the mechanisms of transcriptional control is a necessary prerequisite to achieve this major goal in biology. To this end, we need to know more about the substrate of transcription (chromatin), as well as the effectors of transcription (transcription factors). The theme of this issue of *Current Opinion in Genetics & Development* is the mechanism of transcriptional regulation, with an emphasis on latest topics in this rapidly moving area of research. Because chromatin structure deeply affects transcription at multiple stages, a significant portion of this issue is devoted to the mechanisms related to chromatin regulation.

Chromatin assembly

Chromatin is not only the substrate for transcription, but also allows compact storage of the genome. Therefore, faithful chromatin assembly is necessary for cell viability and all biological

processes that take place in nucleus. Despite the importance of this process, however, relatively little has been known about the mechanism of chromatin assembly *in vivo*. **Polo and Almouzni** review recent advances in this field, including identification of evolutionarily well-conserved factors involved in chromatin assembly, and of possible functional links among these factors. They propose a model in which each histone chaperone has an ATP-dependent chromatin remodeling factor as an obligatory partner for chromatin assembly, suggesting a potential general rule in the molecular mechanism of chromatin assembly.

Proper chromatin assembly, both during normal S phase and upon DNA damage, requires the availability of large amounts of histones in short period of time. However, having an excessive amount of free histones in nuclei can be extremely harmful, as they can bind strongly to DNA in non-specific manner thereby wreaking nuclear havoc. Therefore it makes sense that cells have developed active systems to monitor and adjust the levels of free histones. **Gunjan, Paik and Verreault** discuss multiple mechanisms to maintain adequate levels of histones *in vivo*. In particular, active degradation mechanisms for histones, recently found for both canonical core histones and centromere H3 variant, are discussed in detail.

Regulators of chromatin structure

Because chromatin is generally strongly inhibitory to nuclear processes that are dependent on protein-DNA interactions, eukaryotic cells have evolved multiple mechanisms to counteract, modify or take advantage of this negative effect. One such mechanism is the incorporation of histone variants into chromatin. As **Raisner and Madhani** describe, incorporation of the H2A variant, H2A.Z (Htz1 in yeast), near telomeres counteracts the transcriptionally repressive effects of telomere-specific chromatin structure. Their review highlights the surprising recent finding that H2A.Z is located around the transcription start sites of the majority of yeast genes, which suggests the possibility that H2A.Z has biological roles that have not been previously known.

To date, the most extensively studied mechanism of chromatin regulation is the covalent modification of histones. As a result, a large number of histone modifications have been identified, and how these modifications affect chromatin structure is being actively pursued by many laboratories. **Nightingale, O'Neill and Turner** provide a historical perspective as well as reviewing the latest findings on histone modifications, including identification of enzymes that apply or remove modifications. One emerging theme from this review is the complexity of the system, and how much more we still need to know to fully understand the molecular mechanisms by which histone modifications affect chromatin structure.

Since histone modifications play important roles in gene regulation *in vivo*, one would predict that the enzymes that are responsible for these modifications should be essential for normal development of animals. **Lin and Dent** describe numerous examples in mammalians that this is indeed the case.

The pattern of histone modifications is providing one of the few molecular clues to higher order chromatin structure currently available. One of the best studied markers for heterochromatin, lysine 9 methylation of histone H3, has been proposed to exert its repressive effects via recruitment of heterochromatin protein 1 (HP1). **Hediger and Gasser**, however, review the surprising recent findings that subtypes of HP1 play positive roles in transcription in various systems, again highlighting the complexity of the chromatin regulation system.

Another class of chromatin regulators, ATP-dependent chromatin remodeling factors, are typically associated with local changes in chromatin structure, such as disruption or sliding of nucleosomes around promoter regions. However, **Varga-Weisz and Becker** propose and

discuss supporting evidence for an intriguing new model in which some of these enzymes affect higher order chromatin structure by playing rather architectural roles.

Global analyses of chromatin and transcription

As the availability of genome sequence data progresses, so too has the development of sequence-based tools designed for genome-wide analyses. The transcription and chromatin fields have especially benefited by the development of DNA microarray-based technologies, such as transcription profiling and chromatin immunoprecipitation on microarrays (ChIP-chip). **Orian** reviews different genome-wide analysis techniques that have been successfully applied in chromatin profiling, in which localization of nuclear proteins on chromatin is determined in a genome-wide fashion. This review focuses mainly on the powerful new DamID method and compares it to other commonly used chromatin profiling techniques.

When genome-wide analyses are performed, we are often swamped by the data because of its overwhelming volume. Indeed, the data analysis steps, rather than the experimental steps, are usually rate limiting in genome-wide analyses. Therefore, development of efficient tools for systematic analyses of data is desperately needed. One of the most frequently performed analyses after transcription or chromatin profiling is to identify common *cis*-regulatory elements among genes that are identified in the analyses. **Ochoa and Small** compare and contrast different approaches that have been used for this purpose, as well as providing a look into potential future directions for these approaches.

We have two reviews on areas of research that have been greatly facilitated by genome-wide analysis tools. **Giresi, Gupta and Lieb** review the relationships between DNA sequence and nucleosome occupancy. It has been known that primary DNA sequences strongly affect nucleosome positioning. However, it has been impossible to predict nucleosome positioning based on DNA sequences. Recent development of new tools including high density DNA microarrays is now allowing the mapping of nucleosome positions on a global scale. Because a very large number of DNA sequences that are assembled into nucleosomes *in vivo* can now be identified by genome-wide analyses, there is hope that accurate prediction of nucleosome positions based on DNA sequence will be feasible in the near future.

The relationships between DNA replication timing and transcription have been studied since the early days, but systematic analysis has been technically impossible until recently. **Schwaiger and Schübeler** discuss how genome-wide analysis tools have made these kinds of analyses feasible. With interesting exceptions in single cell eukaryotes, chromosome regions enriched with actively transcribed genes have a strong tendency to replicate early. Possible underlying mechanisms and the physiological relevance of this coupling are also reviewed.

Different flavors of transcriptional regulation

In studies of transcriptional regulation, much of the effort has traditionally been concentrated on the initiation of transcription, especially at the steps involving DNA binding of "typical" transcription components, namely sequence-specific DNA binding proteins and their associated cofactors. Recent advancement in this field has uncovered multiple new mechanisms affecting transcriptional events that underscore the diversity of the regulatory mechanisms governing normal developmental processes.

Transcriptional elongation has been studied for quite some time. However, as **Eissenberg and Shilatifard** describe, direct association of specific patterns of histone modification with transcriptional elongation have been recently revealed. One of the most surprising recent results is that transcriptional elongation by RNA polymerase II associates with methylation of histone H3 at lysine 9, leading to recruitment of heterochromatin protein 1-gamma to actively transcribed

genes in mammalian cells. This result suggests a previously unidentified mechanism of transcription elongation.

Over the years, multiple laboratories have reported the identification of actin and myosin in nucleus, but their roles in the nucleus, if any, have been unclear or even dismissed as artifact. As **Grummt** reviews, recent studies are providing compelling new evidence that nuclear actin and myosin are required for transcription by RNA polymerases I, II and III. Furthermore, actin and actin-like proteins have been identified as integral components of numerous chromatin regulators. These results suggest that actin and myosin play critical roles in transcriptional regulation in multiple ways.

It has not been appreciated until recently that the proteasome is involved in essential steps in transcription. **Collins and Tansey** go over the history of the discovery as well as the latest development in the field. Curiously, the proteasome seems to have at least two mechanistically distinct ways to affect transcription. It can facilitate transcription via its proteolytic function to increase turnover of transcription factors or via a non-proteolytic function - most likely chaperone activity - to facilitate folding and/or exchange of nuclear proteins.

Gene regulation mediated by microRNAs (miRNA) is being studied very actively by many laboratories ever since it recently exploded onto the scene. **Carthew** summarizes the recent findings in the field, with an emphasis on the strategies being utilized to identify direct targets of miRNAs. This review illustrates that miRNAs are capable of mediating signaling, and play critical roles in development and physiology of multiple animals.

Thus, the past few years have witnessed an explosion of new information from the identification of new players, the onset of global analyses approaches, and the development of new technologies to harvest, assimilate, and integrate this new information. In combination with the rapidly paced elaborations on previously defined transcriptional substrates and machineries, these studies are expected to contribute greatly to our understanding of transcriptional regulation pathways, in both their roles in normal development, as well as in their contribution to disease states.