Epigenetic Mysteries Unravelled: The Zinc-Finger Proteins

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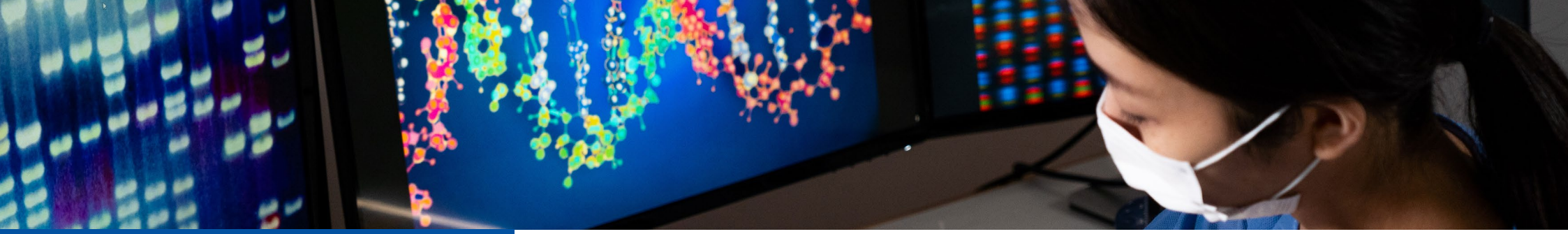

Epigenetic Mysteries Unravelled: The Zinc-Finger Proteins

Exploring the complex mechanisms of cell development processes and DNA structure is critical to understanding how certain diseases, such as cancer, can arise. Professor Danny Reinberg and Dr Havva Ortabozkoyun from the University of Miami in Florida, USA, work to reveal the epigenetic mechanisms at play during cell division and development and, in turn, disease processes. Together, they are discovering new protein molecules involved in genome organisation, deepening our understanding of how cancers and other related conditions can develop.

Exploring Epigenetics

Cancer is one of the leading causes of death around the world, accounting for almost one in six deaths, according to the World Health Organization. Although it can occur in any part of the body, cancers have one thing in common – they are made up of fast-growing abnormal cells that can spread to other areas/tissues, meaning that something is going wrong with the cell division process and the genetic material stored within the cell. The rapidly growing field of epigenetics aims to study the changes in gene expression, the turning 'on' or 'off' of certain genes, which can result in changes to DNA and lead to diseases like cancer, among others.

Professor Danny Reinberg and Dr Havva Ortabozkoyun are based at the John T. MacDonald Department of Human Genetics, at the Miller School of Medicine and Sylvester Comprehensive Cancer Center of the University of Miami. Their groundbreaking work is driving a better understanding of the epigenetic mechanisms occurring not only during development that lead to cell diversity but also when these processes go awry, as in diseases like cancers and certain inherited conditions.

Novel Approaches

Professor Reinberg has a keen interest in the epigenetic processes by which the DNA structure is created during development in mammals, and how they result in different types of tissues in the body. Understanding how the specificity of cells is maintained when they divide and grow, and how diseases can arise due to alteration of this process is of fundamental importance. Along with Dr Ortabozkoyun, he focuses on the epigenetic basis of gene repression – the switching off of certain genes – to understand the cell lineages (Figure 1).

The aim is to identify and understand the role of particular molecules and their impact on DNA and gene activity.

The University of Miami researchers study the mechanisms within cells which result in the compartmentalisation of certain repressed genes, and how their three-dimensional structure is created and maintained. Without this maintenance, these repressed genes could be expressed inappropriately, leading to the production of excess and/or unwanted proteins being produced, a process that can occur in cancerous cells. Research conducted at Professor Reinberg's laboratory is set apart from mainstream work in that the team focuses on mechanistic approaches to understanding the intricate processes occurring.

Organising the Developing Embryo

The precise regulation of gene expression is vital to ensure the proper development of the embryo. In addition to the DNA sequence, the structure of the chromatin and spatial organisation of the genome have a role in regulating gene expression. The genomes of organisms, such as mammals, are tightly folded and packed into the cell nucleus. When this occurs, it is packed along with various proteins to help condense the genetic material further, forming the chromatin (Figure 2). These proteins act like spools for the long DNA molecule to wrap and fold around – much like a thread. The formation of chromatin is pivotal in enabling many different cell processes to happen, such as cell division and DNA replication.

The partitioning of genetic material into chromatin happens using molecules called insulators, and the main insulator protein in mammals is the CCCTC-binding factor, also called CTCF. This insulator, found in the majority of cells, is critical for development

and the three-dimensional organisation of the genome. The CTCF affects certain genes within Hox gene clusters (Figure 3). The Hox genes are involved in the early stages of embryo development, helping to specify where certain structures form on the developing body, for example, where the wings and legs grow on a fruit fly or where the limbs grow on a person. They are organised in clusters, affecting different regions of the body, turning off and on, at different stages of embryonic development in a particular order. This tight sequence of gene activation and deactivation ensures the body of the organism develops in the right way.

Identifying MAZ

Professor Reinberg and Dr Ortabozkoyun carried out an investigation using CRISPR and biochemical screens, resulting in the identification and understanding of the role of a vital substance called Myc-associated zinc-finger protein or MAZ. Utilising CRISPR screening allows small numbers of certain genes or genetic sequences to be identified from within a huge number of genetic sequences, even a complete genome. The genes influencing particular physiological outcomes can be uncovered using this screening process.

They identified MAZ to be involved in CTCF-mediated insulation at Hox clusters. It acted as a cofactor, an additional substance vital for the activity of another. Dr Ortabozkoyun devised a special CRISPR genetic screen and carried this out alongside biochemical approaches to identify the critical proteins and insulators on the chromatins. They studied the effects of MAZ loss in genetically engineered cells, where it altered the cellular identities of motor neurons. Similarly, they were able to study the effects of MAZ deletion in genetically altered mice, where it resulted in homeotic transformations. In this developmental mutation, one type of organ cell transforms into another.

They discovered MAZ's role in insulating chromatin boundaries along with CTCF, preventing them from spreading at Hox clusters, and were able to demonstrate that MAZ is integral to appropriate gene expression and organising the architecture of the genome during motor neuron differentiation and development (Figure 2-3).

Rings, Loops, and Clusters

Continuing their investigations involved looking more closely at the partitioning of chromatin in mammalian cells. Activated and deactivated areas of the chromatin are separated in a way unique to a cell type, resulting in cell-type-specific patterns of gene expression. The CTCF is unchanged at the developmentally vital Hox clusters, and so the changes at the chromatin boundaries must entail other activities. Like CTCF and MAZ, there is another molecule essential for maintaining genome organisation, the ring-shaped cohesin complex.

The loss of CTCF, MAZ or the cohesin complex disrupts the looping forms of chromatin. Indeed, the chromatin loops are the main building blocks of the three-dimensional genome architecture. With this in mind, the team examined cohesin-based chromatin structure but without the presence of known insulators such as CTCF and MAZ – the focus of their earlier research. They hypothesised that other proteins must exist that work with cohesin to create appropriate chromatin boundaries that reflect the progression of the cell differentiation process during development. This spurred the team's next major study, where they made important discoveries into the mysteries of the genome.

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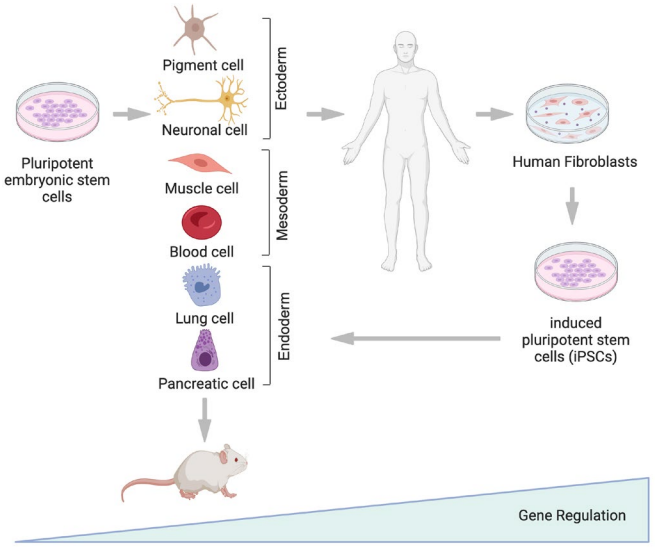
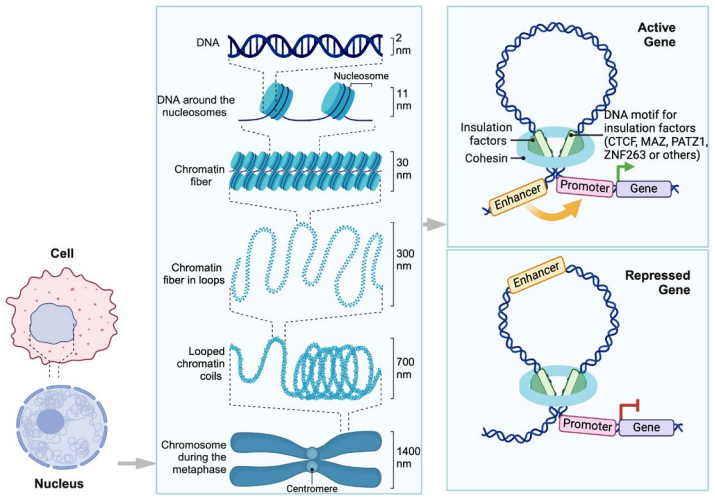
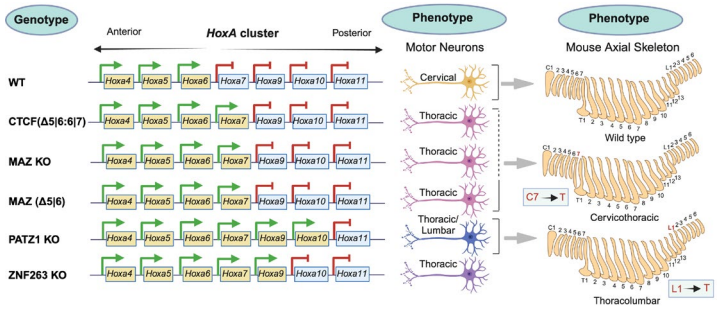


How to study gene regulation? The alterations in gene regulation perturb cellular differentiation and development of an organism, ultimately leading to diseases in human. Created in BioRender. Ortabozkoyun, H. (2025) https://BioRender.com/p42j589.



Insulation factors are involved in gene regulation. The changes in enhancer-promoter interactions in loops affect the expression of target genes in a specific cell-type. Created in BioRender. Ortabozkoyun, H. (2025) https://BioRender.com/r23q277.



***Hox* gene regulation impacts positional identities of cells during development**. The loss of insulation factors including MAZ, PATZ1 and ZNF263 or lack of their binding at *HoxA* cluster leads to changes in motor neuron identities in cells and skeletal structures in mice. Created in BioRender. Ortabozkoyun, H. (2025) https://BioRender.com/d73a529.

A Family of Zinc-Finger Proteins

Proteins are formed from long chains of amino acids, which then fold back and around themselves to form a complex-shaped molecule. The final shape of the protein depends on the sequences of amino acids it contains and also the presence of other chemicals, each forming a unique structure required for it to carry out its vital job within the living organism. Some proteins like CTCF and MAZ have different copies of the 'zinc-finger' feature, which is when a zinc ion stabilises a fold in the protein, creating a finger-like projection on the protein molecule – giving rise to the name.

A genomics analysis proved the researchers' hypothesis on the existence of other molecules to be correct, and they identified sections of DNA corresponding to various new proteins. This investigation resulted in the discovery of a family of zinc-finger proteins, ZNFs, some of which were found to be expressed (produced) only in specific cells or tissues. The ZNFs carry out their functions at chromatin boundaries, determining the identities of the developing cells. Two particularly interesting zinc-finger proteins were identified: PATZ1 and ZNF263. These were both found to regulate distinct Hox gene borders in types of nerve cells called motor neurons. More interestingly still, the loss of the PATZ1 protein resulted in a reduction of the loops forming, significantly impacting genome organisation in the cells. Additionally, loss of this ZNF led to changes in the skeletal patterning in mice, and homeotic transformations occurring in the skeleton (Figure 2-3).

Pushing the Boundaries

The studies were conducted using genetically altered mice, meaning that the impact of certain genes being switched on or off, and the production of particular proteins and their effects on development could be tracked. Noteworthy findings surrounded the critical developmental impact of PATZ1 and ZNF263. PATZ1 was found to be vital to the thoracolumbar boundary, where differentiating developing motor neurons from the upper and lower body meets, whilst ZNF263, like MAZ, focused on cell differentiation at cervicothoracic boundaries between the neck section of the spine and the upper back portion (Figure 3). It was proposed that the proteins act with cohesin and CTCF. It is postulated that ZNF combination with or without CTCF ensures the precise development of cells in the correct positions within boundaries.

Professor Reinberg and Dr Ortabozkoyun's findings are likely to be broadly applicable to other borders, both *Hox* and non-*Hox* controlled, and the fate of cells during development. Their groundbreaking work in epigenetics and cell development brings us another step closer to understanding how disease states such as cancers could occur, paving the way for novel treatment approaches and future research.

Article written by Luisa Postlethwaite, MPharm

MEET THE RESEARCHERS

Professor Danny Reinberg
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Professor Danny Reinberg obtained his BS in Biology from the Catholic University in Chile. He completed his MS and PhD in Molecular Biology at the Albert Einstein College of Medicine in New York, USA. Professor Reinberg has been a Principal Investigator for over three decades, having worked at the UMDNJ-Robert Wood Johnson Medical School in New Jersey, and also the New York University School of Medicine, mentoring numerous graduate students over the years. As Distinguished Professor at the University of Miami, his laboratory work focuses on the epigenetic basis of gene repression to understand the generation of different cell lineages. This includes the study of mechanisms underlying the cellular compartmentalisation of repressed genes and how their three-dimensional structure is derived and maintained. Professor Reinberg has received numerous honours and awards, including from the National Academy of Sciences in 2015. He was the American Association for the Advancement of Science Fellow in 2015 and the International Blaise Pascal Chair at the Curie Institute, Paris, France, in 2017.

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Dr Havva Ortabozkoyun obtained her BSc in Molecular Biology and Genetics from the Middle East Technical University in Turkey and her MSc in Cancer Genomics and Developmental Biology from Utrecht University in The Netherlands. She completed an MSc and PhD in Stem Cell Biology at the New York University Grossman School of Medicine. Currently a Postdoctoral Fellow in the laboratory of Professor Reinberg, Dr Ortabozkoyun studies the regulation of distinct chromatin boundaries by members of an array of zinc finger proteins, including MAZ, PATZ1, ZNF263, and other zinc finger proteins implicated at boundaries, and their role in gene regulation and genome architecture during differentiation and development. Dr Ortabozkoyun serves as a guest editor in JoVE in CRISPR-based gene editing and offers scientific consultation in CRISPR-editing in mice during the establishment of a Functional Genomics Facility at the University of Miami. She aims to set up an interdisciplinary research programme to understand epigenetic mechanisms leading to cellular diversity during development and disease processes, including cancer.

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http://umiamihealth.org/sylvester-comprehensive-cancer-center/research/labs/reinberg-lab

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FURTHER READING AND RESOURCES

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