Molecular Biology

A unifying model of epigenetic regulation
Single-cell tracking reveals a common “algorithm” of operation used by chromatin regulators

By Albert J. Keung1 and Ahmad S. Khalil2,3

Expression of the genome is controlled by an intricate “web” of proteins, chemical modifications, and RNA that together organize genomic DNA into chromatin. Molecular studies of the various forms and levels of chromatin organization are advancing rapidly, revealing an increasing number of connections between chromatin and cellular and disease processes, as well as a fast-expanding web of known chromatin factors (1). On page 720, Bintu et al. take a radically different approach to dissecting chromatin, focusing not on molecular but on “algorithmic” chromatin biology (2). By studying how individual chromatin regulators (CRs) operate to produce distinct gene expression outputs within individual cells, they find that chromatin’s complexity can be reduced into an elegant and unifying model of gene regulation.

In eukaryotic organisms, gene expression states are established in part by a system of CRs that act on chromatin in diverse ways (3). The principal questions posed by Bintu et al. concern how these CRs modulate gene expression, how permanent these expression changes are, and how the CRs’ “operations” relate to one another. To study this, the authors developed a framework for quantitatively interrogating the input-output relationship between the presence (or absence) of a CR and gene expression in live mammalian cells. The framework combines artificial recruitment of CRs to a target reporter locus with precise temporal control—an increasingly popular strategy for focusing the activity of CRs (4–8)—with time-lapse microscopy to track reporter activity in individual cells.

The authors used this framework to compare four repressive CRs that use distinct chromatin modifications. The embryonic ectoderm development (EED) protein of Polycomb repressive complex 2 catalyzes histone H3 lysine 27 (H3K27) methylation. The Krüppel associated box (KRAB) protein promotes H3K9 methylation. DNA methyltransferase 3b (DNMT3B) catalyzes DNA methylation. The histone deacetylase 4 (HDAC4) enzyme directs histone deacetylation. Previous work has shown that different types of repressed chromatin are generally associated with distinct time scales of repression. For example, DNA methylation is widely associated with heritable gene repression (9), whereas histone acetylation is typically transient (10). However, by comparing these distinct CRs side by side, Bintu et al. were able to bring them under a unified quantitative model.

What is key to the authors’ approach are the longitudinal measurements they make of single cells via time-lapse microscopy. In this way, they could track not only changes in reporter protein levels in individual cells, but also changes in protein production rate during and after recruitment of the CRs to chromatin. This approach revealed that all the CRs silence the reporter gene in all-or-none fashion, as characterized by an abrupt change in protein production rate in individual cells. Moreover, when the CRs are released from chromatin, reactivation of the reporter gene also occurs in all-or-none fashion. This leads to a general and simplified model in which cells are stochastically switching between active and silent states, rather than transitioning gradually through different activity levels.

The key distinction among the four CRs is how quickly a proportion of the cells in a population transition to the silent state. To complete the model, the authors extended their tracking of silent cells over longer times to quantify the heritable stability of the silent state. This revealed a third state, ultimately yielding a model in which cells can stochastically transition between active, reversibly silent, and irreversibly silent states. Consistent
with other studies (5), the proportion of cells entering the final, irreversibly silent state is controlled by varying the duration of time that the CR is recruited to the gene. From conceptually simple but technically exquisite experiments, the authors have produced an elegant model for capturing the dynamics of epigenetic regulation. What is compelling about this work is the minimalism of the three-state model, and the fact that CRs with diverse molecular identities and mechanisms can be collapsed into it. Dynamic control of fractions of a population would seem to be an effective way of transmitting and recording environmental signals, and suggests distinct advantages of CRs over canonical transcriptional networks. It is intriguing to consider how cells potentially exploit this rate control mechanism to link specific CRs and associated chromatin modifications with specific cellular processes, such as selectively turning off genes in the right cells during development. Additionally, we know that CRs and modifications rarely exist or act in isolation (17). Thus, it will be interesting to see how this rate control framework extends to combinatorial contexts.

The simplicity of the model is less suited for problems involving a higher level of biochemical detail, given that there is little direct connection to molecular mechanisms. Connecting aspects of the model to the wealth of existing chromatin data (derived from chromatin immunoprecipitation sequencing (ChIP-seq), biochemistry, etc.) will represent an exciting area of investigation. Yet this work is a critical step in our efforts to find logic within the regulatory complexity of chromatin. Distilling complex processes into phenomenological models has been historically central to our understanding of biology. For example, simplified descriptions such as the FitzHugh-Nagumo model have been used for decades to describe the behavior of neurons without full molecular details (12). Finally, this work nicely illustrates the importance of synthetic approaches for providing a functional view of biology, in this case revealing CRs as sophisticated “devices” inherently capable of controlling the time scale and epigenetic memory of gene expression.

REFERENCES
5. N. A. Hathaway et al., Cell 149, 1847 (2012).

10.1126/science.aaf1647

INFLAMMATION

Modulating pulmonary inflammation

Neuroepithelial cells suppress pulmonary inflammation and alveolar remodeling

By Jeffrey A. Whitsett* and Edward E. Morrissey*

The human respiratory tract transports millions of liters of gases throughout life. Because the conducting airways are exposed to countless microbes, particles, and toxicants, the tract has evolved an immune system that protects lung structure and function (1). Ventilation is primarily controlled by neuromuscular activity in the diaphragm and other muscles, and by sensory inputs from relatively rare pulmonary neuroepithelial cells. These cells cluster and form neuroepithelial bodies (NEBs) at branch points along the lung’s airways. On page 707 of this issue, Branchfield et al. (2) reveal how NEBs arise during lung morphogenesis and clarify how their role in inflammation and tissue remodeling is relevant to the pathogenesis of chronic lung diseases that affect children.

The ability to sense, interpret, and integrate complex environmental stimuli debated as individual neuroepithelial cells and evolved into the complex nervous systems typical of vertebrates. Ancient neuroepithelial sensors, such as those in mollusks and insects, are found in cellular niches that direct the homing of hemocytes and immuneocytes to recognize and engulf pathogens (3). Phylogenetic remnants of these ancient sensors remain in vertebrates, represented by neuroepithelial plexuses in the lung. The findings of Branchfield et al. and of other recent studies by Kuo et al. (4) and Noguchi et al. (5) provide new insights into the mechanisms by which neuroepithelial cells migrate and cluster in the mammalian lung to form highly innervated NEB plexuses. These NEBs sense metabolic and other environmental signals, which they transmit to the central nervous system via postganglionic parasympathetic neurons and the vagus nerve (6).

Neuroepithelial cells constitute less than 1% of the airway epithelium. They exhibit ultrastructural and cellular characteristics of neural cells, and they release a unique set of neuroendocrine peptides and bioactive compounds, such as calcitonin gene–related peptide (CGRP), bombesin-gastrin releasing peptide, and 5-hydroxytryptamine (serotonin), with a range of functions including control of vascular tone and permeability. They are also innervated by a rich network of neural fibers (6). Despite their rarity, neuroepithelial cells have been implicated in several important roles in the postnatal lung. Pediatric patients with chronic lung diseases, including cystic fibrosis and bronchopulmonary dysplasia, share alterations in the number or localization of pulmonary neuroepithelial cells, along with deregulation of $O_2$ and $CO_2$ sensing and ventilation; such alterations have also been observed in cases of sudden infant death. Secretory cells that contribute to regeneration of the airway epithelium after injury reside in close proximity to NEBs (7). However, lineage tracing studies indicate that neuroepithelial cells do not contribute to this process (8). Thus, despite clinical and experimental insights, the function of neuroepithelial cells in the lung has remained a long-standing mystery.

Branchfield et al. demonstrate that, similar to the migration of neurons in the central nervous system, the membrane protein Roundabout (Robo) and its ligands from the Slit family of secreted proteins direct the migration of pulmonary neuroepithelial cells to airway branch points. The authors found that Slit1 and Slit2 are released by a subset of neuroepithelial cells within clusters in the mouse lung; Robo expression appears restricted to rare epithelial cells in the airways that express CGRP. The authors also observed Slit3 expression in the vascular smooth muscle of pulmonary a-