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Geometry of the nucleus: a perspective on gene expression regulation

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Gene expression control results from the combined interactions of the nearly hundred proteins forming the pre-initiation complex, thousands of transcription regulators, and genomic DNA. In the recent years, new technologies have revealed several key aspects of nuclear spatial organization that showed a fine interplay between the function of nuclear proteins, their 3D organization, and their dynamics. Here we review several concepts that link biochemical reactivity in the nucleus to its 3D spatial organization. We present the analogies between the emerging understanding of nuclear organization in the field of cell biology, and the more established disciplines of heterogeneous catalysis and the physics of random walks. We provide several recent examples showing how nuclear geometry affects protein reactivity in the nucleus.

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Introduction

Regulation of eukaryotic transcription and control of gene expression are two key questions in today's cellular and molecular biology [1]. The understanding of their physical and chemical principles is essential in many areas of applied science. Clear examples are cancer research, biological engineering, regenerative medicine or pharmacology.

Gene expression is regulated by transcription factors (TFs) interacting at specific *loci* to trigger gene activation. Through this interaction, the assembly of the pre-initiation complex (PIC) at promoters' sites leads to RNA polymerase II (Pol II) engagement in elongation. Our current understanding of this process includes the high mobility of diffusing TFs reaching for specific DNA sequences (referred as target-search) and the combinatorial assembly of the PIC. However, the spatial and geometric constraints that encompass protein–DNA and protein–protein interactions are often overlooked and not properly understood [2]. In addition, all biomolecular processes relevant to gene expression take place in a crowded and complex environment where regulation mechanisms operate at different levels of complexity.

The target-search of TFs in the nucleus is governed by diffusive processes. And while in yeast it has been shown that the search time of upstream TFs determines the gene activation rate [3], pure Brownian diffusion of TFs falls short to fully describe the efficiency and complexity of the gene expression process [4••,5–7]. Gene expression must thus be regulated by several other parameters spanning from exploration of the nuclear space to exploration of the space of protein conformations: variation of global and local concentrations, diversity in the target-search patterns and in space exploration, regulated docking affecting the conformation of both TF and its substrate.

The problems of target-search and reactivity have been formalized in different fields. Since more than a century, chemists have investigated the field of heterogeneous catalysis [8], accounting for diffusion and reaction on surfaces of reduced dimensionality. Likewise, following the seminal work of Pierre-Gilles de Gennes [9,10], physicists have developed formalisms accounting for the diffusivity of molecules in random or disordered systems [11], potentially modifying their reactivity.

In this review we evaluate recent achievements in the understanding of the influence of geometrical factors on the regulation of transcription. We survey and compare the different formalisms used in biology, chemistry and physics in order to draw their similarities and differences. We aim to foster cross-disciplinary interactions among these fields, potentially leading to a more unified usage of these concepts.

Available space in the nucleus

While the mechanisms behind the regulation of gene expression are far from being fully understood, its very first step requires two or more biomolecules to interact at a given moment of time in a given position of the space. In a first approximation to this problem, we can consider the nucleus as a closed container in which a number of reactants diffuse prior to engage in a chemical reaction.

In this idealized system, the kinetics of the reaction can simply be derived from the law of mass action (given that the system were in equilibrium). As such, the reaction rate is proportional to the product of the concentrations of the participating molecules. To evaluate the reaction kinetics when a small number of reactants are involved, as often the case in gene expression [12], the first step is to assess the probability of encounter between reactants. In this scenario, the diffusion properties of the molecules, given by the Einstein–Smoluchowski equation, determine the first-encounter time [12,13].

With such a simplified model of gene expression, it is easy to imagine the role of crowding, molecular exclusion, and local concentration in the kinetics of this process (Figure 1), and by extension in all the biochemistry of the cell. High molecular weight components in the nucleus, such as prominently but not exclusively chromatin, effectively reduce the accessible volume in which TFs are free to diffuse, potentially regulating the process of gene expression. A 'rule of thumb' for the volume of a DNA is 1 nm^3/bp.[1] Thus, neglecting adsorbed water, the volume of human DNA is $\sim 2 \times 3 \times 10^9 = 6 \times 10^9$ nm^3. Similarly, the exclusion volume of nucleosomes can be computed,[2] leading to an estimated volume of chromatin of ~ 25 μm^3, which is a fraction of 12% of the volume of a human nucleus (~ 6 μm diameter[3]). Other estimates (10% in [15], 20–50% in [16]) give similar order of magnitude. In a simple model of first order reaction, such exclusion volume would at most change by a mere factor of two the rate of homogenous biochemical reactions. We must thus take into consideration other characteristics such as the complex geometry of nuclear organization or the heterogeneity of local molecular concentration. The former, as discussed below, renders the calculations of exclusion volume invalid; regarding the latter, many nuclear components do not show a homogeneous spatial distribution in the nucleus [17], and it has been shown that the local concentration of Pol II is regulated, giving rise to significant differences at the local level throughout the nucleoplasm [18].

The complex geometry of the nucleus affects diffusion

An additional layer of complexity can be added to the target-search problem of TFs when taking into consideration the complexity of DNA packing in the nucleus. DNA exhibits a hierarchy of structures that spans from the molecular level up to the size of the nucleus. This not only includes coiling, wrapping, supercoiling, etc. of the DNA polymer but also the non-random organization of the genetic information in the nucleus and the existence of chromosomal territories [1,19–21]. In recent years, growingly solid experimental evidence demonstrates that chromatin exhibits characteristics of a fractal structure [16,22,23] with a measurable fractal dimension (see Table 1, Figure 2 and [24•]), which had been hypothesized almost thirty years ago [25,26].

With these considerations in mind, the question of how much volume is excluded by chromatin becomes crucial. Indeed, fractal objects are characterized by self-similarity across a wide range of scales: a similar spatial pattern can be observed almost unchanged at various magnifications. These fractal objects exhibit interesting mathematical properties. Among those is the fact that a structure of low dimensionality can 'fill' a space of higher dimensionality (for instance, a highly tortuous 1D curve can exhibit space-filling behavior), while having a null volume. These properties can be summarized by computing the so-called fractal dimension, a number that extends the traditional topological dimension (i.e.: 1D, 2D, 3D) to non-integer ones, accounting for such a space-filling behavior. Mathematically, the complementary of a fractal displays the dimensionality of the fractal-embedding space (3D in our case) [27]. A single-point diffusing molecule in the complementary space would therefore display the same characteristics than in a three-dimensional volume. On the other hand, a particle with finite size can have an accessible space that is a fractal.

Even though computing the exclusion volume of a fractal (characterized by its fractal dimension d_f) requires strong assumptions, extensive work in the field of heterogeneous catalysis provides analytical and computational tools to address this question [28–30,11]. Most of the current models in the field take two parameters into account: the fractal scaling regime (δ_{min}, δ_{max}) (i.e. the range of scales where the object can be regarded as fractal) and the size δ of the diffusing molecule. Exclusion volumes and diffusion properties of the molecules can then be derived. Under these assumptions, the available volume A for a diffusing molecule scales as a power of its size ($A \propto \delta^{2-d_f}$ [8]). Thus, the relevant parameter to estimate diffusible space is no longer the volume of nucleus constituents but its fractal dimension d_f.

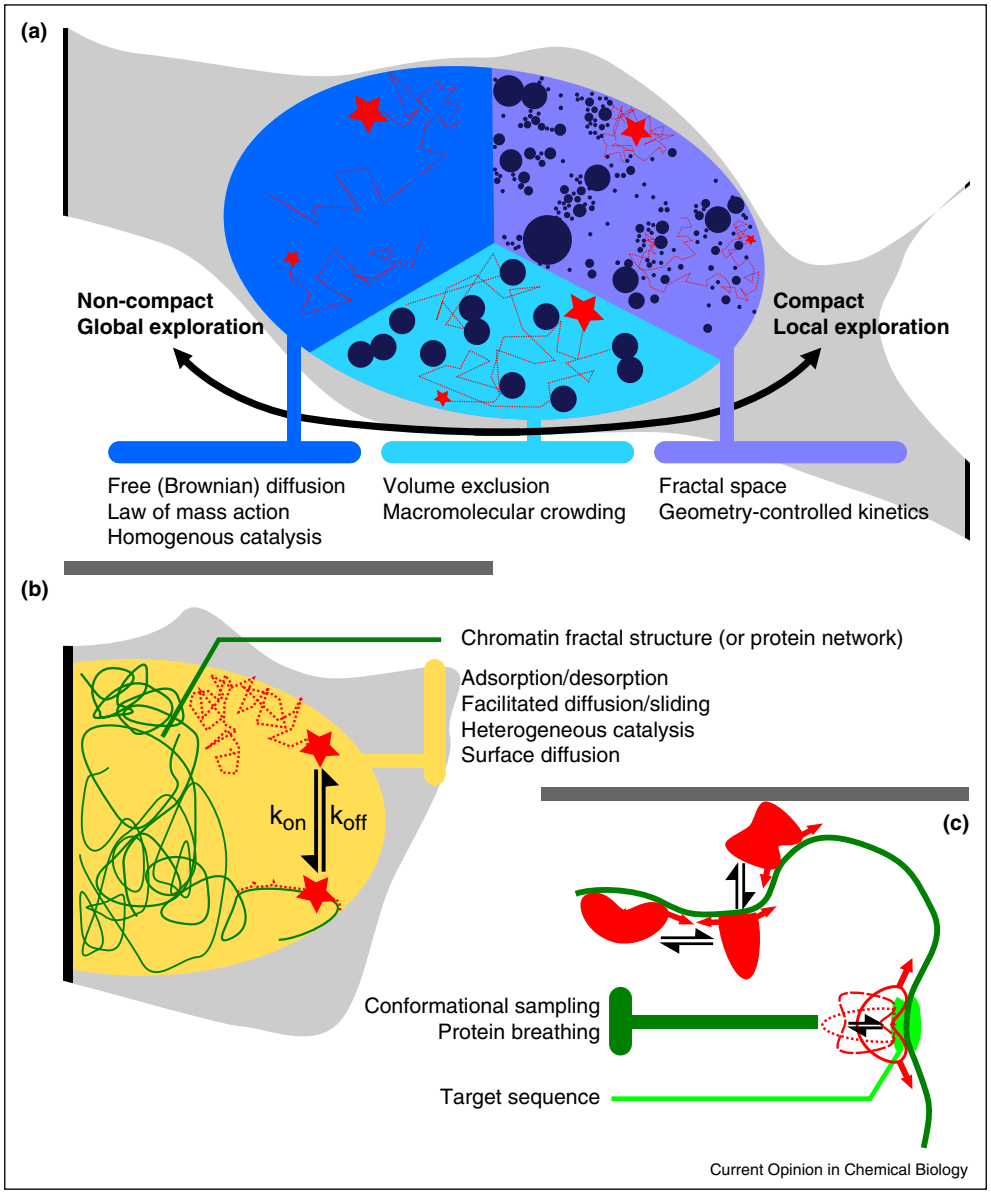
An important question to elucidate is how the fractal structure effectively influences the diffusion of TFs. From a theoretical point of view, diffusion in a fractal structure is characterized by a deviation from the free, Brownian diffusion (Figure 1a, left) to an anomalous, subdiffusive behavior (Figure 1a right), for instance observed by computing the mean square displacement (MSD) on single particle tracking (SPT) experiments (Table 1). In the context of the nucleus, several studies report anomalous diffusion [16,31,32•], thus suggesting a fractal organization of the nucleus as one possible explanatory mechanism.

[1] Bionumbers http://bionumbers.hms.harvard.edu/, accession number: 103778.

[2] Crystal structure of the human nucleosome core, doi:10.2210/pdb2cv5/pdb, NDB ID: PD0676, derived from [14] and Bionumbers, accession numbers: 102977 and 102987.

[3] Bionumbers, accession number: 105995.

Figure 1



TFs exploration patterns in the nucleus are highly diverse. (a) Diffusion of TFs can occur in a space of *reduced volume*. This spans from free diffusion (thus performing a non-compact, global walk) (left), to diffusion in a fractal medium, showing obstacles at all scales and realizing a recurrent, compact walk (right). **(b)** Diffusion of TFs can occur on a space of *reduced dimensionality*, here represented through binding and facilitated diffusion on a nuclear macromolecular network (such as DNA or proteins). TFs oscillate between 3D and less-than-3D diffusion. **(c)** TFs diffuse in the *conformational space*, and sample available conformations, exhibiting 'protein breathing'.

Diffusion of TFs is altered by chemical interactions

Even though diffusion of a TF in the chromatin exclusion volume, a complex, possibly fractal medium, is an accurate representation of the nucleus, target-search models usually consider the fractal chromatin as an inert surface. In this scenario, apparent diffusion coefficients are only determined by the size of the TF (throughout exclusion volume and the scaling of diffusion coefficients with the radius), leaving little room for regulation since TFs exhibit very similar Stokes radii, in the order of a few nanometers. These models are also inconsistent with recent SPT observations, where TFs of comparable sizes show different exploratory behaviors [32•], which cannot be fully accounted for by the fractal organization described above.

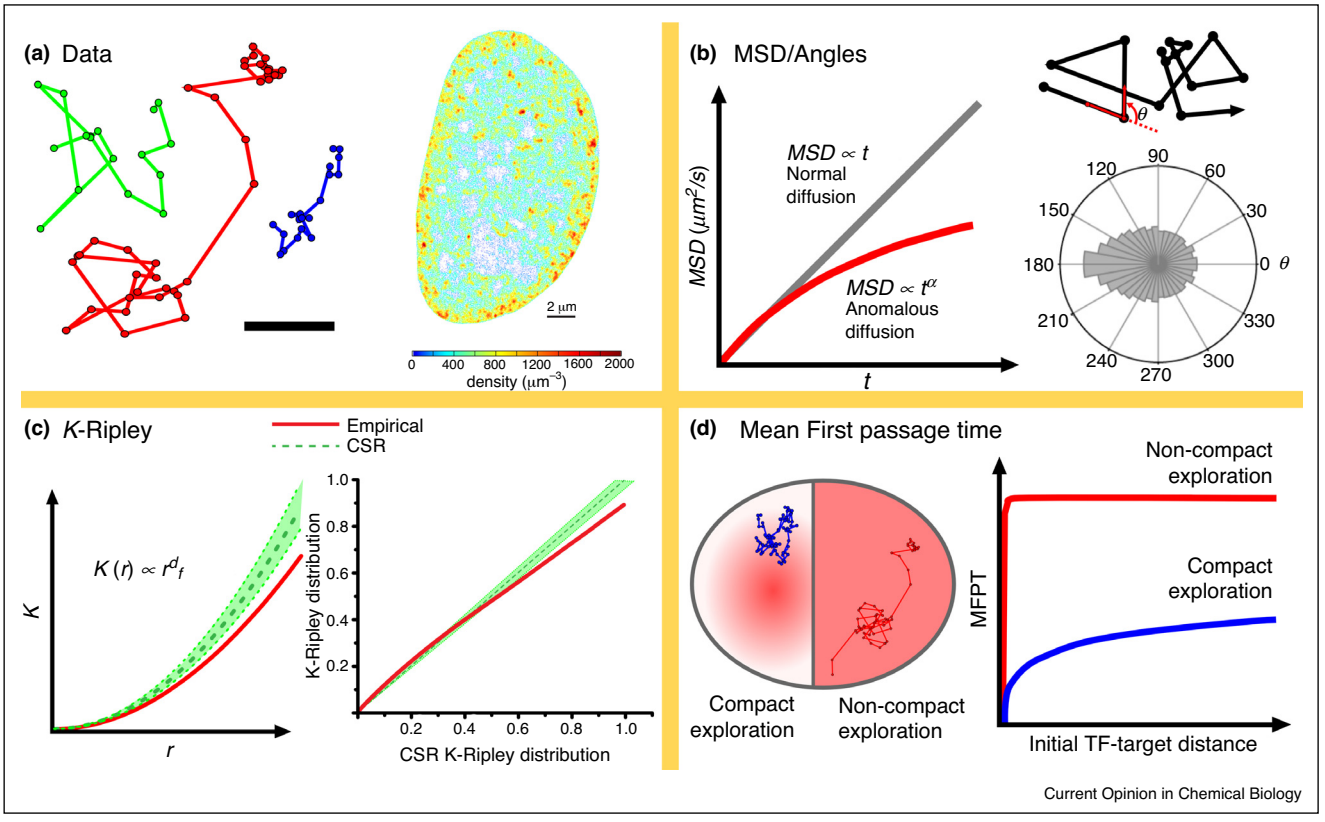
Table 1

Useful computational and mathematical tools to analyze superresolution microscopy datasets and their main bias

Measure	Origin	Formula	Explanation	Provides	Main Bias
MSD (mean square displacement); Figure 2b, left	SPT	$MSD(t) = \langle [x(t) - x(0)]^2 \rangle = 2dD_c t^{\alpha} \propto t^{2/d_w}$ where $x(t)$ stands for the particle coordinates at time t, d is the dimension of the space (1, 2 or 3), D_c is the diffusion coefficient of the molecule, d_w is the dimension of the walk	– The MSD is widely used to determine (apparent) diffusion coefficients. But its scaling α coefficient also contains information about the way the particle explores the space. – When $\alpha > 1$, the particle undergoes a superdiffusive or directed motion. If $\alpha = 1$, it is a Brownian diffusion. $\alpha < 1$ is indicative of a subdiffusive behavior, which is the signature of a constrain in space or time in the diffusion of the particle. – See [32•] and [28].	D_c, α, d_w	– In a heterogenous mix of different diffusive behaviors, overly detection of slow versus fast moving particles may induce a bias on the shape of the $MSD(t)$ curve. – Very hard to distinguish transitions in the dynamics of the diffusing particle.
Angular distribution; Figure 2b, right	SPT	Histogram of angles between consecutive translocations.	– Bridges the dynamics properties of the particle (measured traces) with its geometrical properties (the way it explores the space). – Offers information about the underlying space where the diffusion takes place. – See [32•,58].		Requires a high number of translocations to build the histogram and its evolution with increasing Δt.
K-Ripley; Figure 2c	PALM/ STORM	Distribution of number of neighbors within radius r from every detected particle, ie. the cumulative histogram of distances between points.	– In an unconfined geometry (no edge effect) with points uniformely dispersed (complete spatial randomness), $K(r)$ scales as πr^2 or πr^3 for 2D and 3D, respectively. – $K(r)$, its derivatives or other K-related functions (such as the normalized expression $H(r) = \sqrt{K(r)/\pi} - r$ [59]) give insight about the size of clusters (if any), or the fractal dimension when it scales as a power law ($K(r) \propto r^{d_f}$). – See [24•,60].	d_f, CSR test	– Requires 3D data to derive fractal dimensions larger than $d_f = 2$. – Can be tested against CSR. – Data have to be border-corrected in case of confined geometries.
MFPT (mean first passage time); Figure 2d	d_f, d_w, enclosing geometry	$\frac{\langle T_{TS} \rangle}{\langle \overline{T} \rangle_T} = \Pi_{TS} \propto \begin{cases} 1 - \kappa(\frac{1}{r})^{d_f - d_w} & (\text{non} - \text{compact}) \\ (\frac{r}{R})^{d_w - d_f} & (\text{compact}) \end{cases}$ where $\langle T_{TS} \rangle$ denotes the mean first passage time of a molecule starting its walk from S and reaching a target T at a distance r. $\langle \overline{T} \rangle_T$ denotes is the mean of $\langle T_{TS} \rangle$ respective to all starting points in the geometry. Π_{TS} is a function of the geometry and of the position of both the starting site and the target. κ is a constant and R is the characteristic scale of the geometry.	This gives insights on the distance-dependence of the mean first passage time. – Where compact refers to $d_w > d_f$ and non-compact to $d_w < d_f$ (see section 'Diffusion of TFs is altered by chemical interactions'). – For analytical derivation/simulations, see [46••].		The geometrical factor Π_{TS} has to be computed numerically. Requires prior knowledge of the geometry of the enclosing volume.

Illustrations can be found in Figure 2. SPT, single particle tracking; PALM, photoactivated localization microscopy; STORM, stochastic optical reconstruction microscopy; CSR, complete spatial randomness.

Figure 2



Useful representations to analyze single molecule microscopy experiments. (a) Samples of single molecule data acquired during various types of experiments. (*left*, from Ref. [32•]) Single particle traces, 10 ms resolution. Scale bar: 500 nm (*right*, from Ref. [24•]) PALM — photoactivated localization microscopy — reconstruction of histone H2B fused to the photoconvertible fluorescent protein Dendra2. **(b)** Representation of single particle tracking (SPT) data. *(left)* Mean square displacement (MSD). (*right*, from Ref. [32•]) (top) Computation of angles between successive steps and the subsequent histogram (bottom). **(c)** Representation of PALM/STORM data, from Ref. [24•]. (*left*) Empirical *K*-Ripley function compared to the null model of complete spatial randomness (CSR). (*right*) Same data, but the empirical histogram is now plotted against the average of CSR, allowing a better determination of the fractal dimension d_f. Envelopes represent 95% confidence intervals over simulations of the CSR. **(d)** Analytical tools allow for the computation of mean first passage times (MFPT) of a particle looking for a specific target and starting from a given position (see Refs. [32•,46••]). In the case of compact exploration, the TF is a local explorer, recurrently visiting neighboring sites. Conversely, in the non-compact case, distance from the target is not a relevant parameter and each point is visited with equal probability.

Indeed, such models neglect the widely described regulated interactions of TFs with DNA and other proteins [33••,34,35]. Binding and unbinding rates (k_{on} and k_{off}) of these interactions can dramatically affect the apparent diffusion coefficient of molecules, a phenomenon recently evidenced in single-molecule studies in living cells [32•,36–38]. On the other hand, in the context of heterogeneous catalysis, the adsorption of reactants in intricate geometries has been well characterized. In this framework, molecules undergo successive binding/unbinding events on a surface (referred as chemisorption). During this process, both the TF and the adsorbed surface (DNA or protein network) experience conformational rearrangements [39], modifications that are analogous to the enzyme–substrate co-adaptation described in Koshland's induced fit model [40].

In addition, adsorbed TFs are not necessarily statically trapped: they can diffuse on the adsorbent, thus switching from a 3D space exploration to a 'surface' of reduced dimensionality. This mechanism is known as facilitated diffusion in biology (see [41,42] for theoretical considerations, and [43–45] for experimental evidence) and can be seen as a beautiful example of heterogeneous catalysis in living matter. Indeed, diffusion on a surface of reduced dimensionality increases encounter probabilities, thus reactivity. From a physical point of view, and following the nomenclature introduced by de Gennes [9], TFs can switch from a 'non-compact' to a 'compact' exploration (cf. Figure 2a, right and Figure 2) [46••]. In a compact exploration, the molecule oversamples the explored space and visits a previously accessed site multiple times, thus performing a 'recurrent walk' [47].

It is noteworthy to point out that facilitated diffusion can occur within any structure of reduced dimensionality. The adsorbent structure for TFs can be chromatin (of fractal dimension between two and three), but could also be any protein domain susceptible of forming a network in the nucleus, such as the C-terminal domain (CTD) of Pol II, histone tails, nuclear lamina, etc. Indeed, interacting proteins can form gels [48] or polymeric networks [49]. Furthermore, live cell experiments suggest the coexistence of intricate networks influencing the diffusion of TFs [32•].

In addition to such geometry-controlled diffusion, taking into account biological reactivity is of particular relevance. Numerous post-translational modifications (such as phosphorylation, ubiquitylation or multimerization) affect TFs [40]. These regulations trigger dramatic changes in the space-exploring properties of the TF (plausibly switching between compact and non-compact modes of exploration).

TFs undergo explorations in the conformational space

When the TF finally reaches its target, the consequent reaction (whose final step can be transcription initiation) is a stochastic process [3,50,51]. In bacteria, the *lac* repressor repeatedly slides over its *lac* operator before binding [45]. Also, experiments on transcription elongation by Pol II show that, once bound to its target DNA sequence, elongation exhibits a high failure rate larger than 90% [52]. All in all, these examples indicate that the problem of transcription regulation cannot be reduced to a target-search process, even though it is an important first step in a complex sequence of events.

The bound TF has to overcome an activation energy barrier (E_a) to proceed to the final step of the reaction. At a molecular scale, the protein can be seen as a polymer diffusing in a conformational space of high dimensionality (this dimensionality being determined by the number of conformations accessible to the peptide chain [53]). Although this high dimensionality should prevent efficient conformational sampling, not all the conformations have the same energy, thus defining a so-called potential landscape. Within this potential landscape, some conformations with a too high energy are practically never sampled: the electrostatic interactions between the amino acids considerably narrow the space available for target search, in a similar manner to the exclusion volume encountered in the 3D nuclear space. Furthermore, recent NMR experiments followed by modeling show that the potential landscape even exhibits a reduced dimensionality, where the movements of the protein are highly constrained in a potential 'valley' [54].

From this perspective, attempts to characterize the 'target size' [55] of the target-search process (or effective cross section of interaction) are reduced to a chimera. Such a size reflects the conformational sampling of the protein in a space of very high dimensionality (defined by the positions of the amino acids in the protein) more than its diffusive motion. Rather than a size, this measure should be considered as a reaction probability reflecting the potential landscape sampling of the protein.

Conclusion

In this review, we have presented several formalisms used to describe diffusion in complex geometries, chemical adsorption, facilitated diffusion and molecular docking. Although each of them originated from unrelated works in the fields of biology, physics and chemistry, we highlight their common cornerstones in order to gain insight into eukaryotic gene expression regulation. Even though concepts still lack unification, we believe that in the near future, delving in the parallelisms between these fields will be fundamental to a deeper understanding of transcription.

In the nucleus, each TF senses a (sometimes dramatically) different environment depending on its physical and chemical properties, paving the way for highly diverse regulation of gene expression. Compact, local explorers can exhibit inhomogeneous concentrations throughout the nucleus, enabling concentration-based regulation processes. On the other hand, non-compact, global explorers such as c-Myc [32•] can mediate global effects on the genome, which is consistent with its described role as a 'global genome amplifier' [56] and 'global chromatin remodeler' [57].

Furthermore, protein–DNA and protein–protein interactions are highly regulated and dynamic. A TF constantly switching between chromatin-bound and unbound states can jump from a DNA chain to another, thus escaping simple 1D sliding: it will diffuse on a surface of fractal dimension higher than one. Post-translational modification of the TF affinity for a biomolecular network in the nucleus (such as DNA, Pol II CTD, etc.) can lead to fundamental differences in diffusive behavior, possibly influencing the patterns of gene expression.

When the TF has found its 'geometrical' target, a second, conformational target-search takes place before the TF proceeds through the chemical reaction. This conformational search is realized in a parameter space of high dimensionality. This dimensionality is further increased if we consider the ordered, combinatorial binding of coactivators to the TF.

All these space-exploring behaviors, assemblage routes, and regulatory processes are far from being mutually exclusive. Complex gene expression regulation in the nucleus actually arises from the coexistence of biochemical and biophysical mechanisms acting at all levels

of gene expression. Nonetheless, from a genomic perspective, this complexity is required to tune the expression of ~20 000 genes at a single gene resolution all along highly diverse processes such as cell cycle or differentiation. Conversely, from a TF's point of view, the nucleus should be regarded as a multiverse, where different proteins experience different landscapes with multiple scales, while being in the same space. Thus, the words of the French surrealist Paul Éluard seem more than appropriate: «il y a un autre monde mais il est dans celui-ci »(there is another world, but it is in this one).

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In this work, the authors develop a mathematical model to investigate the influence of the exclusion volume of chromatin on the target-search time of individual molecules. Using three-dimensional structured illumination microscopy images of a DAPI stained nucleus (from Schermelleh *et al.*, Science, 2008), Isaacson and colleagues build an effective potential energy field that tends to impede the diffusing molecule to enter high-density chromatin regions. With this model, they find that when the target is located in regions of low chromatin density, a moderate value of volume exclusivity (the strength of the potential field) leads to faster target-search times. Interestingly, when the DAPI image is randomized (but conserving the density inhomogeneities), this reduction of the time required to find the target is lost. This indicates that the efficiency improvement of the target-search process due to volume exclusion is related to the spatial correlation of chromatin, i.e. its geometry in the nucleus.

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In this work, the authors use photoactivated localization microscopy (PALM) combined with adaptive optics to retrieve the 3D position of hundreds of thousands of H2B histone molecules of the chromatin structure in the nucleus of human cells, with a localization accuracy of ~15 nm in *XY* and ~30 nm in *Z*. Besides observing chromatin density inhomogeneity at the single cell level with molecular resolution, they develop a computational approach to retrieve the so-called *K*-Ripley distribution of these histone molecules, corrected for the enclosing envelope of the nucleus and for bias of detection along the optical axis. The *K*-Ripley distribution is the distribution of distances between every two H2B molecules of the chromatin structure. When compared to a randomized distribution of points in the same nuclear envelope (complete spatial randomness — CSR — test), they observe that the ratio between them scales with a power law within the observable range (30 nm to to 3 μm), which is indicative of a fractal distribution of chromatin. From this computation, they retrieve a direct measure of the fractal dimension of chromatin $d_f = 2.7$, a consistent value across the cell population.

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Using single-molecule tracking of transcription factors (TFs) in live human cells, the authors show how different TFs (P-TEFb and c-Myc) explore their surrounding space in a fundamentally different manner, thus coining the concept of 'protein-specific geometry of the nucleus'. A combination of analytical approaches and numerical simulations is used to interpret the experimental data in the context of compact and non-compact exploration. With this approach, the authors provide a novel framework to study nuclear proteins with a wide range of mobility, and a better understanding of the basis for spatial cooperation and assembly of macromolecular complexes in the nucleus.

33. •• Kwon I, Kato M, Xiang S, Wu L, Theodoropoulos P, Mirzaei H, Han T, Xie S, Corden JL, McKnight SL: **Phosphorylation-regulated binding of RNA polymerase II to fibrous polymers of low-complexity domains**. *Cell* 2013, **155**:1049-1060.

When translocating the products of the low-complexity (LC) domains of three RNA-binding proteins (FUS/EWS/TAF15), they function as transcription activation domains. The authors use this system to demonstrate that the network of polymeric fibers formed from these domains binds to the C-terminal domain (CTD) of RNA polymerase II. By mutation analysis, they also show that the binding between the CTD and the polymers correlates with the strength of transcriptional activity. Moreover, the binding between the CTD and the fibers formed by the LC domains is reversible upon phosphorylation of the CTD, suggesting a model where RNA polymerase II is recruited to active genes and released for elongation after phosphorylation of the CTD. This and the previous works from the McKnight lab show how protein (polymeric) networks in the nucleus can induce a certain degree of organization implicated in the regulation of transcriptional activity.

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46. •• Bénichou O, Chevalier C, Klafter J, Meyer B, Voituriez R: **Geometry-controlled kinetics**. *Nat Chem* 2010, **2**:472-477.

In this theoretical work, the authors derive analytically the distribution of times that it takes for a diffusing molecule to reach a given target, i.e. the first passage time (FPT), in realistic confined geometries. Bénichou and coworkers demonstrate the emergence of two universality classes, namely compact and non-compact exploration, for any diffusion process. The authors show that only two parameters (the fractal dimension d_f and the dimension of the walk d_w) determine the universality class of the search process and, consequently, the stark dependence or lack of thereof of the FPT with the initial distance between the searcher and its target.

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