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Genome organization in the nucleus: From dynamic measurements to a functional model

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ABSTRACT

A biological system is by definition a dynamic environment encompassing kinetic processes that occur at different length scales and time ranges. To explore this type of system, spatial information needs to be acquired at different time scales. This means overcoming significant hurdles, including the need for stable and precise labeling of the required probes and the use of state of the art optical methods. However, to interpret the acquired data, biophysical models that can account for these biological mechanisms need to be developed.

The structure and function of a biological system are closely related to its dynamic properties, thus further emphasizing the importance of identifying the rules governing the dynamics that cannot be directly deduced from information on the structure itself.

In eukaryotic cells, tens of thousands of genes are packed in the small volume of the nucleus. The genome itself is organized in chromosomes that occupy specific volumes referred to as chromosome territories. This organization is preserved throughout the cell cycle, even though there are no sub-compartments in the nucleus itself. This organization, which is still not fully understood, is crucial for a large number of cellular functions such as gene regulation, DNA breakage repair and error-free cell division. Various techniques are in use today, including imaging, live cell imaging and molecular methods such as chromosome conformation capture (3C) methods to better understand these mechanisms.

Live cell imaging methods are becoming well established. These include methods such as Single Particle Tracking (SPT), Continuous Photobleaching (CP), Fluorescence Recovery After Photobleaching (FRAP) and Fluorescence Correlation Spectroscopy (FCS) that are currently used for studying proteins, RNA, DNA, gene loci and nuclear bodies. They provide crucial information on its mobility, reorganization, interactions and binding properties. Here we describe how these dynamic methods can be used to gather information on genome organization, its stabilization mechanisms and the proteins that take part in it.

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1. Introduction

Microscopy methods are now one of the key tools for studying biological systems. Many years ago, Richard Feynman made the jocular comment that "it is very easy to answer many of these fundamental biological questions; you just look at the thing!" [1]. Certainly, numerous information and unprecedented discoveries have been made by observing the structure of cells as a result of the immense progress of optical microscopy, fluorescent probes, labeling techniques and digital imaging during the last century.

Nevertheless, the era of molecular biology has created new challenges that call for high-resolution observations that can seldom be met even with modern super-resolution tools [2]. Moreover, some of the biochemical and biophysical mechanisms on the cellular level are dynamic by nature and cannot be observed simply by measuring the structure.

Here we focus on the use of dynamic methods in live cells as a way to explore the fundamental biophysical mechanisms of cellular processes and demonstrate their power. As a test case, we describe the use of dynamic methods to study the organization of the genome in the nucleus. This is an excellent example of a system that cannot be observed by optical microscopy because of limited resolution, nor by electron or ion microscopy since these techniques are restricted to fixed samples, conductive specimens, or require conductive coating of the samples.

At the same time, molecular methods have also evolved to a level where they can provide insights into the organization of chromatin in the nucleus and its functional roles. These include the chromosome conformation capture (3C) family of techniques supported by the revolution in ChIP and sequencing-based approaches [3–5].

Nevertheless, these methods provide limited information, since they are only feasible for fixed cell studies and they are based on homogenized cell population that provides a statistical average of DNA that origin from millions of cells. Heterogeneity does in fact exist even within small cell populations, and it is difficult to assess with these methods. Single cell techniques, on the other hand, provide unprecedented opportunities to quantify both the average of the measured population, as well as the differences and variability within the population. Actually, with some effort, single cell studies can provide the distribution of various parameters, information that is immensely important for understanding the nature of the biophysical mechanisms of the studied phenomena. Ultimately, the combination of dynamic methods with other imaging modalities may make it possible to elucidate the structure and functions of biophysical mechanisms.

1.1. Chromatin organization

The nucleus of eukaryotic cells is a membrane-bound organelle that contains almost all of the cellular genetic information embedded in the DNA (apart from a few other genes encoded in the mitochondria DNA). Chromatin is composed of DNA studded with proteins, enzymes and RNAs. It is compacted by a few orders of magnitude to fit into the nucleus and is organized into chromosomes and there is a consensus that the spatial organization of the chromatin is crucial to the functional properties of the genome [6]. Chromosomes fold into a confined space of the nucleus and form distinct territories [7] that are not randomly distributed. Furthermore, the DNA is wrapped around nucleosomes such that it shortens the ~2 m long DNA by approximately 6-fold to form a 10 nm wide 'bead on a string' fiber. In addition, different length-scales of DNA are looped together, from sub-mega to several mega base pairs (bp) termed topologically associated domains (TADs). These domains have been found for both inter- and intra-chromosomal regions and exhibit significant cell-to-cell variability. However, there are still open questions as to the nature of this organization and the rules that govern it.

The structural proteins in the nucleus form a network that provides mechanical support to the nucleus of the cell, and play a role in the regulation of cellular events [8]. Nuclear lamins are intermediate filament proteins that polymerize to form the nuclear lamina on the inner side of the nuclear membrane. Mammalian nuclear lamina contains lamins A and C, together with lamins B1 and B2. B-type lamins are expressed in all cells, whereas A-type lamins are developmentally regulated [9]. A- and B-type lamins form separate, functionally distinct networks of intermediate filaments that concentrate near the peripheral lamina network [10].

It was shown that lamins incorporated into the lamina have little or no mobility, whereas a fraction of the nucleoplasmic lamin is mobile [11]. Lamins can interact with chromatin either directly or

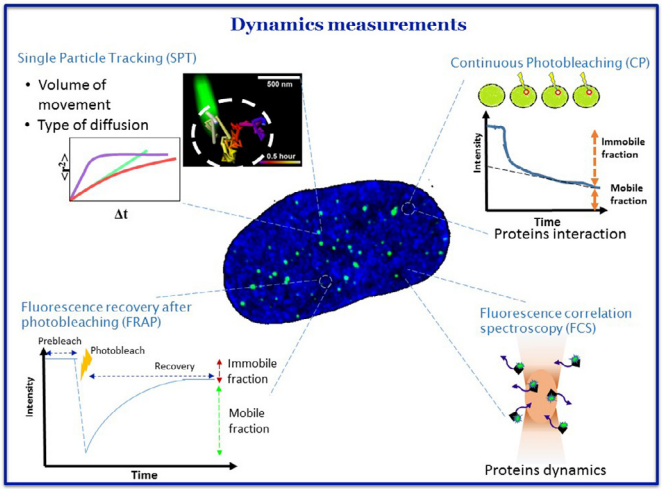

Fig. 1. Dynamic methods in live cells for exploring the structure of the genome. Four of these methods are described here. All the methods require labeling the protein or entity under study. Its interaction with the environment can be determined as well as its diffusion type (SPT and FCS), its sub-populations, such as free and bound fractions (CP), and its binding/unbinding reaction rates (FRAP, CP).