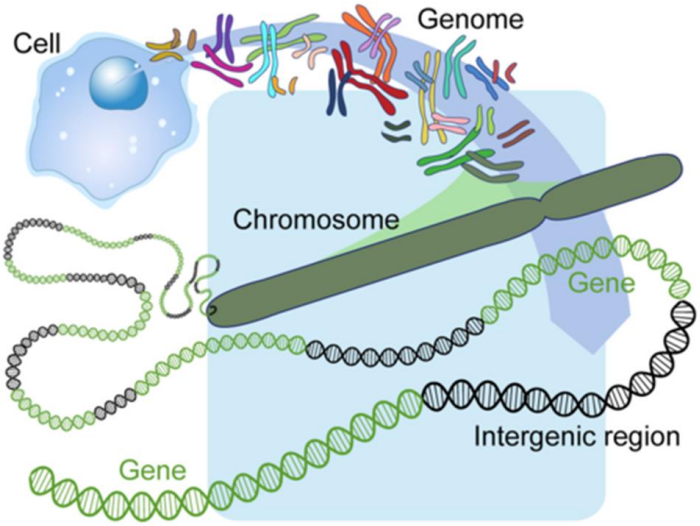
EpiSwitch™ Science: Chromosome Conformations

A 21st Century tool for understanding structure-function relationships in the genome

It has been over 115 years since the Chromosome Theory of Inheritance, the proposal that chromosomes carried the factors of Mendelian inheritance, was articulated by Walter Sutton and Theodor Boveri. Since then, we have seen a remarkable evolution in our understanding of how the genome relates to human health and disease. As is the often case with scientific inquiry, the pace of discovery proceeded in fits and spurts. 50 years passed between when the structure of DNA was solved and the human genome was sequenced. Nonetheless, a combination of human curiosity, ingenuity and persistence coupled with the development of increasingly sophisticated technologies to probe the genome have brought us to an exciting new era in genomic research.

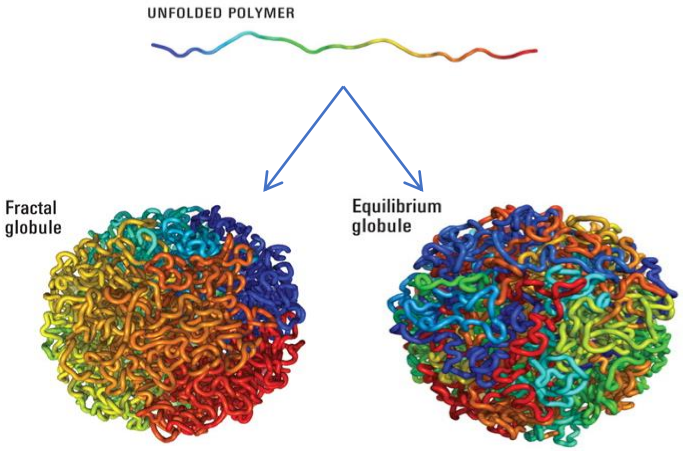
Anyone who took an introductory biology course prior to 2000 had been given the basics of the genome and how it's organized. The rules were simple. Within a cell is a structure called the nucleus that houses our genome on 23 pairs of chromosomes. Chromosomes themselves are made up of molecular "building blocks" that form the famous Watson & Crick double helix. Stretches of these building blocks along the linear space of a chromosome define genes, which encode for proteins that are the workhorses of cellular function: **DNA makes RNA makes Protein**. Any region that does not define a gene (intergenic regions) is "junk DNA" and can be written off as worthless. Besides, the view was that it was likely that there was not much junk DNA anyway.

This dogma took a 180 degree turn in the early 2000's with two major breakthroughs. The first was the sequencing of the human genome and the second was a better understanding of how chromosomes are organized within the nucleus.



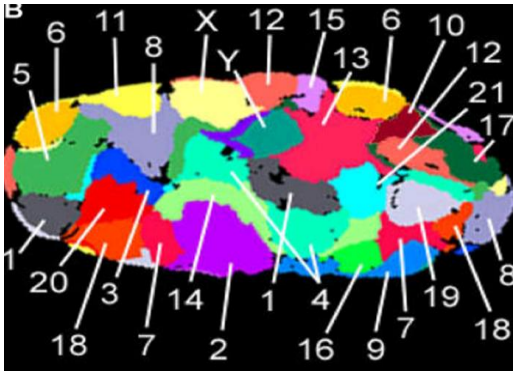
Basic view of cellular organization (Image from ck12.org)

When the human genome was sequenced in 2001, one of the biggest surprises was that the proportion of the genome that actually encoded proteins was very small (around 2%), leaving the need to explain why 98% of the genome was not coding[1]. In parallel, a set of technologies emerged that allowed scientists to visualize the organization of chromosomes within a nucleus. The nucleus is a 3-dimensional structure and chromosomes themselves take up a space within this structure. Undertaking the conceptual thought exercise of imagining chromosomes as unfolded polymers, there are two main ways that that they could self-organize. The first is a random assembly of chromosomes distributed in 3-dimensional space (the equilibrium globule) and the second would be as an ordered structure where chromosomes arrange themselves into distinct compartments within the nucleus (the fractal globule)[2].



Hypothetical organizational states of the genome (Images taken from Erez Lieberman-Aiden et al.)

Using a combination of spectral imaging and molecular biology techniques that could assess the positions of genomic regions in relative proximity to each other in 3-dimensional space, it was observed that in fact, chromosomes did occupy distinct nuclear territories[3]. The concept of the genome operating as a fractal globule within the nucleus was solidified.



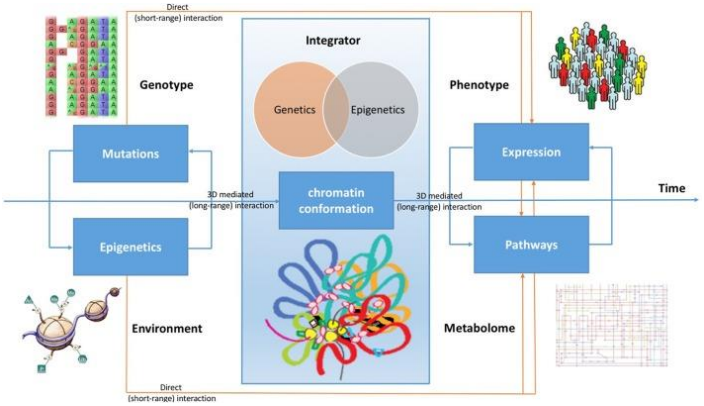
Actual localization of chromosomes in a nucleus (Image from Bolzer et al.)

Out of this two-fold recognition (that most of the genome did not encode genes and that the genome itself possessed a high degree of spatial order) emerged the concept that there was something beyond the linear DNA code itself driving biological function, thus the field of epigenetics was born.

The dictionary definition of epigenetics is "a stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence", but what that really means is that the way chromosomes are arranged within a cell has an effect on what that cell does and that this characteristic can be passed on.

Studying this phenomenon involves looking at the functional consequences (function) of alterations in genomic organization in 3-dimensional space (structure). This represents a dramatic departure from the legacy reductionist thinking of viewing the genome in a purely linear sense. It also places a larger emphasis on understanding the role of intergenic regions in regulating how chromosomes are arranged in 3-dimensional space, or chromosome conformations. Clearly, some new rules and techniques have started to come forward.

The new thinking about genomic regulation reinforces the concept that chromosome topology plays a critical regulatory role in maintaining the normal cellular state (homeostasis). Rather than the gene-centric view that had been at the crux of earlier conventional thinking, this new model places genomic organization, in the form of chromatin conformations, at the center[4]. The 3-dimensional genome acts as the integrator of external environmental stimuli and cellular responses (i.e. gene expression, protein production and the maintenance of metabolic homeostasis). While this principle is conceptually easy to understand, it is less easy to address experimentally.



Chromosome conformations as integrators of biological signals. (Image from Tordini et al.)

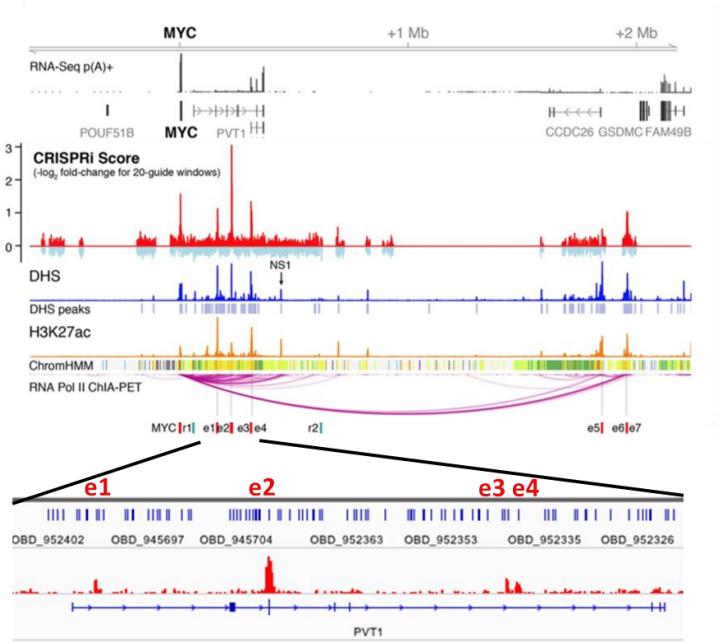
The sheer size of the non-gene-encoding genome (in excess of 3 billion base pairs) and the complexity of three–dimensional genome topology makes asking questions about the function of these regions a particular challenge. What knowledge we have of the rules governing this "dark matter" comes from over 20,000 peer reviewed studies analyzing individual bits of genome topology, one at a time and looking at the effects,

typically by looking at the expression of a handful of genes as a functional readout.

In 2017, three systematic studies published in the prestigious journals *Nature* and *Science*, provided further evidence of the functional links between changes in chromatin structure and cellular function [5-7]. Two of the studies were undertaken by teams at the Broad Institute, Cambridge MA. Using complementary approaches, the teams used screening with CRISPR (a molecular biology tool used to target and edit sections of the genome) to systematically affect thousands of noncoding intergenic DNA regions and look at the downstream functional consequences. Using this approach, both groups identified several interesting genetic regulators, some located millions of bases away from the genes they control. Importantly, the functional outcomes monitored in these studies were not limited to the expression of a few chosen genes, but were fundamental systemic cellular responses - cell proliferation, cell differentiation, resistance to chemotherapeutics.

The lead author in one of the studies, Neville Sanjana, commented on the importance of the new vision and the limitations of the traditional gene reporter approaches: "The screens interrogate the sequences in their endogenous context…Reporter assays can be very helpful, but they lack the 3D conformation or local chromatin environment of the native genomic context. Here, the regulatory sequences undergo all of their normal interactions."

Today the biology of genome structure-function topology is rapidly moving towards development of new focused and efficient techniques that provide practical tools to discover, screen, evaluate, validate and monitor key arrangements of genomic topology. These approaches are a significant step forward compared to the limitations and challenges of traditional methodologies. The leading and most advanced methodologies, *EpiSwitch*, developed by Oxford BioDynamics, out of Oxford University, provides a simple, rapid and reproducible readout of chromosome conformations. Based on molecular measurements in biofluids (blood samples or liquid biopsies), *EpiSwitch* has shown a high degree of concordance between primary disease sites and biofluid readouts.



Chromosome conformations as regulators of cellular function. Looking in the genomic region around the gene for the transcription factor MYC, a key regulator of cell cycle progression and proliferation, the research teams at the Broad Institute identified seven enhancer regions (e1-e7) that formed long range chromosome interactions, in some cases spanning a distance of over a million base pairs. When these conformations were disrupted experimentally, there was an immediate and pronounced effect on cell survival.

(Image from C.P. Fulco et al., Science, 2016)

The application of *EpiSwitch* to clinical samples provides investigators in translational medicine with a powerful tool to aid in the stratification of complex physiological phenotypes, helping with the understanding of disease onset and progression, planning drug development programs, and patient stratification in pre-clinical and clinical settings[8-9].

For more background on *EpiSwitch* technology, its read-out formats and applications in biomarker discovery, target discovery, and patient stratification, see our ***EpiSwitch* Technology White Papers**.

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Authors

Willem Westra, PhD
Ewan Hunter, PhD
Alexandre Akoulitchev, PhD

Oxford BioDynamics Plc
Oxford, United Kingdom
info@oxfordbiodynamics.com

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