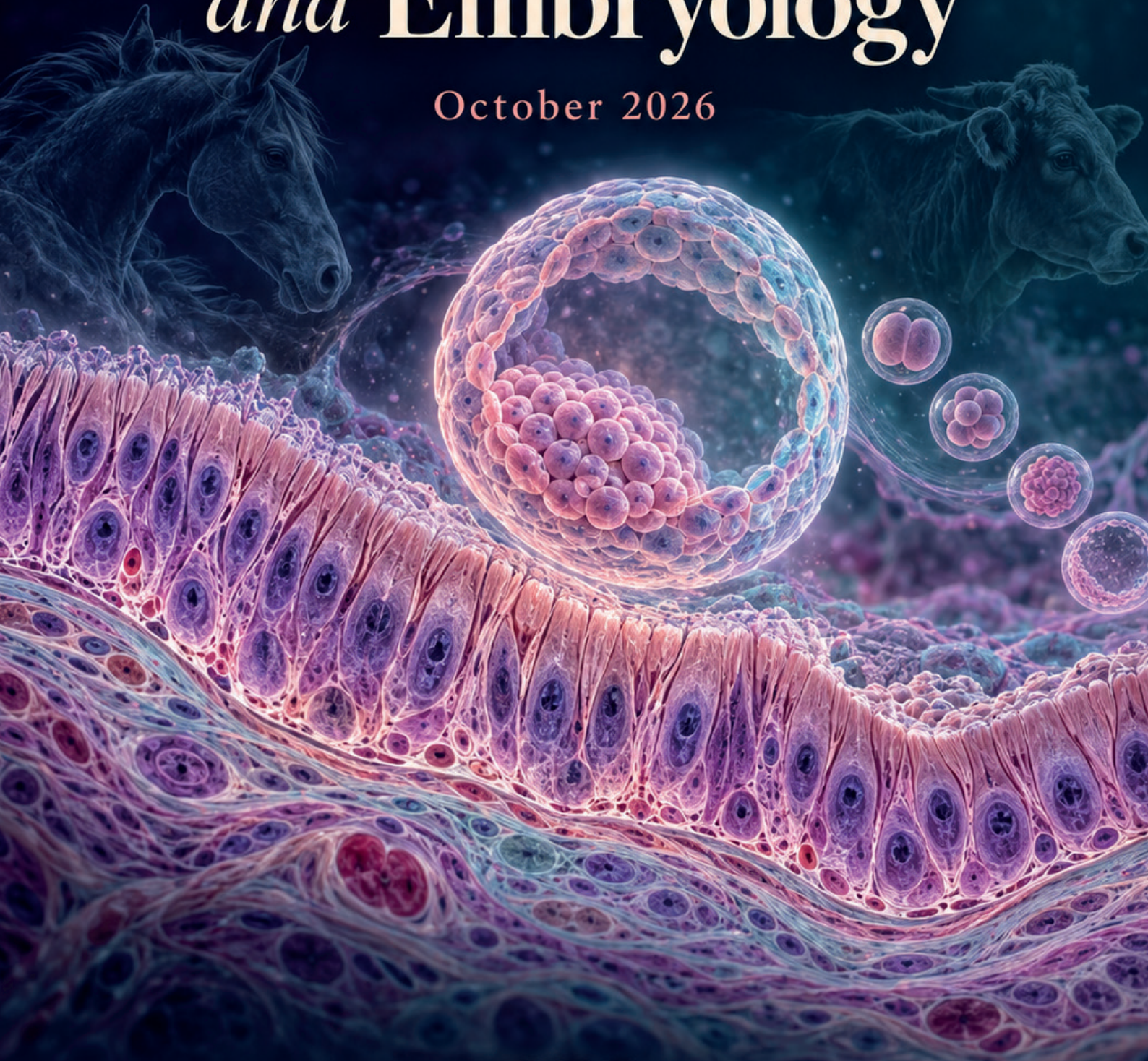


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— *in the field of* —
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and Embryology**

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— EDITOR —

Prof. Dr. Ali BAYRAKDAR

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**INTERNATIONAL STUDIES
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CHAPTER 1

THREE-DIMENSIONAL GENOME ORGANIZATION IN EMBRYOGENESIS AND DEVELOPMENTAL GENE CONTROL

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1. Introduction

Embryogenesis is a complex process in which a single fertilized egg cell gives rise to numerous distinct cell types. In this process, all cells carry the same DNA sequence, yet they express different genes at different times and at different levels. Studies conducted in recent years have shown that this difference cannot be explained by the DNA sequence alone. The three-dimensional arrangement of the genome within the nucleus plays a fundamental role in the control of gene expression (Liu et al., 2023). Linear DNA undergoes a series of compaction and folding events in the nucleus and forms structural units such as the chromosome territory, the compartment, the topological domain and the chromatin loop (Liu et al., 2023). These structures undertake important tasks in the regulation of gene expression, in cell differentiation and in disease processes (Liu et al., 2023).

Three-dimensional genome architecture is not a static structure. It changes in an orderly manner throughout development, and this change directly affects the gene programmes that determine the fate of cells (Bonev et al., 2017). In embryogenesis, specific genes must be activated or repressed. This choice is closely related to the spatial arrangement of the genome (Bonev et al., 2017). For this reason, understanding how the genome folds and how this folding changes has become one of the central questions of developmental biology.

The aim of this chapter is to explain three-dimensional genome organization during embryogenesis and its relationship with developmental gene control in plain language. The chapter first introduces the structural levels of the genome. It then addresses the mechanisms by which these structures are established. It subsequently examines embryonic gene control, developmental reorganization and research methods. Finally, it touches upon future research directions.

2. Structural Organization of the Three-Dimensional Genome

In mammalian cells, linear DNA folds at several interrelated levels. These levels are the chromosome territory, the compartment, the topological domain and the chromatin loop (Liu et al., 2023). Contact maps obtained by methods such as Hi-C make this hierarchical order visible at different scales (Paulsen et al., 2018). Each of the structural levels operates at a different scale and affects gene expression in different ways (Jerković & Cavalli, 2021).

2.1 Chromatin Loops

Chromatin loops are the smallest-scale structural units of the genome (Liu et al., 2023). These loops are formed by the physical approximation of two DNA regions that lie far apart from one another (Kim & Yu, 2020). Loops are mostly established within topological domains (Davidson et al., 2023). In mammalian cells, each topological domain contains more than one chromatin loop, and these loops are preferentially anchored at convergent CTCF motifs (Kim & Yu, 2020). The size of the loops can reach the level of hundreds of kilobases (Stevens et al., 2017). Chromatin loops ensure that a gene and the regulatory elements that control it come into the same spatial proximity (Panigrahi & O'Malley, 2021). For this reason, loops function directly in the regulation of gene expression.

2.2 Topological Domains

Topological domains are chromatin regions that interact densely within themselves and are relatively insulated from neighbouring regions (Oudelaar et al., 2020). These domains are generally between 0.1 and 1 megabase in size and are bounded by convergent CTCF binding sites (Oudelaar et al., 2020). Each domain is separated from its neighbour by distinct boundary regions at which chromatin contacts are abruptly interrupted (Kim & Yu, 2020). Topological domains have been associated with cell differentiation and gene regulation (Kim & Yu, 2020).

Topological domain boundaries are generally established early in development and remain relatively constant throughout differentiation (Oudelaar et al., 2020). In contrast, the interactions within domains are extensively reorganized in differentiating cells, and specific interactions are established between enhancers and promoters (Oudelaar et al., 2020). This finding shows that one part of the structure is stable while another part is dynamic (Oudelaar et al., 2020). The formation of topological domains occurs partly independently of transcription in organisms such as the mouse, *Xenopus* and *Drosophila* (Ling et al., 2022). This observation suggests that the structure can also be established before gene expression.

2.3 A and B Compartments

At the megabase scale, Hi-C experiments divide the genome into two compartments called A and B (Stevens et al., 2017). These compartments segregate according to transcriptional status (Wong et al., 2021). The A compartment is mostly located in the interior of the nucleus and contains active chromatin. The B compartment, by contrast, accumulates near the nuclear envelope and is enriched in repressive histone modifications such as H3K9me2/3 and H3K27me3 (Wong et al., 2021). Interestingly, the A and B compartments persist even when

topological domains disappear (Wong et al., 2021). This situation indicates that compartmentalization rests on a mechanism that is independent of topological domain architecture.

The B compartment is itself divided into subdivisions (Paulsen et al., 2018). The B1 subcompartment contains facultative heterochromatin marked by H3K27me3. B2 comprises pericentric heterochromatin and is associated with both the nuclear lamina and the nucleolus. B3, in turn, is enriched at the nuclear lamina but is not found around the nucleolus (Paulsen et al., 2018). Regions associated with the nuclear lamina, namely the areas called LADs, constitute approximately forty percent of the mammalian genome. These regions are defined by broad blocks of CpG hypomethylation and by the accumulation of H3K9me2/3 and H3K27me3 (Siegenfeld et al., 2022). Studies have shown that compartmentalization is not dependent on the nuclear lamina alone. Even in inverted nuclei, compartmentalization is preserved, and heterochromatin itself directs compartmentalization (Falk et al., 2019).

3. How Genome Architecture Is Established

The structural levels of the three-dimensional genome do not form spontaneously. These structures are established and maintained by specific molecular mechanisms (Kim & Yu, 2020). Among these mechanisms, loop extrusion, boundary elements, histone modifications and DNA methylation stand out.

3.1 Cohesin and the Loop Extrusion Model

The strongest model explaining the formation of chromatin loops is the loop extrusion model. According to this model, ring-shaped factors such as cohesin bind to chromatin and progressively pull DNA to form gradually growing loops. These factors stop when they encounter boundary proteins, and the loop is fixed (Fudenberg et al., 2016). Loss of the core cohesin subunit SCC1 leads to the loss of topological domains. In contrast, loss of the cohesin release factor WAPL causes chromatin loops to grow (Li et al., 2020). It has been demonstrated directly in in vitro experiments that cohesin complexes really do pull and enlarge DNA loops (Nora et al., 2020). This evidence strongly supports the loop extrusion model.

3.2 CTCF and Boundary Elements

The protein CTCF plays a central role in the formation of topological domain boundaries. CTCF is found at topological domain boundaries and is required for the formation of these domains and for the accumulation of cohesin at the boundaries (Davidson et al., 2023). CTCF

contains eleven zinc finger regions and recognizes the DNA sequence asymmetrically. For this reason, CTCF is positioned on DNA in a directional manner (Davidson et al., 2023). Most CTCF binding sites are located in convergent orientation. This orientation indicates that CTCF behaves like an asymmetric barrier against cohesin-mediated loop extrusion (Davidson et al., 2023).

The direct interaction of CTCF with cohesin has also been resolved. The N terminus of CTCF binds directly to the SA2-SCC1 subcomplex of cohesin. Substitution of key amino acids at this interface eliminates the loops anchored at CTCF (Li et al., 2020). This finding shows that CTCF is not only a barrier but also a factor that stabilizes the loop. The directional nature of CTCF binding is a fundamental principle of genome folding, and inversion of a CTCF motif can lead to displacement of the relevant DNA loop (Nora et al., 2020).

3.3 Histone Modifications

Histone modifications play a key role in the organization of chromatin throughout embryonic development. These modifications affect the interaction of transcriptional regulators with chromatin (Xu et al., 2020). After fertilization, the re-establishment of histone 3 lysine 14 acetylation and histone 3 lysine 9 trimethylation is critical for the timely activation of zygotic genome activation genes (Dai et al., 2024). The SWI/SNF complex also acts as a chromatin remodeler and undertakes an important task in the activation or repression of tissue-specific transcription factors (Dai et al., 2024).

Parental gametes carry different epigenetic marks (Shi & Wu, 2009). In spermatozoa, somatic linker histones are replaced by testis-specific variants and most of the histones are replaced by protamines. In the oocyte, by contrast, the H3K4me3 level may be higher owing to meiotic entry (Shi & Wu, 2009). These different marks are transmitted to the embryo after fertilization (Xu et al., 2020). In recent years, methods such as low-input ChIP-seq and CUT&RUN have made it possible to map the reprogramming of histone modifications in mammalian early embryos at high resolution (Xu et al., 2020).

3.4 DNA Methylation

DNA methylation is an important epigenetic modification that regulates the function of the genome. In mammals, methylation occurs predominantly at symmetric CpG dinucleotides (Reik et al., 2001). The Dnmt1 enzyme prefers hemimethylated substrate and ensures the maintenance of methylation patterns across cell divisions. The Dnmt3a and Dnmt3b enzymes, by contrast, establish new methylation (Reik et al., 2001). In mammals, there are two

developmental periods in which methylation patterns are reprogrammed genome-wide. These periods are the germ cells and the preimplantation embryos (Reik et al., 2001). This reprogramming plays an important role in the acquisition of totipotency and pluripotency, in the control of imprinting and in the supervision of transposons (Feng et al., 2010).

In human preimplantation development, it has been shown that the paternally derived genome is extensively demethylated. In this process, a transient global hypomethylation state arises and most CpGs are affected (Smith et al., 2014). The oocyte already displays global hypomethylation and transmits to the embryo its own distinct set of methylated regions, which include CpG island promoter regions. These regions are preserved in the early embryo but disappear during cell specialization (Smith et al., 2012). In mouse embryos, demethylation takes place in a stepwise manner during the first cleavage stages, and this process is associated with the exclusion of the Dnmt1o protein from the nucleus (Dean et al., 2001). Imprinted genes, however, escape this global demethylation and their methylation marks are maintained through Dnmt1 (Hirasawa et al., 2008).

The loss of methylation coincides with the global decrease in repressive histone modifications (Halstead et al., 2020). In mouse zygotes, histone mobility increases, chromatin acquires a dispersed appearance and chromosentromeres disappear. After fertilization, three-dimensional chromatin architecture is largely absent and is progressively established throughout preimplantation development (Halstead et al., 2020). This observation indicates that epigenetic reprogramming is intertwined with the establishment of architecture.

4. Embryonic Gene Control

Genome architecture forms the ground on which developmental gene control rests (Ramasamy et al., 2023). At the centre of this control are the interactions between enhancers and promoters and the transcription factors that establish these interactions.

4.1 Enhancer and Promoter Interactions

Enhancers are short, non-coding DNA sequences that direct the expression of their target genes (Panigrahi & O'Malley, 2021). In mammals, enhancers can be located far away from the genes they control, either upstream or downstream. In order to activate genes, enhancers interact with promoters within three-dimensional chromatin structures (Ramasamy et al., 2023). For this reason, enhancer-mediated gene activation is closely related to the three-dimensional organization of the genome (Ramasamy et al., 2023). The Mediator and cohesin complexes contribute to these interactions (Ramasamy et al., 2023).

The current model that explains the enhancer and promoter interaction combines several components. According to this model, cohesin-mediated loop extrusion produces higher-level structures bounded by CTCF. Transcription factors on the enhancer and the promoter establish phase condensates composed of coactivators such as BRD4 and Mediator. The structure bounded by CTCF and cohesin creates a smaller DNA compartment and increases the frequency of random interaction between the enhancer and the promoter (Crump et al., 2021). Studies have shown that an enhancer can recruit cohesin to itself, create contact domains and interact with CTCF sites. At short distances, approximately between 0 and 100 kilobases, mechanisms independent of cohesin can also support gene activation (Rinzema et al., 2022).

Not all of these interactions result in gene expression. Enhancer and promoter interaction and transcriptional activation are two successive but distinct processes. Interactions are generally established before activation, but not every interaction leads to activation (Yang & Hansen, 2024). During differentiation, cell type-specific enhancer and promoter contacts are established simultaneously with gene expression (Bonev et al., 2017).

4.2 Transcription Factors and Pioneer Factors

In embryonic development, different combinations of transcription factors are required to change cell fate (Iwafuchi & Zaret, 2016). Within these groups, a subset called pioneer factors stands out for their ability to bind silent and unmarked chromatin. These factors initiate the recruitment of other regulatory proteins and give new function to regulatory DNA sequences (Iwafuchi & Zaret, 2014). Pioneer factors can bind to their target sequences on nucleosomal DNA and initiate regulatory events in silent chromatin (Zaret & Mango, 2016). Following pioneer factor binding, the local chromatin can open, be repressed or become transcriptionally active (Zaret & Mango, 2016). These factors also play a critical role in cellular reprogramming and are important for understanding how developmental lineages are established (Iwafuchi & Zaret, 2016).

Studies carried out in the *Drosophila* embryo have shown that chromatin accessibility is determined by a two-layered process. The pioneer factor Zelda opens accessibility depending on motif affinity. Factors involved in axis patterning, in turn, increase accessibility in the contexts in which they mediate enhancer activation. Accordingly, accessibility is first provided through pioneering, and this stage renders enhancers accessible but does not necessarily make them active. The second stage is the stage in which enhancer activation takes place with the correct combination of transcription factors (Brennan et al., 2023). It has been proposed that

the Dux factor may play a similar pioneering role in mouse embryos. It has been shown that half of the accessible regions gained in two-cell embryos are bound by Dux (Ladstätter & Tachibana, 2018).

4.3 Transcriptional Condensates

Transcription takes place in the complex physicochemical environment of the nucleus. Studies conducted in recent years have shown that transcription components can form distinct compartments within the nucleus through liquid-liquid phase separation (Demmerle et al., 2023). These dynamic assemblies are called transcriptional condensates (Ryu et al., 2024). These structures are formed by multivalent interactions between component proteins (Ryu et al., 2024). The Mediator complex, numerous transcription factors, coactivators and RNA polymerase II form condensates during transcription (Panigrahi & O'Malley, 2021).

The products of transcription also regulate condensate formation through feedback. In regulatory DNA elements, low RNA levels support condensate formation, whereas high RNA levels can dissolve condensates (Panigrahi & O'Malley, 2021). In the nucleus, the phase separation of the transcriptional coactivator BRD4 with Mediator directs the formation of transcriptional condensates at large enhancer clusters called super-enhancers and facilitates gene activation (Ling et al., 2022). The nucleosome, the basic functional unit of chromatin, is formed by the wrapping of approximately 146 base pairs of DNA around a histone octamer (Wang et al., 2021). This structure constitutes the physical ground of the processes regulated by condensates. In this field, it is proposed that a distinction be made between the definition of physical phase separation and the complex biomolecular assemblies required for productive gene expression (Demmerle et al., 2023).

5. Developmental Reorganization

Three-dimensional genome architecture does not remain constant throughout embryogenesis (Pecori & Torres-Padilla, 2023). It is re-established after fertilization and is reorganized in a cell type-specific manner during differentiation.

5.1 Zygotic Genome Activation and the Establishment of Architecture

Zygotic genome activation is a critical event that marks the onset of gene expression, and it takes place together with the re-establishment of three-dimensional chromatin (Ling et al., 2022). After fertilization, topological domains and compartments are either absent or not as distinct as in somatic cells in most of the vertebrates and invertebrates examined. The insulation

score of topological domains and compartment strength increase progressively as development proceeds (Pecori & Torres-Padilla, 2023). Zebrafish is the only known exception to this rule, and topological domains and compartments are distinct even before zygotic genome activation (Pecori & Torres-Padilla, 2023).

In the mouse, zygotic genome activation takes place in the two-cell embryo (Ling et al., 2022). Inhibition of zygotic genome activation did not prevent the formation of topological domains. This finding suggests that topological domain formation precedes the transcriptional control function. Accordingly, topological domains first function as building blocks of architecture and then become transcriptional regulators (Boltsis et al., 2021). In human embryos, by contrast, the genome is in an unstructured state at the two-cell stage. Compartments and topological domains begin to appear by the eight-cell stage and become more distinct at the blastocyst stage. Topological domain boundaries are established mainly after the onset of zygotic genome activation, with a sharp increase in CTCF expression (Ling et al., 2022). In human embryos, CTCF is expressed at a high level at the zygotic genome activation stage, and this coincides with the time at which topological domains are observed (Xu et al., 2020). In human embryos, the establishment of topological domains is dependent on zygotic genome activation (Xu et al., 2020).

There are also differences between species. In the mouse and *Drosophila*, structural features such as A/B compartments or topological domains are either severely weakened or absent before zygotic genome activation. These structures emerge at or immediately after the onset of zygotic genome activation (Li et al., 2020). In zebrafish, chromatin structure displays a systemic pattern of loss and reacquisition, and structured organizations disappear during zygotic genome activation. In humans, by contrast, topological domains are progressively established while compartments disappear in the two-cell embryo and are re-established throughout development (Li et al., 2020).

5.2 Cellular Differentiation

During cell differentiation, genome architecture changes markedly. In this process, a shift from the A compartment towards the B compartment is observed and the interactions within the B compartment increase. The number of topological domains decreases while the size of the domains increases (Bonev et al., 2017). Tissue-specific enhancer and promoter interactions are established in accordance with cell-specific gene expression (Ling et al., 2022).

A comprehensive study on mouse neural differentiation showed that several factors affect chromatin interactions throughout differentiation. In this study, transcription was found to be related to chromatin insulation and long-range interactions. However, dCas9-mediated activation is not sufficient to create new topological domain boundaries. The study also detected long-range contacts between the gene bodies of exon-rich active genes in all cell types. During neural differentiation, contacts between active topological domains weaken while inactive domains interact more strongly with one another. A widespread Polycomb network in stem cells is disrupted, while dynamic interactions emerge between neural transcription factors under in vivo conditions (Bonev et al., 2017).

5.3 Lineage Specificity and Hox Genes

Hox genes are master developmental determinants that organize structures along the trunk and limb axes (Deschamps & Duboule, 2017). In mouse limb development, Hoxd genes are expressed in two waves. The early wave determines the arm and the forearm, while the late wave forms the digits. The transition between early and late regulation involves a functional switch between two opposing topological domains. Some Hoxd genes located in the middle of the cluster first interact with the telomeric domain, then orient towards the centromeric domain and establish new contacts there (Andrey et al., 2013). This transition reflects the modular structure of the limbs and the separate evolutionary histories of their different parts. At the same time, it ensures the formation of an intermediate region with low HOX protein content in the region that we develop as the wrist (Andrey et al., 2013).

The molecular details of this regulatory strategy have also been resolved. HOX13 proteins close the telomeric topological domain through a global repressive mechanism. At the same time, they interact directly with the distant enhancers of the centromeric topological domain and sustain the activity of this domain. This dual mechanism explains how the two regulatory regions operate sequentially and in a mutually exclusive manner (Beccari et al., 2016). It has been shown that the HoxD cluster lies between two opposing topological domains and that the three-directional domain is active early, after which this domain is closed and the five-directional domain is activated (Boltsis et al., 2021). The expression of HoxA genes in developing limbs is likewise controlled by extensive three-dimensional chromatin interactions. This architecture is tissue-specific, is established independently of enhancer activity and is strengthened when the enhancer becomes active (Berlivet et al., 2013).

The topological organization of Hox clusters also carries an evolutionary dimension. In slowly evolving invertebrates such as amphioxus, the ancestral Hox locus is organized as a single topological domain containing all Hox genes and regulatory enhancers. At the origin of vertebrates, this single domain was divided into two domains located on either side of the cluster. This division enabled the separation of the spatial and temporal regulatory inputs that explain the collinearity of Hox genes and made possible vertebrate evolutionary innovations such as paired appendages (Tena & Santos-Pereira, 2021). This arrangement is also related to developmental timing. The open state of the Hox cluster is spatially retained in memory during elongation of the body axis, and limb bud cells inherit this state (Deschamps & Duboule, 2017). Topological domains allow more enhancer and promoter contacts during development and differentiation, and this is important for directing specific cell programmes (Boltsis et al., 2021).

6. Research Approaches

The study of three-dimensional genome organization rests on molecular and imaging-based methods that complement one another (Jerković & Cavalli, 2021). Each method has its strengths and limitations.

6.1 Genomic Methods

Chromosome conformation capture technologies have provided important information about genome folding (Bonev et al., 2017). Methods such as Hi-C are suitable for detecting compartments and topological domains. However, the resolution of these methods is directly related to the restriction enzymes used and to sequencing depth. For this reason, even when Hi-C libraries are sequenced with billions of reads, the unbiased detection of local interactions such as enhancer and promoter contacts depends largely on the distribution of restriction sites. This distribution is not uniform across the genome (Jerković & Cavalli, 2021).

To overcome this problem, methods such as Micro-C have been developed. Micro-C replaces the restriction enzymes used in Hi-C with micrococcal nuclease digestion. This approach produces a fairly uniform fragmentation down to the nucleosome level and increases local resolution. In addition, Micro-C preserves information about nucleosome positioning, and this information can be analysed together with chromatin contact data (Jerković & Cavalli, 2021). Single-cell Hi-C is the first single-cell chromatin analysis technique to be successfully established (Jerković & Cavalli, 2021). This method made it possible to determine genome-wide structures at the 100 kilobase scale in a single G1-phase mouse embryonic stem cell (Stevens et al., 2017). The development of low-input and single-cell Hi-C methods has made it

possible to examine chromatin structure genome-wide in early embryonic development (Li et al., 2020).

In addition to chromosome conformation capture-based methods, new approaches have also been developed. Methods such as genome architecture mapping and SPRITE have provided information about the genome-wide three-dimensional spatial structure and physical proximity of DNA in the interphase nucleus (Tomikawa, 2024). The field is continuously supplied with new methods in order to obtain increasing resolution and throughput (Übelmesser & Papantonis, 2019).

6.2 Imaging Methods and Their Limitations

Chromosome conformation capture-based methods indirectly measure the proximity between two or more genomic loci through molecular processing and sequencing steps. For this reason, the extent to which the results obtained reflect the true three-dimensional structure in the cell must be carefully verified (Tomikawa, 2024). Microscopy-based methods, by contrast, can image the appearance of the target directly (Tomikawa, 2024). Imaging determines the true positions and distances between any two loci in three dimensions and therefore offers a more complete picture than chromosome conformation capture methods. Hi-C is a destructive method, requires fixed cells and has a resolution of approximately 2 kilobases (Shaban et al., 2020). Today, the simultaneous imaging of numerous loci is carried out in fixed cells, with a few exceptions. However, this approach has the potential to progress towards analysis in living cells in the future (Shaban et al., 2020).

7. Conclusion and Future Research

This chapter has addressed the structural levels of three-dimensional genome organization during embryogenesis, the mechanisms of its establishment and its relationship with developmental gene control. It is seen that the genome is organized into hierarchical units such as the chromosome territory, the compartment, the topological domain and the chromatin loop (Liu et al., 2023). While some of these structures are established early in development and remain relatively constant, the interactions within domains are extensively reorganized throughout differentiation (Oudelaar et al., 2020). Topological domains first function as building blocks of architecture and then become transcriptional regulators (Boltsis et al., 2021).

Zygotic genome activation is closely related to the re-establishment of three-dimensional chromatin, and the timing of this process differs between species (Li et al., 2020; Pecori & Torres-Padilla, 2023). Histone modifications, DNA methylation and chromatin remodelling

accompany this establishment process (Halstead et al., 2020). Enhancer and promoter interactions are explained by a model in which cohesin-mediated loop extrusion and transcriptional condensates are combined (Crump et al., 2021; Panigrahi & O'Malley, 2021). In the example of Hox genes, how transitions between topological domains direct developmental patterning has been shown in detail (Andrey et al., 2013; Beccari et al., 2016).

Several directions stand out for future research. The first is a more detailed examination of the role of phase separation in the establishment of three-dimensional chromatin. How liquid-liquid phase separation forms transcriptional condensates and what its function is in early embryogenesis have still not been fully elucidated (Ling et al., 2022). The second is the development of single-cell and live-cell imaging methods. Since Hi-C is a destructive method, the evolution of the chromatin structure of a single cell over time cannot be obtained with this method (Shaban et al., 2020). The third is a better understanding of the relationship between disruptions in genome architecture and disease (Krumm & Duan, 2018).

The disruption of topological domains has been defined as an important mechanism in developmental diseases. The testing of copy number variations for diagnostic purposes has become part of the diagnosis of diseases such as congenital malformation and intellectual disability. With the high-resolution array comparative genomic hybridization method, a disease-causing variant can be identified in 10 to 20 percent of cases in large studies (Lupiáñez et al., 2016). Disruption of TAD boundaries by structural variations can lead to the reorganization of the interactions of neighbouring genes with their enhancers. Rearrangements in the WNT6, IHH, EPHA4 and PAX3 region disrupt normal topology and cause inappropriate interactions and misexpression (Lupiáñez et al., 2015). Similarly, genetic or epigenetic disruption of CTCF binding sites at boundary regions can lead to the fusion of two neighbouring domains, and this situation affects the genes contained within them (Krumm & Duan, 2018). In the EPHA4, SOX9, IHH and Pitx loci, structural variations have been shown to disrupt TAD boundaries and to reorganize gene and enhancer interactions (Middelkamp et al., 2019). These mechanisms are not limited to developmental disorders. It has also been shown that deletions at TAD boundaries lead to the activation of oncogenes (Huynh & Hormozdiari, 2019).

In conclusion, three-dimensional genome organization is an inseparable part of gene control in embryogenesis. Understanding the spatial arrangement of the genome is necessary both to answer the fundamental questions of developmental biology and to explain the molecular basis of developmental diseases. Genomic and imaging methods of increasing resolution are rapidly expanding the body of knowledge in this field (Übelmesser & Papantonis, 2019).

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CHAPTER 2

HISTOLOGICAL ORGANIZATION AND FUNCTIONAL CHARACTERISTICS OF BIOLOGICAL BARRIERS

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1. Definition and Basic Functions of Biological Barriers

Biological barriers are specialized cellular and extracellular structures that regulate the passage of substances and cells between different compartments of the organism via tissue-specific mechanisms (Fröhlich, 2002). These barriers do not merely form a physical boundary; they also contribute to selective permeability, immune defense and the maintenance of tissue homeostasis (Constant et al., 2021; Zihni et al., 2016).

Biological barriers have three fundamental functions:

- **Protection:** Epithelial tissues that function as barriers form a physical boundary on the organism's surfaces in contact with the external environment- such as the skin, respiratory tract and gastrointestinal tract- thereby constituting a key component of the first line of defense against pathogens and other potentially harmful agents. Consequently, epithelial cells not only act as a physical barrier but also play a significant role in the body's natural defense mechanisms (Constant et al., 2021). Tight junctions are of critical importance in this barrier function (Anderson & Van Itallie, 2008; Günzel & Yu, 2013).

- **Selective permeability:** The barriers formed by epithelial tissue are not completely impermeable structures; rather, they are dynamic structures that regulate the exchange of substances between different compartments in a controlled and selective manner. In particular, the intestinal epithelium, despite consisting of a single layer of cells, forms a selectively permeable barrier that supports the absorption of nutrients and the secretion of metabolic waste whilst preventing the leakage of potentially harmful luminal contents (Kuo et al., 2022). The passage of substances through barriers can be facilitated by various mechanisms, such as the transcellular pathway through cells or the paracellular pathway between cells. The permeability of these pathways and the transport mechanisms employed vary depending on the tissue in which the barrier is located and the properties of the substance being transported (Günzel & Yu, 2013). Tight junctions regulate the paracellular pathway between neighboring epithelial cells, thereby limiting the passage of substances between cells and contributing to the barrier's selective permeability (Anderson & Van Itallie, 2008; Günzel & Yu, 2013).

- **Maintenance of tissue homeostasis:** For different tissues to maintain their physiological functions, the preservation of their specific microenvironmental conditions is required. Biological barriers regulate the passage of substances by forming a selectively permeable boundary between different compartments, thereby contributing to the maintenance of tissue homeostasis (Balda & Matter, 2023). Tight junctions between epithelial and endothelial cells play a key role in regulating the exchange of substances between

compartments by controlling the paracellular passage of ions and solutes (Zihni et al., 2016). Thus, biological barriers support the maintenance of tissue-specific physiological conditions and the continuity of cellular functions (Förster, 2008).

2. The Histological and Structural Basis of Barriers

Epithelial and endothelial cells are the primary cell types involved in the formation of biological barriers within the body. The specialized junctional complexes between these cells play a key role in maintaining the structural integrity and function of these barriers (Balda & Matter, 2023). The anatomical and histological organization of these barriers varies depending on the tissue in which they are found. Whilst endothelial cells form the primary barrier structure in the blood-brain and inner blood-retina barriers, Sertoli cells fulfil this role in the blood-testis barrier, and retinal pigment epithelial cells in the outer blood-retinal barrier (Fröhlich, 2002). In addition to the primary barrier cells, the structure of biological barriers also includes various cellular and extracellular components. For example, in the blood-brain barrier, endothelial cells, pericytes, the basement membrane and the perivascular processes of astrocytes function together (Dotiwala et al., 2023). Tight junctions between cells in epithelia and endothelium, meanwhile, regulate the paracellular passage of substances, thereby contributing to the selective permeability properties of the barriers (Balda & Matter, 2023).

3. Classification of Biological Barriers

Biological barriers differ according to the anatomical and histological characteristics of the tissue in which they are found, as well as the cellular components involved in barrier formation and their functions. Accordingly, biological barriers can be categorized, based on their fundamental structural characteristics, as endothelial-derived, epithelial-derived, and multicomponent barriers in which multiple cellular or extracellular components act in concert (Fröhlich, 2002).

Examples of structures in which endothelial cells form the primary barrier component include the blood-brain barrier and the inner blood-retinal barrier. In the blood-thymus barrier, however, epithelial reticular cells, as well as capillary endothelial cells, contribute to the structural organization of the barrier (Bruss & Ely, 2023; Fröhlich, 2002). Examples of structures in which epithelial cells perform the primary barrier function include the blood-testis barrier and the outer blood-retinal barrier. The blood-testis barrier is formed by specialized junctional complexes between neighboring Sertoli cells, whilst the outer blood-retinal barrier relies on tight junctions between retinal pigment epithelial cells (Fröhlich, 2002).

Some biological barriers, however, have a more complex organization in which different cellular and extracellular components act in concert. For example, the intestinal mucosal barrier

consists of a single layer epithelial tissue along with components such as mucus and the glycocalyx found on the luminal surface (Capaldo et al., 2017; Zhang et al., 2015). In the glomerular filtration barrier, fenestrated capillary endothelium, the glomerular basement membrane and podocytes all play a role (LeBleu et al., 2007); whilst in the placental barrier, the syncytiotrophoblast layer and foetal capillary endothelium play a role in regulating the exchange of substances between the maternal and foetal circulations (Arumugasaamy et al., 2020).

In addition to these structural differences, biological barriers share common functions such as protection, selective permeability and the maintenance of tissue homeostasis. In the following sections, the major biological barriers in the organism will be discussed individually in terms of their histological organization, basic functions and the structural components involved in maintaining barrier integrity.

4. Major Biological Barriers in the Body

4.1. Blood-Thymus Barrier

The blood-thymus barrier is a specialized structure that forms a selective boundary between the circulating blood in the thymic cortex and the cortical microenvironment where developing T lymphocytes are located (Ribatti, 2015). By largely restricting the passage of circulating macromolecules into the thymic cortex, it contributes to the preservation of the microenvironment necessary for the development and selection of thymocytes (Bruss & Ely, 2023; Ribatti, 2015). However, the blood-thymus barrier does not provide absolute isolation; haematopoietic progenitor cells enter the tissue via specific vascular regions of the thymus and participate in the cellular development process (Bruss & Ely, 2023).

Anatomically, the thymus consists of two lobes surrounded by a connective tissue capsule, and the lobes are divided into smaller lobules. Each lobule comprises two main regions: the cortex, which stains more darkly, and the medulla, which stains more lightly (Bruss & Ely, 2023; Crivellato et al., 2004). Classical studies using electron microscopy have shown that a blood-thymus barrier exists against macromolecules circulating in the blood; however, this barrier is confined to the cortex. In the medulla, there is no blood-thymus barrier as distinct as that in the cortex, and macromolecules in circulation can reach this region more easily. In adults, the blood-thymus barrier exhibits a structural organization comprising five components: capillary endothelial cells, the endothelial basement membrane, the perivascular space, the basement membrane of the epithelial reticular cells, and the epithelial reticular cells themselves (Raviola & Karnovsky, 1972). In this organization, capillary endothelial cells, which are connected to one another by tight junctions, form the innermost component of the barrier, whilst

more externally, flat thymic epithelial reticular cells, connected to one another by desmosomes and tight junctions, are situated (Bruss & Ely, 2023). Furthermore, macrophages distributed throughout the cortical parenchyma contribute to the trapping and phagocytosis of macromolecules that manage to cross the barrier (Bruss & Ely, 2023; Raviola & Karnovsky, 1972). In terms of selective permeability, the blood-thymus barrier largely restricts the access of circulating macromolecules to the thymic cortex; as demonstrated by electron-opaque tracer studies, the small number of macromolecules that can pass through the cortical capillary endothelium via vesicular transport are retained by parenchymal macrophages extending along the capillaries (Raviola & Karnovsky, 1972). The blood-thymus barrier does not provide absolute isolation; it permits the entry of haematopoietic progenitor cells into the thymus. In contrast, maturing T lymphocytes leave the thymus via the vessels in the corticomedullary junction and the medullary region, entering the peripheral circulation (Bruss & Ely, 2023).

The physiological importance of the blood-thymus barrier is linked to the protection of the cortical microenvironment, where thymocyte development and selection take place, from circulating macromolecules (Bruss & Ely, 2023; Crivellato et al., 2004). Thymocytes that undergo positive selection in the cortex migrate to the medulla, where they interact with self-peptide-MHC complexes and undergo negative selection. Thymocytes that recognize self-antigens with high affinity are directed towards apoptosis, thereby contributing to the elimination of autoreactive clones and the establishment of central tolerance (Bruss & Ely, 2023; Crivellato et al., 2004; Takada et al., 2017). In this process, antigen-presenting cells, such as cortical and medullary thymic epithelial cells, macrophages and dendritic cells, contribute to the selection of thymocytes via MHC molecules (Bruss & Ely, 2023). Thymic nurse cells (TNCs) are specialized thymic epithelial cells that temporarily take up immature T lymphocytes, thereby contributing to the processes of development and selection (Reyes García & García Tamayo, 2012).

It has been suggested that disruption of the barrier may have immunological consequences. Bubanovic (2003) suggests that disruption of the blood-thymus barrier may contribute to the entry of tumor- or trophoblast-specific antigens into the thymus, the development of deletion or anergy in antigen-specific clones, and the establishment of acquired thymic tolerance; this, in turn, may play a role in tumor and trophoblast escape mechanisms (Bubanovic, 2003).

4.2. Blood-Testis Barrier

The blood-testis barrier is a selective barrier formed by specialized intercellular junctional complexes between adjacent Sertoli cells within the seminiferous tubules. This

barrier divides the seminiferous epithelium into two distinct compartments: the basal and adluminal compartments (Dym & Fawcett, 1970; Mruk & Cheng, 2015). By regulating the passage of substances between the compartments, it creates a controlled microenvironment for germ cells during the stages of meiosis and spermiogenesis. Furthermore, by limiting the exposure of germ cell antigens to the immune system, it contributes to the maintenance of the testis's *immune-privileged* status (Fijak & Meinhardt, 2006; N. Li et al., 2012).

Anatomically, the testis consists of seminiferous tubules and interstitial tissue. The seminiferous tubules are lined by the seminiferous epithelium, which contains Sertoli cells and germ cells at various stages of development. The epithelium is surrounded by a basal lamina and the tunica propria, which includes peritubular myoid cells. The interstitial tissue primarily comprises Leydig cells, blood vessels, macrophages and other immune cells (Fijak & Meinhardt, 2006). The blood-testis barrier is located in the basal region of the seminiferous epithelium, at the level of the junctional complexes between the lateral surfaces of neighboring Sertoli cells (Mruk & Cheng, 2015). Whilst spermatogonia are found in the basal compartment, preleptotene spermatocytes move into the adluminal compartment following the reorganization of the barrier. More advanced primary spermatocytes and spermatids, on the other hand, are primarily located in the adluminal compartment (Dym & Fawcett, 1970; Mruk & Cheng, 2015). Classical electron microscopic studies have shown that the junctions between peritubular myoid cells partially restrict the passage of substances, whilst the primary permeability barrier is formed by the tight junctions between Sertoli cells (Dym & Fawcett, 1970). Subsequent ultrastructural studies have also supported the view that these junctions are one of the key morphological components of the barrier (Gilula et al., 1976; Luaces et al., 2023).

Structurally, the blood-testis barrier is not composed solely of tight junctions; it also includes basal ectoplasmic specializations (basal ES) and desmosome-like junctions. Basal tubulobulbar complexes (basal TBCs) contribute to the reorganization of these junctions (Mita et al., 2011; Vogl et al., 2014; Wong & Cheng, 2005). Whilst tight junctions restrict paracellular passage, the cytoskeletal and signaling proteins associated with junction complexes ensure the dynamic organization of the barrier. Whilst claudin-11 and occludin are key transmembrane proteins, scaffolding proteins such as ZO-1, ZO-2 and ZO-3 contribute to the linkage of tight junctions to the actin cytoskeleton (Luaces et al., 2023; Mruk & Cheng, 2015).

One of the characteristic features of the blood-testis barrier is its controlled reorganization during spermatogenesis. As preleptotene spermatocytes migrate into the adluminal compartment, new barrier junctions are formed on the basal side of these cells, whilst existing junctions on the adluminal side are dismantled. This ensures the progression of germ

cells whilst maintaining barrier integrity (Luaces et al., 2023; Mruk & Cheng, 2015). Whilst the barrier restricts the uncontrolled passage of circulating molecules and immune cells into the adluminal compartment, it is not entirely impermeable. Carrier proteins located on the membranes of Sertoli cells contribute to the controlled passage of molecules essential for germ cell development (Mita et al., 2011).

The physiological importance of the blood-testis barrier is linked to the maintenance of testicular immune privilege, as well as the preservation of a suitable microenvironment for the advanced stages of spermatogenesis (Dym & Fawcett, 1970; Mruk & Cheng, 2015). As some germ cells begin to differentiate following the establishment of systemic immune tolerance, certain antigens arising during this process may be recognized as potentially foreign by the immune system. Whilst the barrier limits the exposure of these antigens to the immune system, Sertoli cells, testicular macrophages, dendritic cells, androgens and immunomodulatory cytokines also contribute to this protection via local immune regulatory mechanisms (Fijak & Meinhardt, 2006; N. Li et al., 2012).

A breach in the integrity of the blood-testis barrier may contribute to the development of inflammatory and autoimmune responses by increasing the exposure of germ cell antigens to the immune system. Factors such as infection, inflammation and trauma may adversely affect the junctional complexes between Sertoli cells (Mruk & Cheng, 2015; Silva et al., 2014). Impaired barrier function is associated with germ cell damage and suppression of spermatogenesis and, in some cases, may contribute to the development of autoimmune orchitis and male infertility (Lustig et al., 2020; Silva et al., 2014).

4.3. Blood-Brain Barrier

The blood-brain barrier is a highly selective biological barrier situated between the peripheral circulation and the central nervous system, playing a key role in maintaining brain homeostasis (Kadry et al., 2020; Shao et al., 2025). Whilst limiting the passage of blood-derived substances into the brain's extracellular fluid, it regulates the transport of molecules required by the central nervous system via specialized mechanisms (Kadry et al., 2020; Manu et al., 2023; Yoon & Kim, 2008). This selective permeability is associated with tight junctions between brain microvascular endothelial cells, the absence of endothelial fenestrations, low levels of vesicular transcytosis and specific transport systems (Kadry et al., 2020; Manu et al., 2023).

The histological organization of the blood-brain barrier is underpinned by the microvascular endothelial cells of the central nervous system. These cells are closely associated with the basal lamina, pericytes and astrocytic perivascular processes. Together with neurons

and other glial cells, these structures form the neurovascular unit, which plays a role in maintaining the functional integrity of the blood-brain barrier (Chen et al., 2022; Kadry et al., 2020). Astrocyte end-feet contribute to the formation of the glia limitans vascularis, a structure associated with the parenchymal basement membrane, by enveloping a large portion of the outer surface of the brain's microvasculature (Manu et al., 2023). These foot processes are particularly rich in aquaporin-4 (AQP4) and play a role in the regulation of water homeostasis (Manu et al., 2023; Schiera et al., 2024). Furthermore, astrocytes contribute to the maintenance of the barrier properties of endothelial cells via various factors such as GDNF and angiopoietin-1. Pericytes, on the other hand, form close interactions with microvascular endothelial cells and play a role in regulating vascular stability, blood flow and barrier permeability (Ding et al., 2025). It has been demonstrated that a deficiency in pericytes can lead to increased endothelial transcytosis, resulting in heightened permeability of the blood-brain barrier (Armulik et al., 2010).

The primary types of junctions between cerebral microvascular endothelial cells are tight junctions and adherens junctions. Whilst adherens junctions contribute to the maintenance of intercellular mechanical integrity and the establishment of connections with the cytoskeleton, tight junctions limit paracellular permeability, thereby ensuring high transendothelial electrical resistance (Schiera et al., 2024). The key transmembrane components of tight junctions include claudins, particularly claudin-5, occludin and junctional adhesion molecules (JAMs) (Ding et al., 2025; Shao et al., 2025). These proteins contribute to the organization of junction complexes and the maintenance of barrier integrity by binding to the actin cytoskeleton via intracellular scaffolding proteins such as members of the zonula occludens (ZO) family (Balda & Matter, 2023; Günzel & Yu, 2013).

Substance transport across the blood-brain barrier is selectively regulated via paracellular and transcellular pathways. Whilst paracellular transport is largely restricted by tight junctions, transcellular transport occurs via specific carrier proteins and other transport mechanisms. Nutrients and certain molecules essential for the central nervous system can pass through endothelial cells via specific transporters. In contrast, efflux transporters limit the accumulation of specific molecules in brain tissue by facilitating their return from endothelial cells into the blood (Jamieson et al., 2017). ATP-dependent efflux transporters, such as P-glycoprotein (P-gp/ABCB1) and the breast cancer resistance protein (BCRP/ABCG2), are located on the luminal membrane of brain microvascular endothelial cells facing the blood. These transporters limit the passage of various drugs, xenobiotics and certain endogenous

compounds into the brain parenchyma and their accumulation there by using ATP energy to transport them back from the endothelial cells into the bloodstream (Shao et al., 2025).

Through this specialized cellular and molecular organization, the blood-brain barrier supports the stability of the central nervous system microenvironment. Conversely, disruption of barrier integrity or increased permeability may facilitate the entry of blood-derived substances into the brain parenchyma, thereby contributing to pathological processes associated with neuroinflammation and various neurological disorders (Shao et al., 2025).

4.4. Blood-Gas Barrier

The blood-gas barrier (alveolar-capillary barrier) is a specialized structure where the exchange of oxygen and carbon dioxide takes place between the air in the alveolar spaces and the blood in the pulmonary capillaries (Leiby et al., 2020). From an anatomical and histological perspective, the barrier exhibits two distinct structural organizations: a thin zone and a thick zone. In the thin zone, there are fused basal laminae between the thin cytoplasmic processes of type I alveolar epithelial cells (type I pneumocytes) and capillary endothelial cells, whilst in the thick zone, a wider interstitial space containing connective tissue components and supporting cells separates these two cellular layers (Hsia et al., 2016; Leiby et al., 2020; Weibel & Knight, 1964). The fusion of the basal laminae in the thin zone contributes to efficient gas exchange by reducing the diffusion distance for gases (West, 2003). The basal lamina, which contains type IV collagen, supports the mechanical integrity of the barrier, whilst the alveolar epithelium and capillary endothelium play a role in regulating the passage of fluids and solutes. The active ion and fluid transport mechanisms of the alveolar epithelium, meanwhile, help to prevent excessive fluid accumulation in the alveolar spaces, thereby contributing to the maintenance of suitable conditions for gas exchange (Leiby et al., 2020; West, 2003).

The physiological importance of the blood-gas barrier lies in its ability to maintain structural integrity against the mechanical stresses generated during respiration whilst ensuring efficient gas exchange (Leiby et al., 2020; West, 2003). Damage to the barrier or an increase in its permeability can lead to the leakage of plasma fluid and proteins into the interstitium or alveolar space, resulting in pulmonary oedema and impaired gas exchange (Leiby et al., 2020). Furthermore, an excessive increase in transmural pressure in the pulmonary capillaries can lead to a loss of mechanical integrity in the barrier (stress failure) and alveolar haemorrhage (Leiby et al., 2020; West, 2003). Consequently, the blood-gas barrier plays a key role in maintaining the balance between gas diffusion, mechanical integrity and alveolar fluid balance (Leiby et al., 2020).

4.5. Blood-Retinal Barrier

The blood-retinal barrier (BRB) is a specialized barrier system between the systemic circulation and retinal tissue that selectively regulates the passage of substances into the retina (O’Leary & Campbell, 2023). Formed by the retinal vessels and the retinal pigment epithelium (RPE), this barrier controls the movement of fluids and molecules, thereby limiting the uncontrolled passage of macromolecules and potentially harmful substances into the retinal tissue and contributing to the protection of the microenvironment of retinal neurons (Liu & Liu, 2019).

From an anatomical and histological perspective, the blood-retinal barrier consists of two main components: the inner (iBRB) and outer (oBRB) barriers. The inner blood-retinal barrier is primarily composed of retinal microvascular endothelial cells (O’Leary & Campbell, 2023). Similar to the blood-brain barrier, these cells lack fenestrations, and the tight junctions between them largely limit paracellular permeability (Liu & Liu, 2019; Tisi et al., 2021). Retinal capillary endothelial cells contribute to the formation of the retinal neurovascular unit by interacting closely with the basement membrane, pericytes, astrocytes and the perivascular processes of Müller cells (O’Leary & Campbell, 2023; Tisi et al., 2021). In particular, the density of pericytes in retinal capillaries is higher than in the capillaries of many other tissues, and these cells play a significant role in maintaining barrier integrity (Tisi et al., 2021).

The outer blood-retinal barrier, on the other hand, is primarily formed by tight junctions between neighboring retinal pigment epithelial (RPE) cells (O’Leary & Campbell, 2023). Whilst the RPE is situated between the fenestrated choriocapillaris and the neuroretina, Bruch’s membrane, located between the basal surface of the RPE and the choriocapillaris, contributes to the regulation of molecular transport via extracellular matrix components (O’Leary & Campbell, 2023; Tisi et al., 2021). Tight junction complexes include transmembrane proteins such as claudins and occludin, along with intracellular scaffolding proteins such as ZO-1, ZO-2 and ZO-3, which link these transmembrane proteins to the actin cytoskeleton (Tisi et al., 2021). Whilst paracellular transport across the blood-retinal barrier is restricted by tight junctions, transcellular transport is regulated via mechanisms such as specific carrier proteins and efflux pumps (Liu & Liu, 2019).

The physiological importance of the blood-retinal barrier is linked to the maintenance of retinal homeostasis and visual function (O’Leary & Campbell, 2023). The integrity of the barrier contributes to the maintenance of retinal fluid balance and the microenvironment necessary for neuronal function (Liu & Liu, 2019; Naylor et al., 2019; O’Leary & Campbell, 2023). A breach in the integrity of the barrier can lead to the accumulation of blood-derived

proteins and fluid in the retinal tissue, the development of retinal oedema, and an adverse effect on visual function (Naylor et al., 2019; O’Leary & Campbell, 2023). In particular, increased permeability of the inner blood-retinal barrier in diabetic retinopathy and functional impairments of the RPE and associated structures in age-related macular degeneration are among the key pathological processes (O’Leary & Campbell, 2023; Tisi et al., 2021). Consequently, the blood-retinal barrier is a specialized barrier system that contributes to the protection of the retinal microenvironment and visual function by regulating the selective passage of substances (O’Leary & Campbell, 2023).

4.6.Glomerular Filtration Barrier

The glomerular filtration barrier is a specialized structure located within the renal glomeruli, between the blood and Bowman’s space, which facilitates the selective ultrafiltration of plasma (A. S. Li et al., 2020; Petrosyan et al., 2019). This barrier allows the passage of water and small solutes whilst ensuring that blood cells and the majority of plasma proteins, particularly albumin- are retained in the circulation (Fissell & Miner, 2018; A. S. Li et al., 2020; Petrosyan et al., 2019). The selectivity of filtration depends on the size and other physicochemical properties of the molecules, as well as the structural and functional integrity of the barrier (A. S. Li et al., 2020).

From a histological perspective, the glomerular filtration barrier comprises three fundamental components: the fenestrated glomerular endothelium, the glomerular basement membrane and the podocytes with their foot processes and slit diaphragms. The glomerular capillary endothelium contains fenestrations approximately 70-100 nm in diameter, and its luminal surface is covered by an endothelial surface layer rich in glycocalyx. This structure contributes to the regulation of filtration selectivity. The glomerular basement membrane, situated between the endothelium and the podocytes, is a specialized extracellular matrix layer comprising primarily type IV collagen, laminin, nidogen and heparan sulphate proteoglycans. In classical transmission electron microscopy, the glomerular basement membrane is characterized as a three-layered structure comprising the lamina rara interna, lamina densa and lamina rara externa. On the side of the barrier facing the urinary space are podocytes, which envelop the glomerular capillaries from the outside. Podocyte foot processes, which develop from the primary extensions of the podocytes, attach to the glomerular basement membrane via integrins and dystroglycans. The filtration slits between adjacent podocyte foot processes are bridged by specialized intercellular junctional structures known as slit diaphragms (A. S. Li et al., 2020). The slit diaphragm is a molecular complex that plays a key role in maintaining the selective permeability of the glomerular filtration barrier (Fissell & Miner, 2018; Kawachi &

Fukusumi, 2020). This structure, which can exhibit a zipper-like organization under electron microscopy, extends between the foot processes of neighboring podocytes, thereby limiting the filtration of plasma proteins. The key components of the slit diaphragm include transmembrane proteins such as nephrin and NEPH1, as well as proteins involved in the organization of the junctional complex, such as podocin, CD2AP and ZO-1. These proteins contribute to the maintenance of the structural integrity of podocytes and filtration selectivity by mediating the interaction of the slit diaphragm with the actin cytoskeleton. In particular, nephrin plays a role in establishing molecular links between neighboring foot processes, whilst podocin, as a membrane-associated protein, supports the organization and function of the slit diaphragm complex (Kawachi & Fukusumi, 2020).

Impaired integrity of the glomerular filtration barrier is one of the primary mechanisms underlying the development of proteinuria and albuminuria (A. S. Li et al., 2020). Changes in the endothelial glycocalyx, structural abnormalities in the glomerular basement membrane, or podocyte foot process effacement and loss of function of slit diaphragm proteins may adversely affect the selective permeability of the barrier (Fissell & Miner, 2018; A. S. Li et al., 2020). Albuminuria is one of the key indicators of glomerular barrier dysfunction and is used in the assessment of various kidney diseases (Petrosyan et al., 2019). Furthermore, pathogenic variants in the NPHS1 and NPHS2 genes, which encode the nephrin and podocin proteins, are associated with the development of hereditary nephrotic syndromes (A. S. Li et al., 2020).

Consequently, the glomerular filtration barrier is a specialized barrier that enables the selective ultrafiltration of plasma through the coordinated functioning of the fenestrated endothelium and glycocalyx, the glomerular basement membrane and the podocyte slit diaphragms; its structural and functional integrity plays a key role in maintaining renal homeostasis.

4.7. The Intestinal Epithelial Barrier

The intestinal epithelial barrier is a specialized physiological barrier that separates contents such as the microbiota, food antigens and pathogens in the intestinal lumen from the body's internal environment, whilst allowing for the selective absorption of nutrients (Moonwiriya et al., 2023). From a histological perspective, this barrier is formed by a single layer of columnar epithelium, in which neighboring cells are connected to one another by tight junctions. The intestinal epithelium comprises several cell types, including enterocytes responsible for nutrient absorption, goblet cells that secrete mucus, enteroendocrine cells, and stem cells located in the intestinal crypts (Kong et al., 2024). Paneth cells, found in the small intestine, play a specific role in the secretion of antimicrobial substances (Okumura & Takeda,

2024). The microvilli on the apical surface of enterocytes facilitate the transport of nutrients by increasing the absorptive surface area (Aleman et al., 2023). Stem cells located in the crypts ensure the continuous renewal of epithelial cells, whilst differentiating cells migrate towards the villus tips along the crypt-villus axis in the small intestine (Kong et al., 2024). Many cells in the small intestinal epithelium are replaced approximately every 3-5 days (Aleman et al., 2023; Kong et al., 2024).

The mucus layer, located on the luminal surface of the intestinal epithelial barrier, forms a protective physical and chemical barrier between microorganisms and epithelial cells. In the colon in particular, mucus is organized into a dense inner layer, through which bacteria are largely excluded, and a looser outer layer in which the microbiota can colonize (Kong et al., 2024). The glycocalyx, which covers the apical surface of epithelial cells, helps limit microbial adhesion through its carbohydrate-rich structure and includes membrane-bound mucins such as MUC1, MUC13 and MUC17. Tight junctions between neighboring epithelial cells primarily consist of transmembrane proteins such as claudins and occludin, whilst E-cadherin-mediated adherens junctions ensure the maintenance of intercellular mechanical integrity (Okumura & Takeda, 2024). Tight junctions regulate paracellular permeability; some claudin proteins contribute to the formation of ion-selective pores (Aleman et al., 2023; Kong et al., 2024). The absorption of nutrients, meanwhile, occurs via mechanisms such as specific membrane transporters, diffusion and, under certain conditions, paracellular passage (Aleman et al., 2023). Furthermore, antimicrobial substances such as alpha-defensins and lysozyme, secreted by Paneth cells, contribute to the barrier's chemical defense mechanisms alongside the mucus layer (Kong et al., 2024).

The intestinal epithelial barrier plays a key role in maintaining intestinal homeostasis, regulating nutrient absorption and defending against microorganisms. A breach in barrier integrity can lead to increased intestinal permeability and facilitate the passage of microbial products and antigens from the lumen into the mucosal tissues (Yu et al., 2022). Increased intestinal permeability, sometimes described using the term 'leaky gut', is associated with various gastrointestinal disorders, such as inflammatory bowel diseases (Kong et al., 2024; Yu et al., 2022). Furthermore, the relationships between intestinal permeability and conditions such as irritable bowel syndrome, coeliac disease, obesity and type 1 diabetes are being investigated; however, the causal mechanisms underlying these relationships have not yet been definitively established for all these conditions (Aleman et al., 2023). Impaired barrier function may contribute to the dysregulation of mucosal immune responses and the development or maintenance of intestinal inflammation (Okumura & Takeda, 2024).

4.8. Skin Barrier

The skin barrier is one of the fundamental defense systems that separates the organism from the external environment and contributes to the maintenance of internal homeostasis. Its primary functions are to limit the uncontrolled loss of water and electrolytes and to prevent pathogens and harmful chemical agents from entering the body. The stratum corneum, the outermost layer of the epidermis, forms the basis of the epidermal barrier. This layer significantly restricts the passage of water and water-soluble substances (Proksch et al., 2008). The stratum corneum consists of terminally differentiated keratinocytes (corneocytes) that have lost their nuclei; its thickness and the number of cell layers vary depending on the anatomical region. Corneocytes are embedded within an intercellular lipid matrix comprising primarily ceramides, cholesterol and free fatty acids (Nadaban et al., 2023). The ‘brick-and-mortar’ model, in which corneocytes represent the bricks and the intercellular lipid matrix the mortar, explains the fundamental barrier organization of the stratum corneum. Connections between corneocytes are maintained via corneodesmosomes (Kahraman et al., 2019).

Lamellar bodies in the keratinocytes of the stratum granulosum play a significant role in the formation of the epidermal barrier. The lipid precursors and enzymes secreted by these organelles contribute to the formation of extracellular lipid lamellae at the junction between the stratum granulosum and the stratum corneum (Proksch et al., 2008). In the viable epidermis beneath the stratum corneum, tight junctions, particularly those located in the stratum granulosum, form an additional permeability barrier. Various tight junction proteins, notably claudin-1, ensure the maintenance of epidermal barrier integrity by regulating paracellular transport between neighboring keratinocytes. It has been demonstrated that claudin-1 deficiency can severely impair the epidermal permeability barrier (Furuse et al., 2002).

The functional integrity of the skin barrier is closely linked to the organization of intercellular lipid lamellae and the physicochemical properties of the skin surface. The lipid matrix in the stratum corneum plays a key role in limiting transepidermal water loss (Kahraman et al., 2019). The slightly acidic pH of the skin surface creates a microenvironment known as the ‘acid mantle’ and contributes to the regulation of epidermal barrier homeostasis, stratum corneum integrity and cohesion, and antimicrobial defense. The stratum corneum is constantly renewed through the processes of epidermal differentiation and desquamation. During desquamation, proteases such as kallikrein-5 and kallikrein-7 facilitate the removal of corneocytes from the surface by mediating the controlled degradation of corneodesmosomal proteins. This process contributes to the regulation of the layer’s thickness and structural integrity (Ali & Yosipovitch, 2013).

Abnormalities in the composition of epidermal lipids and in keratinocyte differentiation may lead to increased barrier permeability and elevated transepidermal water loss (Nadaban et al., 2023). Impaired barrier integrity facilitates the entry of environmental allergens and irritants into the epidermis, thereby contributing to the development of immune responses and inflammatory processes. These changes contribute to the pathogenesis of several skin diseases, particularly atopic dermatitis, contact dermatitis, ichthyosis and psoriasis (Proksch et al., 2008).

Consequently, the skin barrier is a dynamic structure based on the organization of corneocytes and intercellular lipids in the stratum corneum, tight junctions in the stratum granulosum and coordinated epidermal renewal mechanisms. It limits water loss whilst protecting against harmful environmental factors.

4.9. The Placental Barrier

The placental barrier is a specialized structure that separates the maternal and foetal circulations during pregnancy and regulates the selective exchange of substances between the two compartments. Whilst facilitating the passage of oxygen and nutrients to the foetus and metabolic waste products into the maternal circulation, it limits the passage of various pathogens and potentially harmful substances to the foetus (Aye et al., 2025).

From a histological perspective, the placental barrier is primarily formed by chorionic villi surrounded by maternal blood. The outer surface of the villi is covered by the syncytiotrophoblast, a multinucleated, continuous layer of cells (Burton & Fowden, 2015). In the early stages of pregnancy, the placental barrier consists of the syncytiotrophoblast, cytotrophoblast cells, the trophoblastic basal lamina, the villous connective tissue stroma, the foetal capillary basal lamina and the foetal capillary endothelium (Gauster et al., 2022). As pregnancy progresses, the cytotrophoblast layer largely loses its continuity, the villous stroma thins, and the foetal capillaries approach the syncytiotrophoblast surface (Aye et al., 2025). Particularly in the terminal villi, regions of the vasculosyncytiotrophoblastic membrane develop in which the distance between the syncytiotrophoblast and the foetal capillary endothelium is markedly reduced. In these regions, the trophoblastic and capillary basal laminae may approach one another and fuse in places, thereby reducing the distance required for gas diffusion (Aye et al., 2025; Gauster et al., 2022).

Substances are transferred between the maternal and foetal circulations through different mechanisms, depending on their physicochemical properties and the placental transport systems (Ortega et al., 2022). Whilst some small molecules, notably oxygen and carbon dioxide, are transported by passive diffusion, facilitated diffusion is involved in the

transfer of glucose, and various carrier-mediated mechanisms are responsible for the transport of amino acids (Aye et al., 2025; Ortega et al., 2022). Furthermore, vesicular transport processes such as endocytosis and transcytosis enable certain macromolecules to cross the placental barrier (Ortega et al., 2022). The development of terminal villi and the thinning of the barrier throughout pregnancy contribute to increased efficiency in maternal-foetal substance exchange (Aye et al., 2025).

In addition to substance transport, the placental barrier also performs important immunoprotective functions. The continuous cellular organization of the syncytiotrophoblast and antiviral defence mechanisms limit the passage of various pathogens into foetal tissues. Extracellular vesicles secreted by the syncytiotrophoblast, including those containing cytokines and antiviral microRNAs, also contribute to this defense. Hofbauer cells- macrophages of foetal origin located in the villous stroma-play a role in regulating local immune responses (Aye et al., 2025). However, the placental barrier does not provide absolute protection; certain pathogens, such as the Zika virus, human cytomegalovirus and rubella virus, can cross the placenta, leading to foetal infections and developmental abnormalities (Cruz-Holguín et al., 2024). The placenta also provides selective but limited protection against xenobiotics. Certain ATP-binding cassette (ABC) transporters located in the syncytiotrophoblast help to reduce foetal exposure by facilitating the return of specific xenobiotics to the maternal circulation (Aye et al., 2025).

Consequently, the placental barrier is a dynamic structure that physically separates the maternal and foetal circulations whilst regulating the selective passage of gases, nutrients and metabolic waste; it supports foetal development through immune defense mechanisms and transport systems, and undergoes histological and functional remodeling throughout pregnancy.

5. Conclusion

Although biological barriers exhibit unique histological organizations in different organs and tissues, they share common functions such as the regulation of selective permeability, the maintenance of tissue homeostasis and defense against harmful agents. The structural basis of these barriers is formed by epithelial and endothelial cells, intercellular junction complexes, basement membranes and tissue-specific supportive components. The permeability characteristics and molecular regulatory mechanisms of the barriers vary according to the physiological requirements of the organ in which they are located. Any disruption to their structural or functional integrity may contribute to the development of pathological processes associated with inflammation, tissue damage and impaired organ function. Consequently, understanding the histological organization of biological barriers is important not only for normal tissue physiology but also for assessing the pathogenesis of various diseases.

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CHAPTER 3

NUCLEAR ORGANIZATION IN EARLY EMBRYONIC DEVELOPMENT

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1. Developmental Context

Following fertilization, the mammalian embryo carries out fundamental events such as epigenetic reprogramming, the acquisition and loss of totipotency and the first cell fate decision within a short window of time. These events are completed within the approximately three-day period extending from the fertilized egg to blastocyst formation (Borsos & Torres-Padilla, 2016). One of the most striking features of this period is the profound genome-wide changes that arise in nuclear architecture. The position of the genome within the nucleus is re-established according to nuclear landmarks, and this positioning plays a decisive role in the regulation of gene expression (Pecori & Torres-Padilla, 2023). The acquisition of distinct cell identities takes place together with tightly coordinated changes in gene expression and with marked changes in chromatin conformation.

The cell nucleus is organized through three complementary fundamental components. These are the nucleolus, the nuclear lamina and chromatin. The nucleolus functions not only as the centre of ribosome production but also as a compartment that regulates chromatin organization (Leeke et al., 2025). The nuclear lamina is a filamentous network located close to the inner surface of the nuclear envelope, and chromatin is intermittently attached to this network (Skalli & Goldman, 1991). Chromatin, in turn, is dispersed within the nucleus as compacted and open regions, and this distribution changes dynamically throughout development (Ahmed et al., 2010).

The aim of this chapter is to explain in plain language how nuclear organization is established during early embryonic development and how this establishment is linked to developmental gene control. The chapter addresses nucleolar organization, the nuclear lamina, chromatin dynamics and nuclear interactions in turn.

2. Nucleolar Organization

2.1 Structure and Formation of the Nucleolus

The nucleolus is the most prominent structure of the nucleus and is an organelle that is not surrounded by a membrane. This structure, in which ribonucleoprotein particles come together, is devoted essentially to ribosome biogenesis (Lafontaine et al., 2020). The fact that the nucleolus is not surrounded by a membrane distinguishes it from other organelles, and this structure forms spontaneously through liquid-liquid phase separation (Erdmann et al., 2021). The nucleolus is not a single liquid droplet. It is defined as a multiphase condensate composed

of layers with different physical properties. In somatic cells, these layers are arranged as the fibrillar centre, the dense fibrillar component and the granular component. The presence of three subcompartments within the nucleolus is a common ultrastructural feature of membraneless organelles, and this arrangement creates a phase-within-a-phase structure within the nucleus (Sawyer et al., 2018). Proteins such as nucleolin, fibrillarin and nucleophosmin take part in the establishment of nucleolar organization. These proteins direct nucleolar compartmentalization through their distinctive structural features and their multivalent interactions (Dogra & Kriwacki, 2025).

In the early embryo, the establishment of the nucleolus follows a path different from that of somatic cells. In the egg and the zygote, the nucleolus is not transcriptionally active and differs structurally from somatic nucleoli. These structures are called nucleolus precursor bodies (Kyogoku et al., 2018). Nucleolus precursor bodies were long regarded as passive material stores that cannot carry out ribosome synthesis (Fulka et al., 2020). Current findings have changed this view. These structures are indispensable for development, yet the material they contain is largely dispensable for the formation of active embryonic nucleoli (Fulka et al., 2020).

The number and distribution of nucleolus precursor bodies provide information about developmental potential. Multiple nucleolus precursor bodies are found in the pronuclei of human zygotes, and their number and distribution make it possible to predict the developmental potential of the zygote. The speed of movement of these bodies within the pronucleus may also indicate how the embryo will develop and its chromosomal constitution, that is, the euploidy and aneuploidy status (Fulka et al., 2026). In mouse embryos, the number of these bodies decreases throughout the cell cycle and during the transition from the zygote to the eight-cell stage. The classical three-compartment morphology of somatic-type nucleoli gradually appears around this body (Kyogoku et al., 2018). In porcine embryos, by contrast, the most prominent structure in the nucleus during the first two cell cycles after fertilization is fibrillar spheres, and fibrillar-granular nucleoli are established in the third cell cycle (Hyttel et al., 2000).

The structural establishment of the nucleolus and its transcriptional competence do not always proceed simultaneously. In the *Xenopus laevis* embryo, the structural organization of the nucleolus takes place before its transcriptional competence. This observation indicates that nucleolar structure is related to the presence of unprocessed ribosomal RNAs rather than to transcription (Verheggen et al., 1998).

2.2 Ribosome Biogenesis

Ribosome biogenesis is the best-known function of the nucleolus. In this process, ribosomal RNAs are transcribed, processed and matured (Quinodoz et al., 2025). The steps of ribosomal RNA processing are not randomly distributed within the nucleolus and are spatially segregated. Successive maturation is required for the RNA to move outwards through the nucleolar phases. The layered structure of the nucleolus therefore works like a ribosome assembly line (Quinodoz et al., 2025).

Ribosomal RNA constitutes both the skeleton and the substrate of the nucleolus. Defects in small subunit processing change the order of the nucleolar phases and produce inside-out nucleoli that block ribosomal RNA flow (Quinodoz et al., 2025). In the early embryo, ribosome biogenesis is a critical requirement for development. In mouse eggs, the maternal nucleolus is indispensable for zygotic genome activation and early embryonic development (Wang et al., 2019). The nucleolar protein NOP2 is detected in the late two-cell embryo and reaches its highest level at the eight-cell stage. Suppression of NOP2 leads to developmental arrest at the morula-blastocyst transition, and 208 genes are differentially expressed (Wang et al., 2019). Among these genes are numerous lineage-specific genes as well as genes encoding ribosomal proteins. Loss of NOP2 also markedly reduces the amount of ribosomal RNA and lowers developmental potential in bovine embryos as well (Wang et al., 2019).

The function of the nucleolus is not limited to ribosome production. In the early embryo, the nucleolus also serves as a regulator of chromatin organization. Totipotent mouse embryos carry non-classical immature nucleolus precursor bodies, and the conversion of these structures into mature nucleoli is required for the transition from totipotency to pluripotency (Fu et al., 2024). Nucleolus precursor bodies provide a structural platform for the reorganization of centric and pericentric chromatin, and the dynamic change of these structures controls the release of pioneer factors (Fu et al., 2024).

3. The Nuclear Lamina

3.1 Lamina Components

The nuclear lamina is one of the three components of the nuclear envelope. The nuclear envelope consists of the nuclear membrane, nuclear pore complexes and the nuclear lamina (Margiotta & Bucci, 2016). The lamina is a complex polymeric network formed by intermediate filament proteins called lamins, and this network is located close to the surface of the inner membrane that faces the nucleoplasm (Albers & Fuchs, 1992).

Three principal lamin proteins are found in mammalian somatic cells. These are lamin A (70 kDa), lamin B (67 kDa) and lamin C (60 kDa) (Albers & Fuchs, 1992). Lamin A and lamin C are produced from a single gene, and the transcript of this gene generates two different proteins through alternative splicing. Lamin B, by contrast, is encoded by a separate gene. Lamin A and C expression is regulated in mammalian development according to the developmental stage and the tissue type (Albers & Fuchs, 1992). This regulation is developmentally meaningful. Early vertebrate embryos carry only B-type lamins, and the expression of A-type lamins mostly coincides with the major changes in tissue differentiation (Fuchs & Weber, 1994).

Lamins are responsible for nuclear architecture and serve an important binding function for the structural proteins of the nuclear envelope. They also determine the distribution of nuclear pore complexes within the nuclear membrane. Lamins can organize and protect heterochromatin regions and also affect DNA transcription by regulating RNA polymerase II activity (Margiotta & Bucci, 2016). The lamina is a relatively stable structure once it is established. Within the cell, lamins largely remain in place after joining the lamina network and behave as part of a stable supramolecular network (Herrmann et al., 2007).

The establishment of the nuclear envelope and the pore complexes is of particular importance in early development. During fertilization, more than one nucleus may form, and these must fuse into a single nucleus (Hatch & Hetzer, 2014). Nuclear pore complexes are required for normal pronuclear development. Injection of a nuclear pore antagonist into mature oocytes arrests pronuclear development (Šutovský et al., 1998). In early embryos, pore complexes are found not only in the nuclear membrane but also in cytoplasmic membrane stacks called annulate lamellae, and these structures contribute to the rapid expansion of the nuclear membrane (Hampoelz et al., 2016).

3.2 Nuclear Architecture and Lamina-Associated Regions

The position of the genome within the nucleus is not random. Lamina-associated regions, namely LADs, are the best-known example of this order. LADs are regions ranging in size from 100 kilobases to 10 megabases. These regions are defined by high AT content, generally lower transcriptional activity and later replication timing compared with regions outside LADs (Briand & Collas, 2020).

LADs are established dynamically in early development. Genomic interactions with the nuclear lamina have been mapped at high resolution in mouse preimplantation embryos, and it has been shown that nuclear organization is not inherited from the maternal germ line and is rapidly re-

established after fertilization (Borsos et al., 2019). The two parental genomes form LADs carrying different features, and these differences converge after the eight-cell stage. Maternal LADs are more variable between cells and more euchromatic (Briand & Collas, 2020). The mechanism of LAD establishment is not related to DNA replication. In mouse zygotes, paternal LAD formation is prevented by ectopic expression of the Kdm5b protein, and this finding suggests that LAD establishment may depend on the rearrangement of H3K4 methylation (Borsos et al., 2019).

LADs have been defined as the earliest feature of nuclear organization in development. A gain-of-function screen in mouse embryos showed that perturbations affecting histone H3 modifications, heterochromatin and histone content are critical for the establishment of nuclear architecture at the zygote and two-cell stages. Interestingly, embryos with disrupted LADs can re-establish nuclear architecture at the two-cell stage (Pal et al., 2025). There is a balance between lamina association and repressive histone marks. In mouse embryos, preimplantation-specific LAD segregation overlaps markedly with the non-classical broad regions of H3K27me₃, and loss of H3K27me₃ leads to the re-establishment of the canonical LAD profile (Guerreiro et al., 2024).

4. Chromatin Dynamics

4.1 Chromatin Reorganization

The distribution of chromatin within the nucleus changes markedly throughout early development. In mouse embryos, chromatin is extensively dispersed at the one-cell stage and no distinct accumulation is seen at the nuclear envelope. At the two-cell stage, chromatin gathers into discrete compaction blocks and its density at the nuclear envelope increases. At the eight-cell stage and in pluripotent epiblast cells, chromatin is dispersed largely as a widespread network of uncompacted fibres, and this appearance is indistinguishable from the organization in embryonic stem cells. By contrast, lineage-committed trophectoderm and primitive endoderm cells show a higher level of chromatin compaction (Ahmed et al., 2010).

Chromatin mobility has also been associated with cellular potency. In mouse embryos, chromatin mobility has been examined in the inner cell mass and trophectoderm lineages of the blastocyst, and the study suggests that chromatin dynamics may be linked to cellular potency (Bošković et al., 2014). Chromatin compaction is not merely a general feature. At the germ layer stage, in cells whose fate has not yet been determined, highly compacted heterochromatin marked by H3K9me₃ is found at protein-coding genes, and these regions are profoundly

reorganized during differentiation (Nicetto et al., 2019). Disruption of three H3K9me3-associated methyltransferases has shown that H3K9me3 heterochromatin plays a critical role in lineage decision and in the maintenance of lineage identity at the onset of organogenesis (Nicetto et al., 2019).

Changes in chromatin organization can be abrupt. Three-dimensional FISH analyses in mouse embryonic nuclei have shown that there are several abrupt changes in nuclear organization throughout preimplantation development (Aguirre-Lavin et al., 2012). These changes are not limited to the preimplantation period. During cell fate transitions, the dynamic reorganization of nuclear structures plays a decisive role in the transition from totipotency to pluripotency and in other fate decisions (Liu et al., 2024).

Chromatin reorganization is also directly linked to the maternal-embryonic transition. The cleavage-stage embryo first completes the maternal-embryonic transition. In this transition, the embryo degrades egg-derived products and initiates its own transcriptional programme (Halstead et al., 2020). At the early cleavage stages, transcription takes place at a low level, and this situation is called minor embryonic genome activation. Minor activation is followed by major activation, which involves widespread transcription of embryonic genes. ATP-dependent remodelling complexes take part in the opening of chromatin. These complexes use the energy obtained from ATP hydrolysis to alter the interactions between histones and DNA and to render DNA accessible to various factors, including transcription factors. SWI/SNF, ISWI and Mi-2/NuRD are among these complexes (Shi & Wu, 2009).

Maternal Brg1 is a factor that stands out in this process. Brg1 is the catalytic ATPase subunit of the SWI/SNF complex and remodels nucleosomes (Mu et al., 2022). Brg1 is a maternal gene and is required for embryonic development in the mouse. Complete deficiency of Brg1 in mouse eggs leads to reduced transcription in approximately thirty percent of the genes expressed in the embryo (Bultman et al., 2006). Loss of Brg1 does not affect global histone acetylation, but H3K4me2 levels decrease (Clift & Schuh, 2013). Embryos carrying a maternal Brg1 mutation display a zygotic genome activation phenotype and arrest at the two-cell stage (Bultman et al., 2006). These data indicate that maternal remodelling complexes are an important step for zygotic genome activation (Bultman et al., 2006).

4.2 Epigenetic Regulation

The establishment of heterochromatin follows a distinct temporal pattern in early development. In mouse embryos, foci marked by H3K9me3, namely chromocentres, can be detected from the

two-cell stage onwards, and these foci are the classical cytological marker of constitutive heterochromatin (Laue et al., 2019). In mouse zygotes, pericentric heterochromatin covers the prenatal nucleolar bodies and is particularly prominent in the maternal pronucleus. At this stage, there is almost no heterochromatin at the periphery and the chromocentre is not visible (Politz et al., 2013). Shortly after the first cell division, a burst of pericentric transcription takes place, and this burst is simultaneous with the clustering of centromeres into chromocentres after moving away from the prenatal nucleolar bodies. Ribosomal RNA transcription begins at the mid-to-late two-cell stage, and active rDNA genes unite with the nucleolar precursor bodies to form nucleoli. In the same period, heterochromatin begins to appear at the nuclear periphery (Politz et al., 2013).

H3K9me3 levels increase progressively throughout development. Global H3K9me3 levels rise from the eight-cell stage onwards, and at this stage the embryonic constitutive heterochromatin state converts into the classical Suv39h-mediated state (Laue et al., 2019). The timing of heterochromatin establishment in the zebrafish embryo is different. In this organism, foci rich in H3K9me3 are not detected at the 512-cell stage, that is, 2.7 hours after fertilization. Weak and diffuse H3K9me3 labelling is first seen at the oblong stage, 3.7 hours after fertilization. Emerging foci are recorded at the dome stage, 4.5 hours after fertilization. Chromocentres, in turn, are distinct at the shield stage, 6 hours after fertilization (Laue et al., 2019). These findings indicate that the maternal-embryonic transition regulates the genome-wide establishment of heterochromatin (Laue et al., 2019).

Histone modifications and transposon control are intertwined in the establishment of repressive epigenetic marks. In the mouse, most parental H3K9me3 regions are re-established after fertilization, and most of the LTR-enriched H3K9me3 domains form progressively after the four-cell stage (Xu et al., 2020). Transposons are not merely repressed elements in the early embryo. The mouse endogenous retrovirus MERVL is expressed both in two-cell-like embryonic stem cells and in cleavage-stage embryos and directs the expression of many transcripts specific to zygotic genome activation and totipotency. The LINE1 element is also actively transcribed after fertilization, and the LINE1 RNA level reaches its highest value at the two-cell stage (Xu et al., 2020).

The reprogramming of histone modifications is more complex than that of DNA methylation, and no general model has so far been derived from the data obtained. Nevertheless, histone modifications, like DNA methylation, change dynamically throughout preimplantation

development in a stage- and cell type-specific manner, and these changes are required for the fine regulation of gene expression (Xu et al., 2020).

5. Nuclear Interactions

Nuclear components do not work independently of one another. The nucleolus, the nuclear lamina and chromatin form a whole within the nucleus through mutual interaction. The nucleolus is a large and membraneless organelle that gathers heterochromatin around itself, and this gathering takes place in the form of nucleolus-associated domains, namely NADs. Together with the nuclear lamina, the nucleolar periphery constitutes the two principal regions of heterochromatin accumulation (Peng et al., 2023). NADs are B compartment regions showing low gene density and low gene expression and overlap markedly with LADs (Vertii et al., 2019).

The genome-wide distribution of NADs has also been mapped. In proliferating human embryonic fibroblasts, 1,646 NADs have been identified, and these regions cover approximately thirty-eight percent of the annotated human genome (Dillinger et al., 2017). These regions are predominantly heterochromatic and are associated with loci showing late replication. The interactions of NADs are predominant in interphase chromosome contacts within the distance range of 10 to 50 megabases (Dillinger et al., 2017). NADs are regions ranging in size from 0.1 to 10 megabases, relatively gene-poor and rich in satellite repeats (Schöfer & Weipoltshammer, 2018).

The nucleolus is also the largest heterochromatin centre of the nucleus and therefore holds a special place in three-dimensional genome organization (Peng et al., 2023). Lamina-associated regions and nucleolus-associated regions complement one another. Both region types are B compartment regions of low gene density and low expression (Vertii et al., 2019). This overlap indicates that peripheral and nucleolar heterochromatin share a common regulatory logic. Nucleolus precursor bodies are a developmental hub of this interaction network. These structures provide a structural platform for the reorganization of centric and pericentric chromatin, and dynamic changes in nucleolar structure control the release of pioneer factors (Fu et al., 2024). For this reason, nucleolar organization, lamina-associated regions and chromatin dynamics should be evaluated not separately but as an interconnected system.

6. Conclusion and Perspectives

In early embryonic development, nuclear organization is re-established rapidly and in a stepwise manner after fertilization. Nucleolar structures, lamina-associated regions and chromatin regions are the fundamental components of this establishment. As a membraneless and multiphase condensate, the nucleolus spatially regulates ribosome biogenesis and at the same time assumes a regulatory role in chromatin organization (Lafontaine et al., 2020). Nucleolus precursor bodies serve as transient structures in embryogenesis, and the conversion of these structures into mature nucleoli is closely related to the transition from totipotency to pluripotency (Fu et al., 2024).

The nuclear lamina consists only of B-type lamins in early development, and the expression of A-type lamins appears together with tissue differentiation (Fuchs & Weber, 1994). Lamina-associated regions are re-established after fertilization, and this establishment is not inherited from the maternal germ line (Borsos et al., 2019). These regions are the earliest feature of nuclear organization (Pal et al., 2025). The balance between lamina association and H3K27me3 explains the unusual genome organization in the totipotent embryo (Guerreiro et al., 2024).

Chromatin dynamics are closely linked to developmental potential. Chromatin that is dispersed at the one-cell stage forms compaction blocks at the two-cell stage and becomes a widespread network similar to that of embryonic stem cells at the eight-cell stage (Ahmed et al., 2010). Heterochromatin marked by H3K9me3 converts into the classical Suv39h-mediated state from the eight-cell stage onwards (Laue et al., 2019). ATP-dependent remodelling complexes take part in the opening of chromatin, and maternal Brg1 is required for zygotic genome activation (Bultman et al., 2006).

Interactions among nuclear components cannot be fully understood by studying individual components. Lamina-associated regions and nucleolus-associated regions overlap as B compartment regions of low expression (Vertii et al., 2019). For this reason, nucleolar organization, peripheral heterochromatin and chromatin dynamics should be treated as an interconnected system.

Several directions stand out for future research. The first is to examine the establishment and function of nucleolar phases in greater detail. How ribosomal RNA shapes the multiphase architecture and how this architecture is regulated in early embryogenesis are not yet fully known (Quinodoz et al., 2025). The second is to elucidate the functions of nucleolus precursor bodies. The relationship of these structures with chromatin organization and their role in

determining developmental potential remain controversial (Fulka et al., 2026). The third is to develop live-cell imaging methods. The joint examination of nuclear organization in fixed cells and in living cells is necessary for understanding the evolution of processes over time (Pecori & Torres-Padilla, 2023). Solving the effect of nuclear reorganization on cell fate decisions is an important goal for both developmental biology and regenerative medicine (Liu et al., 2024).

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