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Regulated Noise in the Epigenetic Landscape of Development and Disease

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In this Perspective, we synthesize past and present observations in the field of epigenetics to propose a model in which the epigenome can modulate cellular plasticity in development and disease by regulating the effects of noise. In this model, the epigenome facilitates phase transitions in development and reprogramming and mediates canalization, or the ability to produce a consistent phenotypic outcome despite being challenged by variable conditions, during cell fate commitment. After grounding our argument in a discussion of stochastic noise and nongenetic heterogeneity, we explore the hypothesis that distinct chromatin domains, which are known to be dysregulated in disease and remodeled during development, might underlie cellular plasticity more generally. We then present a modern portrayal of Waddington's epigenetic landscape through a mathematical formalism. We speculate that this new framework might impact how we approach disease mechanisms. In particular, it may help to explain the observation that the variability of DNA methylation and gene expression are increased in cancer, thus contributing to tumor cell heterogeneity.

Normal development and its aberrant regulation in common disease involve changes in cellular plasticity. For example, excessive plasticity in cancer makes it difficult to maintain a normal transcriptional program and cellular phenotype. It is well known that chromatin structure and nuclear organization play critical roles in regulating when and where genes are expressed during cell fate determination and normal or abnormal cell function (Cremer et al., 2006; Schneider and Grosschedl, 2007). Here, we explore how classical frameworks and recent experimental data suggest that epigenetic modifications and nuclear architecture also regulate cell plasticity through the modulation of the effects of stochastic noise.

We start by providing an overview of phenotypic plasticity and then stochastic noise, highlighting their links to epigenetic mechanisms. Next, we describe some recent results in both developmental and disease models that connect nuclear architecture, epigenetic structures, and stochastic noise, leading us to propose a new model for how cells could modulate the effects of noise in response to signaling to regulate cellular phenotypic plasticity. We discuss the possibility of formalizing this model with a mathematical framework that has been used to study physicochemical systems undergoing noise-induced phase transitions. Ultimately, this enabled us to propose a modern take on the classical Waddington landscape. It is not our intention to marginalize other well-established mechanisms, such as gene regulatory networks, but rather to put forward a new and unconventional idea that we hope will spur discussion in the field: that the epigenome can modulate the effects of stochastic noise to facilitate phase transitions in development and disease.

Phenotypic Plasticity and Epigenetics

Historically, the classical paradigms of epigenetics have been recognized by their particular phenotypes, such as gene silencing, but they can also be viewed as models of variation. Indeed, the important role that chromatin structure plays in driving phenotypic variation was evident in the earliest genetic studies of position effect variegation (PEV) (Girton and Johansen, 2008). First described in the 1930s in *Drosophila*, PEV was observed in the context of X-ray-induced chromosome rearrangements, when a translocation would bring a locus, such as the *white* gene, from a euchromatic region into repressive centromeric heterochromatin or, more generally speaking, near a euchromatin-heterochromatin junction. The resulting "mottled" phenotype was manifested by eyes with both red and white patches because the *white* gene was repressed in some cells, but not in others. This phenomenon is usually viewed as an example of epigenetic silencing. However, what is most striking about this model is that the variation in silencing is itself titrated by proximity to the point of heterochromatin spreading, such that the strength of the effect is generally inversely correlated to the distance from the breakpoint. Variegation can be further modified by mutations described as enhancers (E(var)) or suppressors (Su(var)) of variegation, which include histone-modifying enzymes and even structural components of the nucleus, such as lamins (Bao et al., 2007; Ebert et al., 2004, 2006). Variegation, from the Latin *varius egare*, meaning literally "variable driving," is thus understood to result from variability of the distance of chromatin spreading along a chromosome.

Two other concepts important to our discussion of epigenetically regulated stochasticity are developmental plasticity and

canalization. Developmental plasticity refers to the fact that a single genotype can result in distinct phenotypes when found in different environments. Canalization describes the ability of development to produce a consistent phenotypic outcome despite being challenged by variable conditions. Waddington, who coined this term, used it interchangeably with the term buffering. He argued that "the genotype can, as it were, absorb a certain amount of its own variation without exhibiting any alteration in development" (Waddington, 1942). He further proposed that mutants are significantly more variable than wild-type organisms, to the point that abnormal types of tissues may arise in pathological conditions. Waddington later predicted that it should be possible to genetically alter the degree of flexibility of the buffering (Waddington, 1959). However, it is important to note that Waddington viewed the epigenetic landscape as controlled (i.e., determined) by genes, with changes arising only through genetic mutation and with new paths carved by the forces of evolution, as illustrated by his later work on genetic assimilation.

An interesting twist to the story of phenotypic plasticity comes from the work of Klaus Gärtner, which demonstrated that progressive inbreeding of animals in a carefully controlled laboratory setting over more than 20 years failed to reduce the variability of quantitative biological traits, such as body weight and fertility in mice and cattle (Gärtner, 1990). Gärtner referred to the source of variation, which was estimated to explain 70%–80% of phenotypic variability, as the "third component"—that is, neither genetics nor the environment.

This notion is illustrated by two outstanding examples of phenotypic plasticity in the context of genetic homogeneity: honeybees and crayfish. (To learn more about this "third component" of variability during reprogramming, see the Perspective by Cherry and Daley on page 1110 of this issue). Social insects in general and honeybees in particular have recently become a model of great interest for studying learning and social interactions. Despite being identical at the DNA level, worker and queen bees differ substantially in their morphology, physiology, behavior, and reproductive potential (Omholt and Amdam, 2004), as well as in gene expression (Barchuk et al., 2007). When DNA methyltransferase Dnmt3 levels were significantly reduced in newly hatched honeybee larvae using RNAi, the relative abundance of queens versus workers reversed. Reduced Dnmt3 levels also changed DNA methylation patterns at promoters of developmentally relevant genes (e.g., dynactin p62) and global gene expression patterns (Kucharski et al., 2008). Marble crayfish are a recently developed model organism consisting of parthenogenetic females that reproduce by apomictic thelytoky, meaning that offspring are generated from unfertilized eggs that have not undergone meiosis. Despite their remarkable genetic stability from generation to generation, marble crayfish display marked variability in their phenotypic traits, including variability in development and growth, life span, reproduction, coloration patterns, and DNA methylation (Vogt et al., 2008).

Other examples of phenotypic differences, despite genetic similarities, include the considerable heterogeneity in allelic silencing in cancer cells (He et al., 1998) and the age-related phenotypic differences that arise in identical twins (Kaminsky et al., 2009). Thus, variability is pervasive in development, and it is intimately linked to epigenetic mechanisms. Furthermore, although the degree of variation can be modified genetically, such as in PEV, variation itself is prominent even in a completely genetically homogeneous background, as illustrated by the above examples on bees and crayfish.

Stochastic Noise, Buffering, and Epigenetic Modulation of Phenotypic Plasticity

Cell individuality was first observed in bacteria in 1976 (Spudich and Koshland, 1976) and has been implicated in generating behavioral variability, as well as determining cell fate, ever since (Korobkova et al., 2004; Maamar et al., 2007; Rao et al., 2002). Many of these observations have also found support in mammalian systems, and there are now many examples of the important role that noise plays in cell fate decisions in organisms ranging from bacteria to humans (Losick and Desplan, 2008). Noise may, in fact, ultimately affect the stability of certain phenotypes, as was recently explored in the context of gene expression noise underlying HIV latency (Miller-Jensen et al., 2011). These authors further indicate that chromatin modifications, which are generally classified as "activating" or "repressive" (i.e., an "on" or "off" switch), might be better conceptualized as altering the rate of transcription between promoter states.

With the progressive inclusion of systems approaches into biology, many questions dealing with the nature of stochastic noise in biological systems are starting to be explored, including how noise gives rise to phenotypic variation (Kaern et al., 2005) and how cells may be able to harness noise for their own benefit (Eldar and Elowitz, 2010; Raj and van Oudenaarden, 2008; Raser and O'Shea, 2005). Stochasticity, referring to the nondeterministic nature of certain dynamic systems, ultimately stems from the fact that biological processes are fundamentally driven by random collisions between small numbers of macromolecules with multiple potential conformational states (Delbrück, 1940; Fedoroff and Fontana, 2002). A few proposed mechanisms for the underlying stochasticity of cellular phenotypes include: "bursting" due to stochastic remodeling (opening and closing) of promoters; variable amplification at the translational level; and influences from upstream components (e.g., morphogens, signaling molecules, and the extracellular matrix), which are often variable across cellular microenvironments and are subject to noise themselves (Eldar and Elowitz, 2010; Kaern et al., 2005). The picture emerging from these recent studies is that noisy systems offer the significant advantage of generating nongenetic cell-to-cell variability—that is, cells that behave uniquely despite being genetically identical to each other. This is not unlike Gärtner's macroscopic observations on the variability of laboratory animals mentioned above.

The question then becomes how to reconcile all of this stochastic noise with developmental robustness, or what Waddington referred to as canalization or buffering. One protein that might be important in both processes is the atypical heat shock protein, Hsp90. Disrupting Hsp90 increases phenotypic variation in most organ systems of the fly (Rutherford and Lindquist, 1998). The specific nature of the phenotypic defects varied depending on the genetic background, leading the authors to postulate that they were seeing previously buffered, cryptic

genetic variation. Later studies have explored the idea that Hsp90's role might be related to variable penetrance rather than, or in addition to, modulation of cryptic genetic variation and that Hsp90 may also play an important role during development in mediating the interaction between genotype and phenotype (Yeyati et al., 2007; Burga et al., 2011). Indeed, inhibiting Hsp90 at low doses (i.e., below the threshold for inducing the unfolded protein response) modulated the penetrance of embryonic malformations in zebrafish either up or down (Yeyati et al., 2007).

Furthermore, studies in *Drosophila* suggested that buffering occurs in isogenic backgrounds as well and is mediated, at least in part, by the epigenetic machinery. Mutations in genes encoding chromatin-remodeling enzymes and treatment with HDAC inhibitors could cause similar abnormal eye phenotypes as Hsp90 mutations (Sollars et al., 2003). Importantly, a direct molecular interaction between Hsp90 and Trithorax has been demonstrated, and this interaction can affect homeotic gene expression in *Drosophila*. Similar effects were observed with Trithorax's mammalian ortholog mixed-lineage leukemia (MLL) (Tariq et al., 2009). Thus, the important buffering factor Hsp90 acts at least in part through epigenetic mechanisms. An additional example of this comes from studies of inbred mice heterozygous for a null mutation in either Dnmt3a or the modifier of epigenetic reprogramming Trim28; these animals exhibited greater variance in traits such as body weight and were at greater risk of developing common disorders, including metabolic syndrome (Whitelaw et al., 2010).

Much remains to be discovered about these mechanisms of phenotypic variability, and the answers will likely involve aspects of multiple models. While the relevance of hiding and releasing cryptic genetic variation is certainly key when discussing evolution (the field has indeed been focused on the importance of generating variability for natural selection to act upon), when dealing with development and disease in an individual organism, the relevant measure is no longer variability for the sake of natural selection but, rather, for modulation of cellular plasticity. We suggest that phenotypic plasticity and buffering/canalization are two sides of the same coin; in other words, they are generally opposing processes that share underlying, epigenetically mediated and developmentally modulated mechanisms. The next section will develop this idea in greater detail by describing data from examples in development and cancer.

Epigenetic Stochasticity in Development and Cancer

When discussing the role of epigenetic mechanisms in cancer, one immediately considers abnormal methylation resulting in the inappropriate regulation of oncogenes and tumor suppressor genes. We propose that the epigenetic regulation of the effects of stochastic noise, although certainly compatible with this idea, is an example of epigenetic mechanisms driving oncogenic transformation in a way that goes beyond the classical genetic paradigms of gene silencing and activation. The preliminary details of our model were significantly shaped by two different sets of studies from our laboratory: one dealing with genome-wide methylation in cancer and the other with epigenome reprogramming during epithelial-to-mesenchymal transition (EMT). Both of these data seemed to bring us to a common conclusion: that the same well-defined chromatin compartments that are characterized by altered epigenetic variability in disease can also get reprogrammed during developmental cell fate transitions. Another important lesson learned from these studies is the growing role of variation as a new dimension in epigenetics that may contribute to addressing cellular plasticity in normal and pathological states.

Previously, we had found that the genome contains differentially methylated regions (DMRs) that can distinguish tissue types from each other and from cancer and that these DMRs appear to be involved in the reprogramming of induced pluripotent stem cells (Doi et al., 2009; Irizarry et al., 2009). Following up on these observations, we recently used a semiquantitative custom methylation array to analyze 151 DMRs in 290 samples, including matched normal and cancer samples from colon, breast, lung, thyroid, and Wilms tumor (Hansen et al., 2011). The variability of DNA methylation at a specific DMR was far greater across a given type of cancer than the variability of the same DMR across the matched normal tissues from the same patients. This increased *variability* in cancer compared to normal tissues was true for all cancers tested and was strikingly higher than the mean differences in DNA methylation between cancer and normal, which is conventionally examined (Hansen et al., 2011). These hypervariable DMRs in cancer also appear to be important in normal cellular differentiation during development because the top 25 most variable loci in cancer could distinguish the five normal types of tissues from each other. These data suggest a biological relationship between normal tissue differentiation and stochastic variation in cancer and that cancer at the epigenetic level may be a disease of generalized dysregulation of the effects of stochastic noise.

We then performed whole-genome bisulfite sequencing of a subset of the samples to address what this loss of epigenetic integrity may look like genome-wide. We found loss of methylation stability in colon cancer involving CpG islands, shores, and large hypomethylated blocks (up to several Mb). These blocks, which mapped to more than half of the genome, had consistent boundaries. Importantly, they largely overlapped with large organized chromatin histone H3 lysine (K)-9 modifications (LOCKs) and regions corresponding to domains associated with the nuclear lamina (LADs) (Figure 1C). In addition, these hypomethylated blocks in cancer were enriched for genes that showed increased variability in gene expression in cancer samples, including functional categories such as mitosis, cell-cycle regulation, and matrix remodeling.

The second study from our group explored EMT induced by the transforming growth factor β (TGF-β) in AML12 mouse hepatocytes (McDonald et al., 2011). EMT is a developmental biology paradigm for studying reprogramming, injury repair, and tumor progression and metastasis. Indeed, EMT involves extreme cell plasticity and reversible changes in cell type (Kalluri and Weinberg, 2009; Thiery et al., 2009). Cells undergoing EMT develop stem-cell traits (Mani et al., 2008), and similar processes are critical during the reprogramming of induced pluripotent stem cells (Li et al., 2010; Polo and Hochedlinger, 2010; Samavarchi-Tehrani et al., 2010). Thus, EMT-like changes may be a general mechanism for increasing plasticity during cell fate transitions, regardless of the direction. In our mouse hepatocyte model, the cells undergoing EMT have enlarged nuclei that are

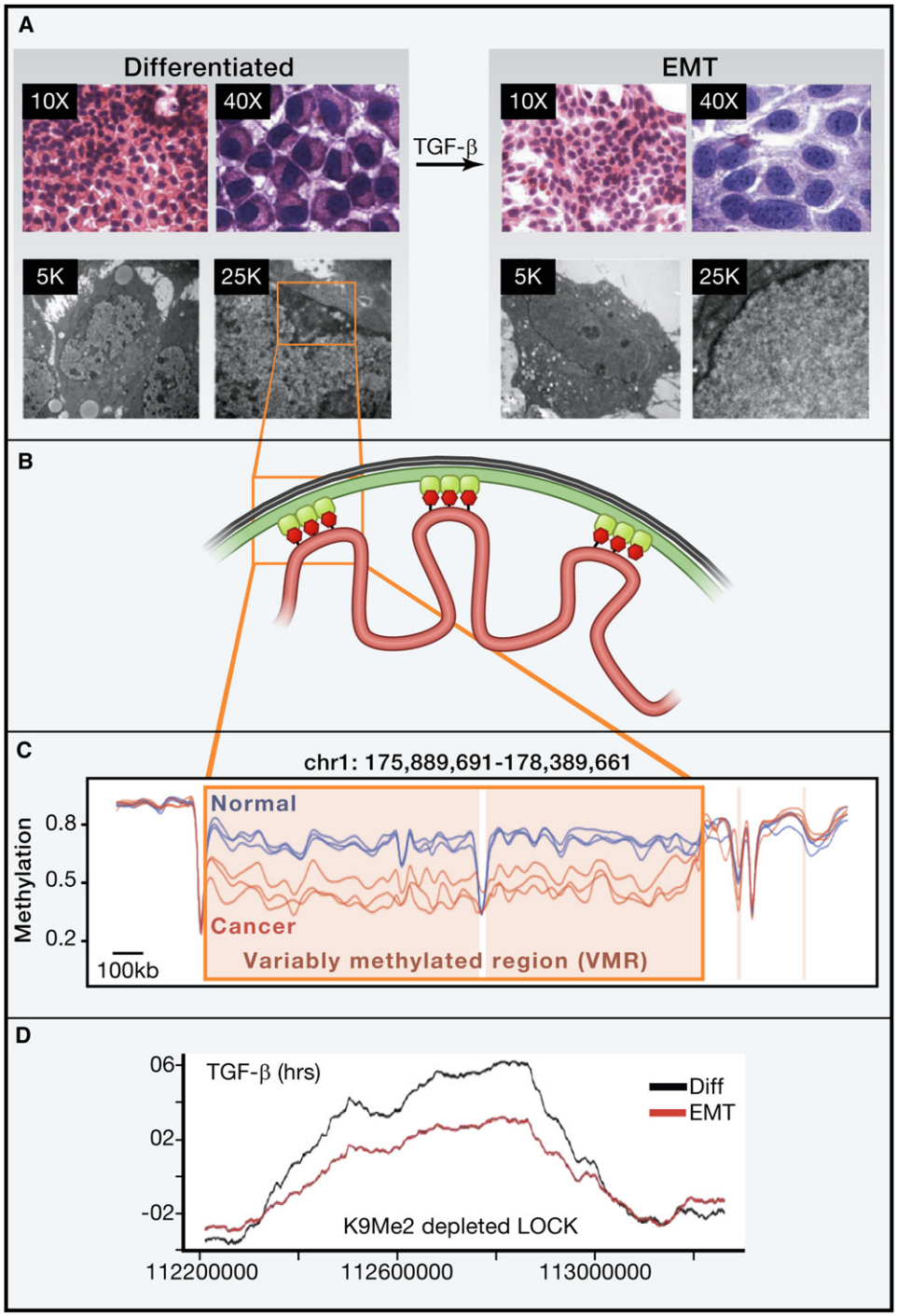

Figure 1. VMRs in Cancer Are LOCKs in Developmental Reprogramming

(A–D) Large variably methylated regions (VMRs) in cancer (C) were identified by whole-genome bisulfite sequencing. These VMRs are hypomethylated in cancer and largely correspond to nuclear lamina-associated large organized chromatin lysine (K)-modifications (LOCKs), which can be visualized by electron microscopy (A, bottom) and native chromatin immunoprecipitation (D). The same well-defined chromatin compartments that are reprogrammed during developmental cell fate transitions display altered variance across many types of cancer (B and C). Images reprinted with permission from Hansen et al. (2011) and McDonald et al. (2011).

hypochromatic (i.e., they are paler under the microscope when stained with hematoxylin and eosin) and have an altered shape (Figure 1A, top). At a molecular level, bulk levels of nuclear histone H3 lysine-9 dimethylation (H3K9me2), corresponding to partial loss of LOCKs, are reversibly reduced by TGF-β (Figure 1D). Loss of nuclear lamina-associated chromatin during EMT

was also demonstrated directly by transmission electron microscopy, suggesting a role of nuclear structure in reversible differentiation plasticity during EMT (Figure 1A, bottom).

What ties the two studies together is that the hypomethylated blocks in cancer correspond in their location to heterochromatin LOCKs (Figure 1B). Furthermore, the genes within the hypomethylated blocks were the most variably expressed genes in cancer (Hansen et al., 2011), and reversible loss of LOCKs during EMT led to gene activation at the LOCK boundaries. Although gene expression variance was not examined directly in that study (McDonald et al., 2011), we predict that future experiments in this system will demonstrate increased gene expression variability upon TGF-β treatment. Other studies in the literature support this prediction. For example, TGF-β has been shown to increase expression variability of reference genes in a renal fibrosis primary culture system (Elberg et al., 2006). More recently, Mukasa and colleagues demonstrated extensive remodeling of the *interferon-gamma* (*Ifng*) and *Interleukin 17* (*Il17*) loci in response to TGF-β and other signals. Their data suggested that the chromatin structure at these loci is unstable, rendering the loci more sensitive to rapid changes in cell-extrinsic factors, such as TGF-β, that are critical in cellular plasticity (Mukasa et al., 2010).

Taken together, these experiments demonstrate the plastic nature of cell fate in both developmental and disease models. These experiments also highlight the shared epigenetic mechanisms underlying this plasticity. This brings us back to the initial observation: that well-defined chromatin compartments displaying altered variability are reprogrammed during developmental cell fate transitions. Although this observation could be extrapolated to many disease models, we propose that an appropriate place to start is a discussion of its relevance in the current cancer stem cell debate. We think that this is particularly relevant, given the current reformulation of the classical hierarchical and unidirectional model of cancer stem cells in favor of the dynamic regulation and phenotypic plasticity that allows cancer cells to reversibly switch into and out of stem cell states (Scheel and Weinberg, 2011). Recent studies point to dynamic chromatin modifications as an independent route to reversing drug resistance in cancer cells enriched for stem cell markers (Sharma et al., 2010). When Sharma and colleagues treated a non-small cell lung cancer line with lethal doses of erlotinib, they observed a persistence of transiently drug-tolerant cells. Interestingly, the drug-tolerant phenotype, which was associated with stem cell markers, was reversibly gained and lost by individual cells at a low frequency. The phenotype also appeared to be linked to alterations in global chromatin, as measured by nonrandom distribution of differentially expressed genes along the chromosome. Furthermore, knockdown of histone demethylase KDM5A interfered with the cells' ability to survive the drug treatment. HDAC inhibitors selectively killed drug-tolerant cells through a mechanism that appears to involve signaling from IGF-1 receptor and downstream chromatin modifications mediated by KDM5A, implicating the epigenetic machinery in the dynamic regulation of phenotypic heterogeneity in drug tolerance.

This idea could be extended to other disease models, such as aging, neuropsychiatric diseases, and metabolic disorders; for instance, to help explain the buffering mediating the incomplete penetrance of mutations (Burga et al., 2011; Raj et al., 2010), the increased variability (Bahar et al., 2006; Southworth et al., 2009; Thompson et al., 2010) and decreased plasticity seen in aging (Peleg et al., 2010), or neuronal plasticity (Nott et al., 2008; Riccio, 2010). Epigenetic mechanisms underlying metabolic disturbances are also growing in importance (Pirola et al., 2010; Tateishi et al., 2009; Vaquero and Reinberg, 2009; Villeneuve et al., 2008). Indeed, Mar et al. recently described changes in the variance of gene expression per se in neural stem cells derived from the brains of patients with Parkinson's disease or schizophrenia (Mar et al., 2011).

A Reformulation of the Waddington Landscape

In 1953, Conrad Waddington offered a metaphor for biological development in which the forces driving a cell toward its ultimate cell fate were like gravitational forces propelling a ball to roll down to a local point of minimum elevation (Figure 2, left). Waddington's often-quoted epigenetic landscape has become a common metaphor used in the field of epigenetics to articulate new insights into the nature of the phenomena that we study. Though much of its success as a metaphor has relied on being necessarily indeterminate at the molecular level, it remains a powerful communication tool that easily resonates with people's intuition. We would thus like to start this section by illustrating our model through a comparison to Waddington's landscape. We then introduce an adaptation of an existing mathematical formalism employed in the study of physicochemical systems to explore noise-induced phase transitions of cells. We consider the adaptation of this mathematical formalism to the topic at hand our attempt to update Waddington's landscape.

Waddington included valleys and hills in his landscape to represent canalization (or buffering); that is, terms describing how cells ultimately end up as one of several discrete cell types, despite environmental perturbation. What some representations of his drawing omit is that Waddington also drew under the landscape a set of pegs and guy rope portraying the influence of genes acting in a static way through symbolic pulleys (Figure 2, left). He would later use this landscape again to illustrate ideas regarding genetic assimilation, which would result in small, incremental changes in the landscape arising by mutation over generations (but not necessarily during development).

In contrast, we suggest that the degree to which the epigenome buffers stochastic noise is itself developmentally regulated. In mathematical terms, this means that noise is not just a constant term to add to the equation but is itself a function of the developmental landscape. When this important yet seemingly small mathematical detail is taken into account, we find that the contour of the hill also changes as the ball rolls down, which is explained more formally below. This dependence happens at two levels. First, pluripotent cells will generally be noisier than more differentiated cells; thus, pluripotent cells would have a landscape that is more flexible and easily changed compared to that of more differentiated cells. Second, noise should be highest during cell fate transitions in development. Having said that, we do not expect noise to be homogeneous across the genome, which will make data interpretation and cell type comparisons rather tricky.

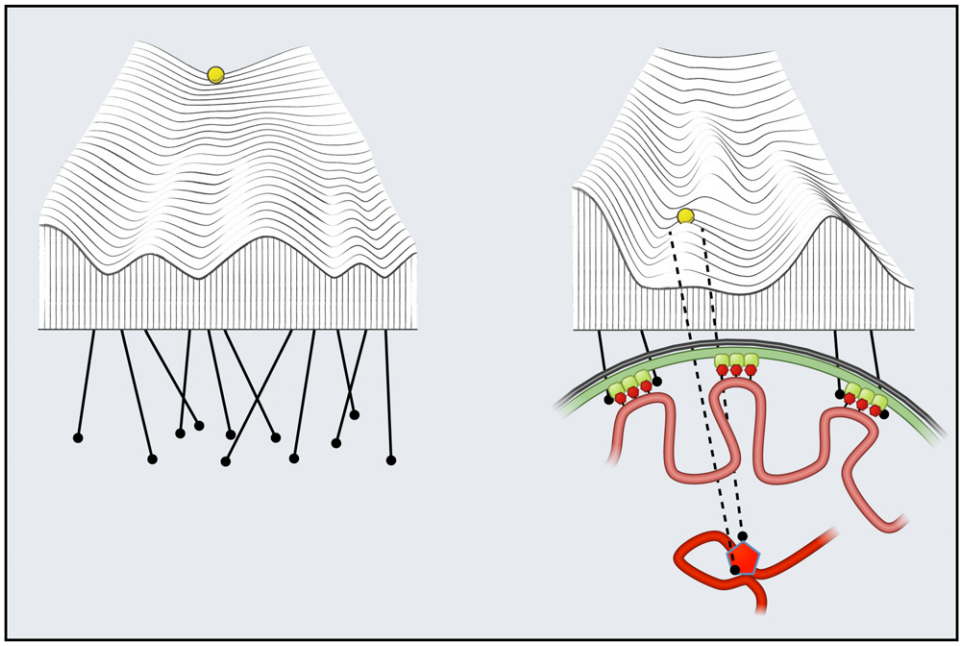

Figure 2. Regulated Noise in a Dynamic Epigenetic Landscape
On the left is a depiction of the classical Waddington representation of canalization, in which the ball rolling down the hill is directed into one of multiple valleys as a consistent endpoint, despite perturbation that might occur on the way. Waddington suggested a deterministic model with genes (small black circles below) pulling on the landscape from below to direct these endpoints. Changes in the landscape would arise by mutations in the genes. On the right, we suggest that modulation of the effects of noise is regulated during development and in response to external cues, which affects the contour of the epigenetic landscape itself. During differentiation, as the ball rolls down the hill, nuclear structure changes in a metastable manner through, for example, structures such as LOCKs and methylated blocks, thus changing the steepness of the valleys. At the same time, new chromosomal interactions could increase localized variability in ways that were not possible at the ground state—in this case, changing the landscape to open an alternative pathway to diversity (new bifurcation shown below the ball). The other shapes represent chromatin modifications (red circles), lamin proteins (green), and chromosome interactome mediators (red pentagon).

Thus, depending on which way the ball happened to roll (i.e., depending on the cell's particular developmental history and current signaling events), the cell's fate and its opportunities for reprogramming would change. Similarly, the cell could affect the landscape of other cells that are fellow travelers by its own influence on the environment within the developing tissue through such mechanisms as nonautonomous cell signaling. Note that it was recently shown that increasing signaling variance in a cell population increases (group) information transduction capacity as much as 4-fold during tumor necrosis factor signaling (Cheong et al., 2011). What we are describing can be seen, in cartoon form, as a revision of Waddington's landscape (Figure 2, right); here, the changing depth of the hills and valleys are governed, in part, by changes in nuclear structure, which could include LADs, LOCKs, hypomethylated blocks, and 3D structural variations of the nuclear lamina. Such structures are continually responding to cues and signals, both intra- and extracellular.

We call attention to the fact that neither we nor Waddington intend for the rolling ball analogy to represent an inexorable pathway from stem cell to end stage differentiation. Waddington himself says, anticipating our own travails as biologists in constructing our model, "A multidimensional phase space is not very easy for the simple-minded biologist to imagine or to think about," (Waddington, 1957), but he is interested in "the course by which [developmental change] gets there," as are we. We are proposing that the epigenome contributes not only to the mean levels of gene expression (as others have discussed), but also to altering variability and how it is affected by stochastic noise; thus, the epigenome facilitates noise-induced phase transitions and the promotion or resolution of pluripotency.

Waddington represented his idea as a system of ordinary differential equations. In reading his "The Strategy of the Genes," one can appreciate that these mathematical constructs were important to how Waddington conceptualized embryology and development, with numerous mentions of phase spaces and steady states. We thus find it appropriate to attempt to extend the mathematical formalism as we attempt to extend the proposed biological mechanisms underpinning the landscape.

We can adopt mathematical language from modern statistical mechanics. The idea that phase transitions (such as states of matter, oscillating chemical reactions, and optical bistability) can be induced by noise has been used in the study of many physical processes, which can be modeled by a stochastic differential equation of the following form:

$$dX_t = f(X_t)dt + \sigma g(X_t)dW_t$$

in which W_t describes a Wiener process, or continuous stochastic process. The transitions between the available states occur across the overall space described by the coordinate X, and X_t reflects a developmental state that may be affected by environmental signals. Thus, the change in state depends on

a deterministic Waddingtonian function $f(X_t)$, e.g., a gene regulatory network modeled by a set of differential equations, and a function $g(X_t)$ that acts stochastically, e.g., in response to signaling that alters the effects of noise through an epigenetic mechanism, such as reduction of LOCKs in EMT. This would be equivalent to reducing the amount of buffering provided by the epigenome. As elegantly reviewed in the mathematical text by Horsthemke and Lefever (Horsthemke and Lefever, 2006), this equation, which is not solvable in a straightforward way, can instead be represented as a "probabilistic" potential of the system through the following expression:

$$p_s(x) = N \exp\left[\frac{-2\tilde{V}(x)}{\sigma^2}\right]$$

in which $p_s(x)$ is the stationary probability density, and the probabilistic potential $\tilde{V}$ is defined as:

$$\tilde{V}(x) = -\left[\int^{x} \frac{f(z)}{g^2(z)} dz - \nu \frac{\sigma^2}{2} \ln g(x)\right]$$

in which ν serves to distinguish between interpretations by Stratonovic ($\nu = 1$) and Ito ($\nu = 2$) (independent derivations by a physicist and mathematician, respectively) and N is a normalizing factor. The maxima of the potential correspond to the peaks and plateaus of the system (and thus minima of p_s) and the minima of the potential correspond to the valleys of the system.

The model that we are proposing argues that g (the stochastic part of the function) is itself a function of X_t; that is, it is regulated by the developmental state of the cell, and it also depends on the cell's microenvironment when it is in that particular developmental state. This means that the epigenome can developmentally regulate the degree to which external (environmental) noise can influence its own landscape and can explain in a theoretical way the high frequency of transitions arising normally during development and disease. A constant or additive external white noise would have only a disorganizing effect on the potential landscape (Horsthemke and Lefever, 2006). The inclusion of noise as a function of developmental state and signaling microenvironment will alter the contour of the landscape itself.

Some Predictions and Implications of the New Model

Our model predicts that pluripotent cells would have a relatively high stochastic variation across the genome as compared to differentiated cells. As cells respond to differentiation agents and environmental cues and start to commit to a given lineage, stochastic noise would decrease in order to allow for the establishment of a stable transcriptional program and the maintenance of a developmental pathway (canalization). In addition, highest noise would be found during developmental phase transitions. In other words, whenever a progenitor cell is faced with the need to destabilize its own transcriptional program so that the daughter cell's transcriptional program may emerge, or when a cell is reprogrammed (e.g., in response to an EMT-inducing external signal), we would expect increased stochastic variation (plasticity). The folding and unfolding of these large 3D chromatin structures (e.g., LOCKs, chromosomal interactions) could potentially constitute a slow step that would provide a basis for metastable states in differentiation and reprogramming. Such a metastable state could help to explain reversible and plastic interconversion between different cell states in genetically homogeneous populations of cells, such as those observed by Sharma et al. (2010). However, we do not expect variability to be homogeneous across the genome or different regions to change in the same direction. Thus, special attention will have to be paid to comparing appropriately across cell types, experimental conditions, and genomic regions.

An appealing mechanism for developmentally regulated epigenetic plasticity is DNA methylation. We recently reported the existence of "variably methylated regions" (VMRs), defined as regions in which DNA methylation varies stochastically across the population, even within the same tissue and even in isogenic mice (Feinberg and Irizarry, 2010). Thus, VMRs correspond to regions of stochastic variation. Surprisingly, these VMRs are found at key loci for development, such as axial pattern formation, neurogenesis, development of the immune system, and development of the gut (Feinberg and Irizarry, 2010). VMR DNA sequences might themselves be subjected to natural selection because specific VMRs present in humans but not mice can be distinguished by differences in moderate CpG-density shores near high CpG-density islands (Feinberg and Irizarry, 2010). Recently, it was found that CpG density and transcription factor binding sites have a major impact on the consistency of methylation of a defined CpG island (Lienert et al., 2011). Thus, small DNA sequences near CpG islands could have a major effect on intercellular variability of DNA methylation and gene expression. Consistent with this idea, dietary exposure to food rich in methyl donors during pregnancy leads to increased gene expression variance in the offspring at some sequences in the genome and not in others. This is also consistent with the idea that the environment may act at specific loci susceptible to normal epigenetic variation (Li et al., 2011). Furthermore, enzymes were recently discovered that cause oxidation and deamination of methylcytosine (Branco et al., 2011). These include the methylcytosine dioxygenase Tet1 and cytidine deaminase AID, which together could provide a potential basis for the destabilization of methylation under physiological or pathological circumstances. Indeed, hydroxymethylation is prominent in the brain, where neurons show global hypomethylation and unexpectedly high interindividual variation (Iwamoto et al., 2011).

By measuring variation in DNA methylation rather than mean values of DNA methylation, we were recently able to distinguish cancer from noncancer across a broad range of tumor types (Hansen et al., 2011). Profiling with this kind of a tool may allow for earlier risk assessment in personalized medicine by providing more quantitative, higher dimensional data, such as methylation status at multiple VMR loci, compared to simply the presence/absence of a SNP or chromosomal aberration. This type of data could also help to segregate a given patient population into groups based on the underlying disease mechanism, thus personalizing prognostic information and predictions of responses to available therapeutic options.

Potential modulators of epigenetic stochasticity could include chaperones such as Hsp90, a buffer that shows developmental change and variability in expression (Burga et al., 2011). It could

also include epigenetic modifiers, like LSD1, that can alter LOCK/LAD stability in response to TGF-β (McDonald et al., 2011) and KDM5A that alters chromatin in response to IGF1 (Sharma et al., 2010). In addition, signaling can result in protein-protein interactions that change the 3D configuration of loci in the nucleus, increasing intrinsic and extrinsic noise (Cai et al., 2006; McCullagh et al., 2010). For example, overexpression of SATB1, which promotes intrachromosomal interactions, also promotes tumor progression (Han et al., 2008). At a more macroscopic level, dynamic nuclear infoldings induced by neural activity were recently reported that result in small subnuclear compartments with differential ability to resolve induced calcium signals (Wittmann et al., 2009). These compartments would be attractive candidates for nuclear structural features that modulate epigenetic stochasticity. An eventual understanding of how given signaling pathways alter this process will free us from being tied to targeting histone modifying enzymes that, because of their pleiotropism, may cause unwanted side effects. Instead, we could target specific signaling events or binding partners that are more context specific in order to affect the epigenetic landscape. Finally, the possibility of predicting or even manipulating how cells dynamically access reversible cell states would be a powerful therapeutic tool, with applications such as sensitizing cancer cells to chemotherapy or nudging pluripotent cells in a direction of interest in laboratory or clinical tissue engineering applications.

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