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Current Opinion in Chemical Biology

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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Current Opinion in Chemical Biology 2014, **20**:112–119

This review comes from a themed issue on **Molecular imaging**

Edited by **Christian Eggeling** and **Mike Heilemann**

For a complete overview see the Issue and the Editorial

Available online 27th June 2014

http://dx.doi.org/10.1016/j.cbpa.2014.05.009

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.