GENETIC MECHANOREGULATING NANOENGINERED CHIPS

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Even if all the cells of a given multicellular organism share the same genetic content, each cell specializes in specific phenotypes, defined by a pool of genes that it can normally express. Nowadays, even if the mechanism that regulates cell differentiation is completely understood, the involvement of genome architecture, more particularly the 3D spatial organization of DNA, in the regulation of gene expression has been studied intensively. Eukaryotic DNA is a two-meter long polymer packed in the cell nucleus in the form of chromatin fibers, a "beads on a string" structure, in which constitutive elements are the nucleosomes, short segment of DNA wrapped around a histones core [1]. Chromatin fibers are organized into 3D higher-order structures classified as euchromatin and heterochromatin domains. Euchromatin regions are active gene-rich domains of decondensed DNA, while heterochromatin mainly comprises highly packaged silenced DNA. However, the chromatin is not static and can change its organization in response to different stimuli. Key players in the chromatin organization are the post-translational histone modifications such as histone acetylation, which is generally considered a marker of euchromatin, and histone methylation highly localized in the heterochromatin regions [2].

Within interphase cell nucleus, chromatin usually folds into characteristic structures called chromosomes located within distinct nuclear region known as chromosome territories (CT) [3-5]. Experimental techniques, such as Chromosome Painting, suggest that the spatial distribution of chromosomes is not stochastic [6]. Gene-rich chromosomes frequently localize in the inner part of the nucleus, while gene-poor chromosomes preferentially are in proximity of the nuclear envelope. On the other hand, chromosome size is anti-correlated with the distance from the nuclear center [3, 7].

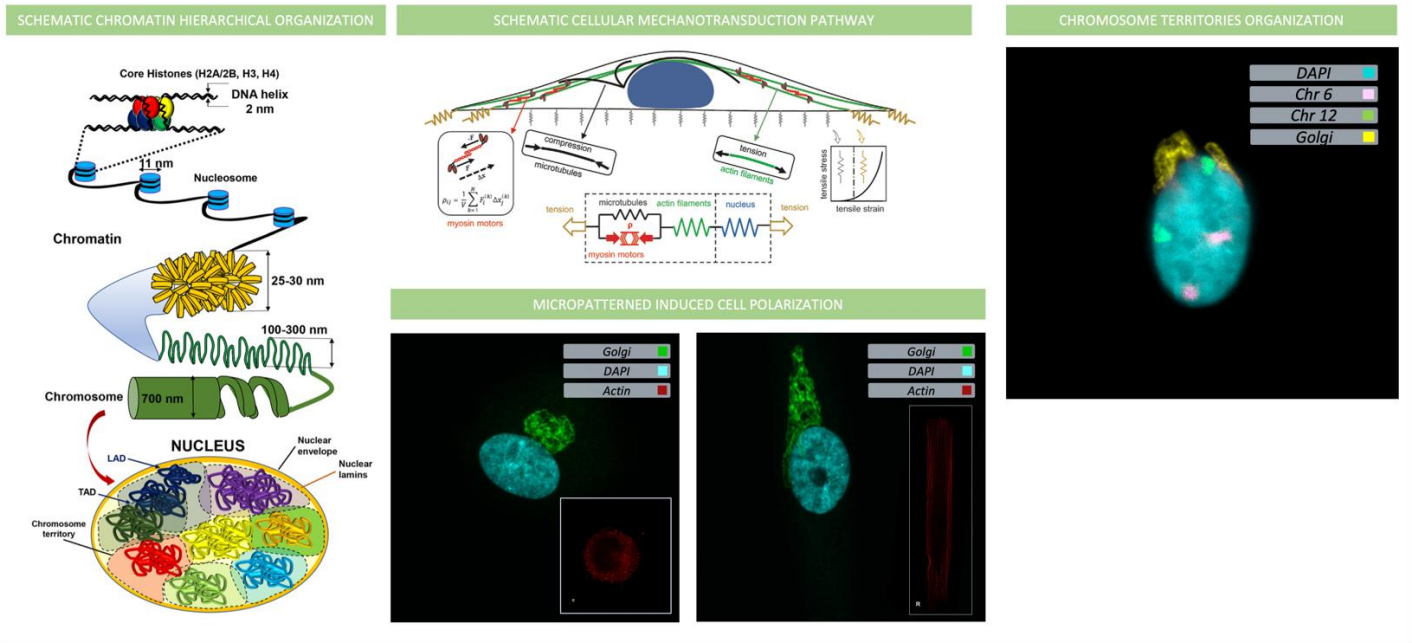
The remodeling or alteration of the 3D organization of chromatin and chromosomes can lead to a variation of the condensation state of the genome, so facilitating or limiting the access of transcription machinery thus resulting in a change of the cell transcriptome profile [1, 8].

Nowadays, it is well known that the mechanical environment in which the cell resides profoundly impacts nucleus content organization. In fact, the cell nucleus is mechanically connected to the external cellular space through the cytoskeleton. So, forces applied on the cell surface can be transmitted to the nucleus interior [8, 9]. Consequently, the mechanical conditioning of nuclear matter could be an effective way to modulate gene expression and hence cell phenotype [10, 11].

Based on this evidence, the current project aims to investigate how mechanical stimuli can be finely tuned and used to activate or repress gene transcription in a quantitative and temporal predictable manner. This approach is central to understanding the geometric control of genetic programs involved in cellular homeostasis and the differentiation process. For example, cell mechanics can be modulated by altering the cellular morphology (shape, size, and aspect ratio) using engineered micropatterned substrates [12]. For example, adhesive islands limiting cell spreading area, strongly impact cytoskeleton's architecture thus inducing an alteration of the cell's mechanical and transcription state [8].

Additionally, we can employ cell confinement to modify the chromosome topology in a non-stochastic way. For example, adherent cells can spontaneously define an axis of polarity typical of migrating cells. This asymmetry of the cytoskeleton propagates inside the nucleus, causing chromosomes to adopt a conserved and non-random arrangement in the nuclear space [13, 14].

In this project, the conformation and 3D organization of euchromatin/heterochromatin domains and CTs into the eukaryotic cell nucleus will be investigated with a multidisciplinary approach comprising: (i) nanoengineered chips, such as fibronectin micropatterned coated substrates, designed for the optimal cell machano-regulation; (ii) immunofluorescent techniques to label and recognize specific histone modifications and cell organelles of interest; (iii) DNA hybridization techniques, such as chromosome painting, to investigate the 3D conformation of specific chromatin domain and chromosome territories positioning. Then, super-resolution microscopy techniques combined with advanced image analysis and mathematical modeling will be employed to extensively investigate the correlation between mechanical stimuli and 3D nuclear conformation.



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