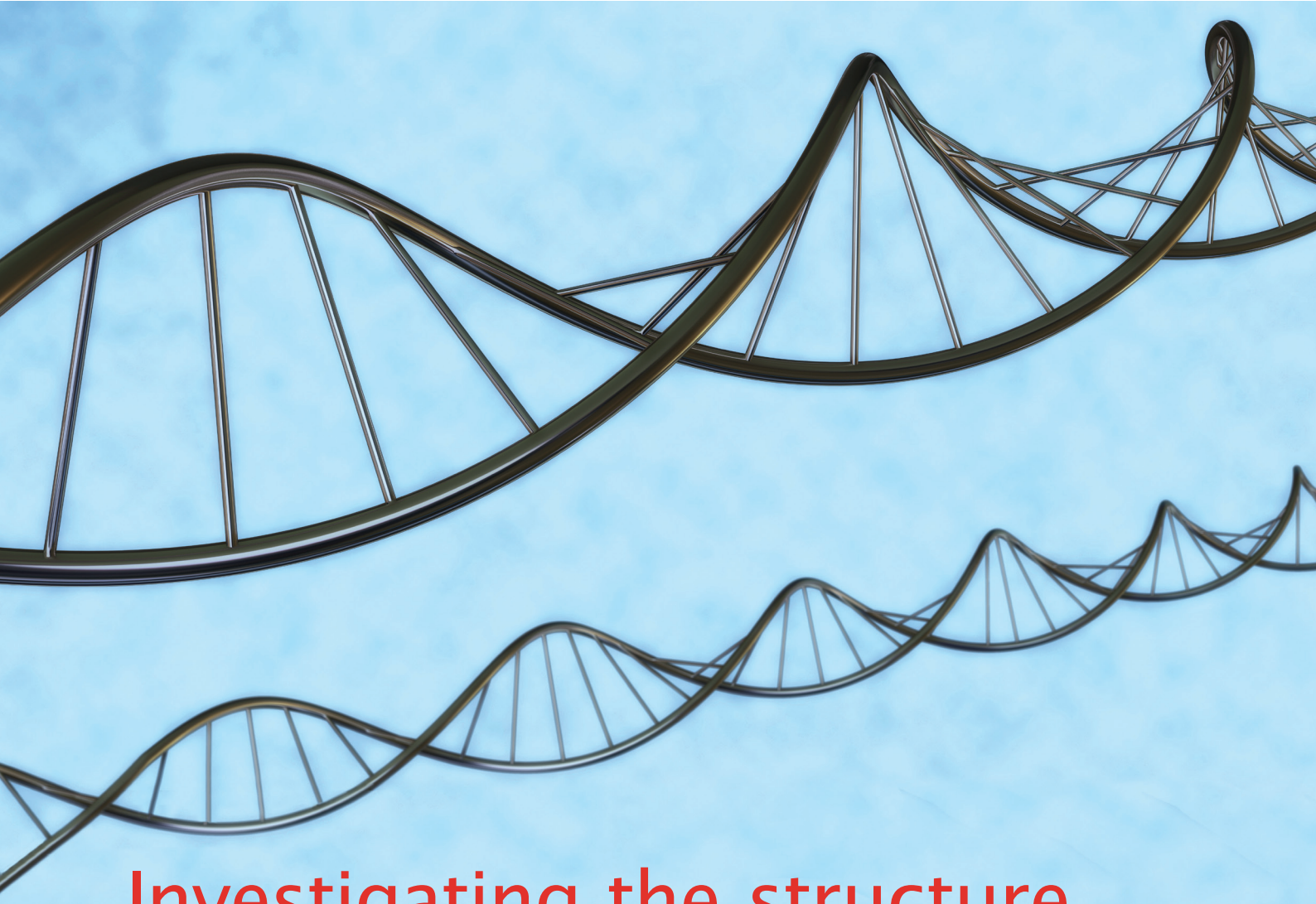


# Investigating the structure and regulation of chromatin

The SMINSULATOR project uses single molecule biophysics and quantitative modelling to investigate the structure and regulation of chromatin, the combination of DNA and proteins that forms the nucleus of a cell. The long-range interactions that occur between different parts of chromosomes are of particular interest, as **Dr Marcelo Nollmann** explains

**Eukaryotic chromosomes are** condensed into several hierarchical levels of complexity: DNA is wrapped around core histones to form nucleosomes, which form a higher-order structure called chromatin, and chromatin is subsequently organised by long-range contacts. The SMINSULATOR project, an EU-funded initiative based at INSERM in Montpellier, investigates the role of long-range interactions in nuclear organisation. "The main aim of our project is to investigate how long-range interactions in chromatin can lead to preferential organisation of the chromosome and transcription regulation," outlines Dr Marcelo Nollmann, the project's coordinator.

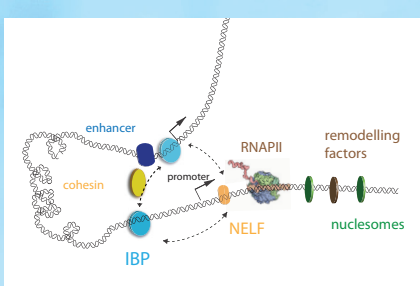
Transcription is the process by which the information encoded in the genome is converted into RNA, the template required to produce proteins. The specific transcription profile of a cell ultimately determines its identity. "Chromatin organises into euchromatin and heterochromatin, which display different physical and molecular characteristics that are key for the regulation of fundamental cellular processes, most notably of transcription," explains Dr Nollmann. "Genes are transcriptionally silenced in heterochromatin (or 'closed' chromatin), which is thought to possess a highly compact structure. Conversely, euchromatin (or 'open' chromatin) is defined as less condensed chromatin where most gene expression takes place during interphase. We are interested in identifying the protein network required to partition chromatin into the euchromatin and heterochromatin domains, and understanding how this partitioning leads to the definition of a cell's identity through its transcription profile."One of the major areas of investigation is insulator factors, a class of regulatory proteins which set up boundaries between heterochromatin and euchromatin.

## Studying transcription regulation one molecule at a time

The project's specific focus is on eukaryotic chromosomes, distinguished from prokaryotes by the presence of a nuclear compartment, within which genetic material is stored. Previously, it was thought that eukaryotic chromosomes were organised into chromatin, and that prokaryotes are not, but recent evidence suggests that may not be the case. "Several bacterial proteins have been identified that may play a role similar to that of nucleosomes," says Dr Nollmann. The problem is that relatively little is known about those factors and the way they work; Dr Nollmann and his colleagues are developing novel approaches, involving the detection of single molecules, to try to determine the roles of insulator proteins in chromatin organisation. "Mechanistically, we can see several parallels between eukaryotes and prokaryotes: they are both organised in chromatin-like structures that play a very important role in the expression of genes," he says.

Researchers are using two kinds of single molecule approaches to investigate these structures. The first involves a bottom-up approach to try to reconstruct the basic functions of insulator proteins in the cell. In this approach, each specific protein is purified *in vitro* and its activity is reconstituted. "This approach allows us to considerably simplify the system, a step usually key to asking highly mechanistic questions. Single-molecule studies, in addition, have the added advantage that they allow for the direct measurements of dynamics and sample heterogeneities otherwise obscured by ensemble averaging when using conventional methods. One example is the study of what specific proteins are required in the establishment of the long-range DNA-DNA interactions that we think happen *in vivo*," explains Dr Nollmann.

In the second approach, researchers



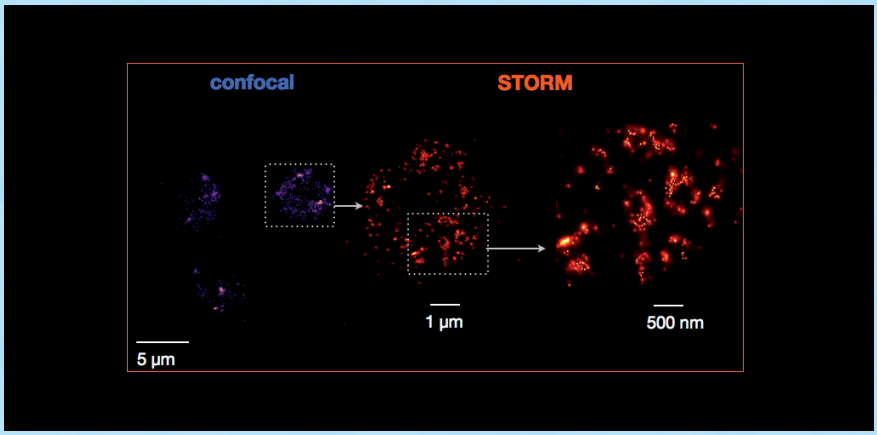

Speculative network of factors leading to long-range DNA interactions between insulators.

can directly test whether long-range interactions observed in the test tube also occur in the cell. "In this case, we develop methods that allow us to detect the position and movement of single protein molecules in live cells," says Dr Nollmann. "These methods do not always provide such detailed insights, as the environment in the cell is much more complex and less controlled, but they are still key as they allow us to directly test the possible differences of protein actions when they are in their real cellular environment."

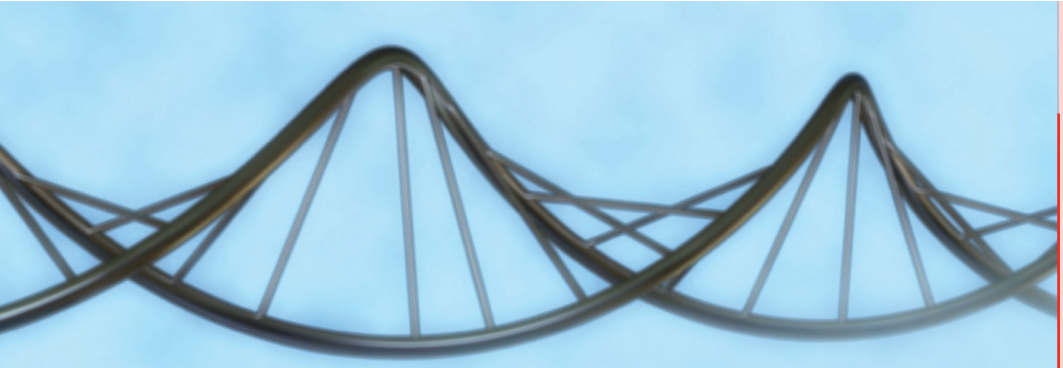
Dr Nollmann says insulators play a vitally important function. "You need to separate the chromatin regions that are heavily expressed in some cell types and not in others, because that essentially is going to give the identity of the cell, and the outlook of proteins that are expressed in that cell," he stresses. Several mechanisms are required to enforce the boundary between euchromatin and heterochromatin and change it when needed, such as if certain cell types are developing. "They may need to change the proteins that are expressed, so they need to go in to some regions of the chromatin and change it, for example from heterochromatin to euchromatin. So they need access to those boundaries, and the proteins that participate in them," explains Dr Nollmann. "In that case it may generate a spread of euchromatin into heterochromatin regions, which may lead to the expression of genes in those heterochromatic regions."

## Quantitative models

Genome-wide methodologies are also very powerful as they allow researchers to measure genomic binding sites of proteins involved in chromatin organisation, and to detect the existence of long-range interactions. These methods are limited however, in that they provide information about a large, mixed population of cells that may have different binding profiles or chromatin conformations and interactions of/between different proteins.




Single-molecule super-resolution microscopy is a novel methodology that is used to obtain images of the structure of chromatin at the nano-scale.



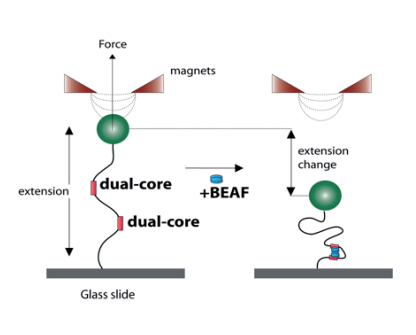
By contrast, single-molecule microscopy gives the unprecedented ability to discover the function of different proteins in chromosome organisation in each individual cell, and to investigate how chromosome organisation is modulated in time and in response to external cues.

The production of quantitative models of chromatin structure and regulation from single-molecule data is an essential part of the project's work, so that researchers can draw comparisons with results obtained from genome-wide approaches. "These comparisons will allow us to determine whether the chromosome is organised and regulated in the same way in different cells and study the role of heterogeneity in cell populations. Also, we will be able to determine the dynamic changes in chromosome architecture and transcription regulation during the life of a cell," explains Dr Nollmann.

**Single-molecule microscopy gives the unprecedented ability to discover the function of different proteins in chromosome organisation in each individual cell, and to investigate how chromosome organisation is modulated in time and in response to external cues**

The higher-order structure of chromatin is highly complex and determines the transcription profile of a cell, so the idea that different cells or cells in different stages of their life cycle would have different higher-order chromatin structure would not be entirely surprising.

This research is mainly fundamental in nature, but Dr Nollmann nevertheless expects that their results will have an impact on the study of disease. The techniques developed in the project are currently being applied to look at very small, simple systems to gain rigorous mechanistic insights, but that could be extended to the study of chromatin architecture and the role of insulator factors in entire animals to gain a wider perspective. "We can do experiments under those conditions and see what is different in the embryos with respect to single cells. In future, we could also look at mature flies and, for example, the expression of those genes in different tissues, to determine how insulator proteins orchestrate the organisation of chromatin and the transcription program leading to cell differentiation. So I hope that by the end of the project we will get a full picture of how these very fundamental mechanisms affect gene expression patterns in the whole organism," says Dr Nollmann. With the project still in its early stages, Dr Nollmann says the methodology could evolve. "I hope that in future we can move more and more towards using these single molecule methods in tissues, even maybe live animals," he says.




Magnetic tweezers are a single-molecule manipulation technique that uniquely report on dynamic changes in DNA extension induced by the formation of long-range DNA interactions induced by the binding of proteins.

## At a glance

### Full Project Title

Unveiling the Roles of Chromatin Insulators in Higher-order Chromatin Architecture and Transcription Regulation one molecule at a time.

### Project Objectives

Our research aims to unravel the mechanism by which insulator bodies dynamically regulate chromatin structure and transcription by using single-molecule biophysics and quantitative modeling. This project has the potential to impact our understanding of several fundamental cellular processes, including transcription regulation, cell-cycle dynamics, higher-order chromatin organisation, and cell differentiation.

### Contact Details

Project Coordinator,
Dr Marcelo Nollmann
Centre de Biochimie Structurale
29, rue de Navacelles
34090 Montpellier
France
**T:** +33 (0)467 417 912
**E:** marcnol@gmail.com
**E:** marcelo.nollmann@cbs.cnrs.fr
**W:** https://sites.google.com/site/smbmontpellier2/insulator-biology

*http://www.ncbi.nlm.nih.gov.gate1.inist.fr/pubmed/21983085*

Dr Marcelo Nollmann

**Project Coordinator**

Dr Marcello Nollmann is a Principal Investigator at the Centre de Biochimie Structurale (CBS) in Montpellier, where he started his own laboratory in May 2008. Its main axis of research is the development and application of single-molecule methods to study the mechanisms by which DNA is moved and organised in the cell. Researchers have so far built a magnetic tweezers setup, and a one-color photo-activated localisation fluorescence microscope (PALM).