Nuclear architecture emerges at the awakening of the genome

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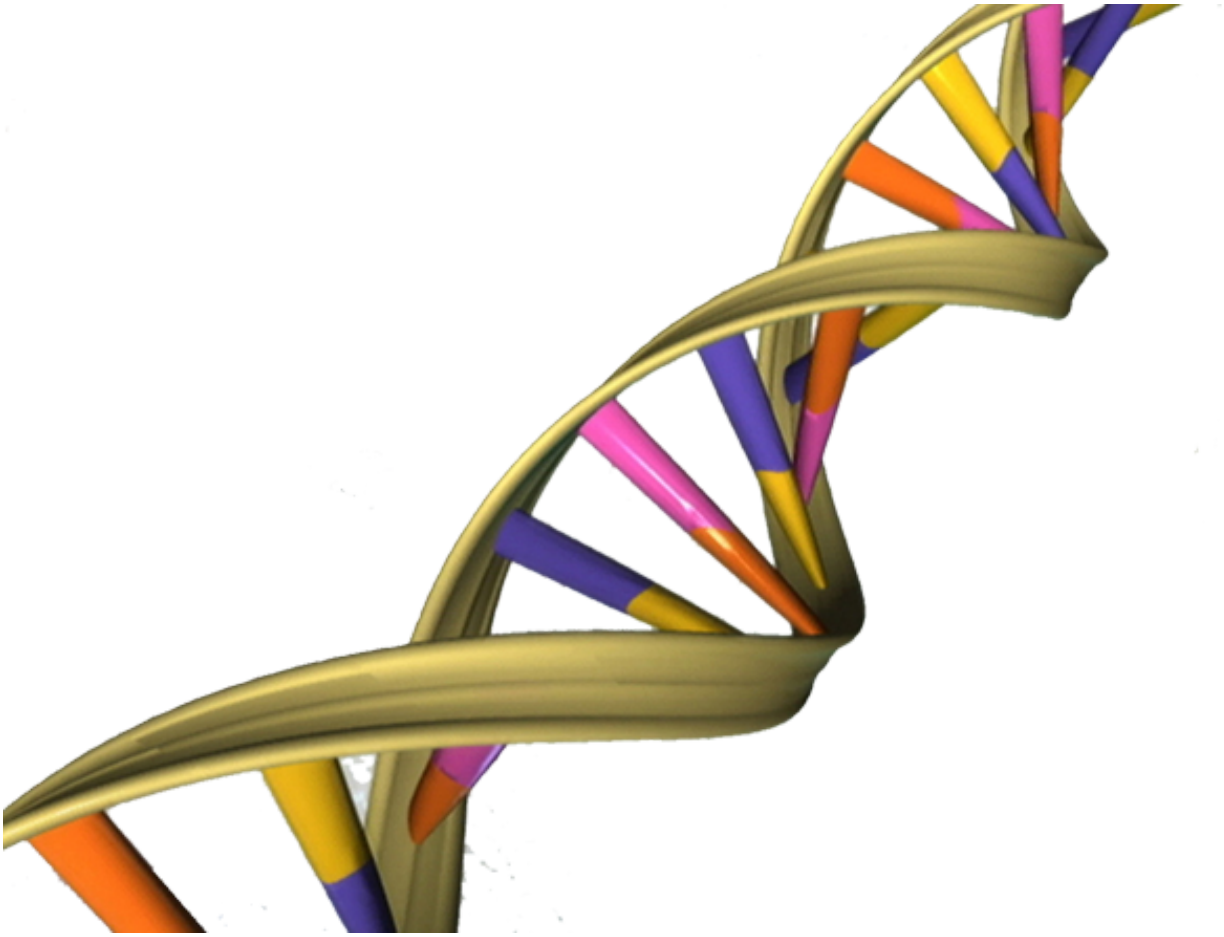

A depiction of the double helical structure of DNA. Its four coding units (A, T, C, G) are color-coded in pink, orange, purple and yellow. Credit: NHGRI

The DNA molecules in each one of the cells in a person's body, if laid end to end, would measure approximately two metres in length. Remarkably, however, cells are able to fold and compact their genetic material in the confined space of the nucleus, which spans only a few micrometres. Importantly, the compaction and arrangement of the genome inside the nucleus needs to be achieved in an ordered fashion that still allows cells to access the genetic information appropriately, for example to produce messenger RNAs for specific proteins, or to replicate the genetic material prior to cell division. When mutations occur that disrupt features associated with the spatial organisation of the genome, this leads to developmental disorders and cancer.

Scientists have had a long-standing interest in examining the spatial organisation of the genome in the cell's nucleus, mostly using microscopy techniques. Recent advances in genomic techniques to measure the 3D organisation of the genome have allowed for an increased resolution of this organisation. However, when the genome gains 3D organisation during development is not known. Now, using early development fruit fly embryos and genomic techniques to measure 3D genome organisation, scientists of the Research Group 'Regulatory Genomics' at the Max Planck Institute for Molecular Biomedicine in Muenster have shown that the 3D organisation of the genome emerges when the early embryo switches on its own genetic programme.

A common image of the cell's genetic material is the rod-like structures of mitotic chromosomes. However, those only exist while cells are undergoing cell division. The rest of the time, the genetic material is found in the form of chromatin fibres - DNA molecules densely wrapped around histone proteins - which are less densely compacted than mitotic chromosomes and occupy the nuclear space.

"One could think of this as a plate of spaghetti, where each individual piece of pasta would correspond to the DNA molecule in each

chromosome", says Juanma Vaquerizas, head of the Max Planck Research Group 'Regulatory Genomics' at the Max Planck Institute for Molecular Biomedicine, who led the study. "A fundamental question in the field was whether each spaghetti would randomly mingle with other pieces of pasta or whether they would occupy a defined space within the plate."

Using microscopy approaches, scientists had determined before that the location of chromatin in the nucleus was not random, and recent advances in our ability to measure chromatin architecture have shown that finer structures, called topologically associating domains (TADs), form part of the basic functional units that determine the 3D organisation of the genome. However, a very puzzling observation has been that when the TAD organisation of the genome is examined in different cell types in an organism or in conserved regions of the DNA between species, this seems to be very similar across samples, despite different parts of the genome being actively used in different cell types. This prompted Clemens Hug and Juanma Vaquerizas to address the question of when during organismal development chromatin architecture is established.

The team turned to early development of fruit flies to perform their experiments. "An amazing feature about fruit fly embryonic development is that upon fertilization, the nuclei synchronously divide every 10-15 minutes for thirteen times without gene activation", says Vaquerizas. Maternally deposited mRNAs and proteins make sure that differentiation and development occur during those initial nuclear cycles. Then, at nuclear cycle 14 - only 2,5 hours after fertilization - the embryonic genome is activated. "Thus, in fruit flies, we can accurately study early chromatin organization at a high temporal resolution", says Vaquerizas.

The choice of organism and its developmental timing proved critical for

the researchers' experiments, since this allowed them to examine 3D genome organisation in nuclei at a stage when transcription is naturally not occurring, and by doing so, decouple genome organisation from the effects of transcription.

By using state-of the-art genomic analyses, the scientists were able to study chromatin organization at a very high spatial resolution. Clemens Hug, PhD student and first author of the study, explains the method they used: "The so-called in situ Hi-C technique allows us to accurately identify those parts of the DNA that interact with each other in the three-dimensional nuclear space and the extent of interaction throughout the genome. We are therefore able to capture the 3D organization of the chromatin at a certain time point and can reveal changes in organisation across early development stages." Strikingly, the team found that at early stages of development the genome lacks defined higher-order chromatin organisation, and that 3D architecture progressively emerges in later stages.

"We found that TAD boundaries - defining functionally distinct chromatin units - arise when the first zygotic genes are transcribed. The number of TAD boundaries reaches a plateau when the complete zygotic genome has been activated", says Hug. "These boundaries are occupied by housekeeping genes that are constantly transcribed in all cell types. Once established, these are maintained throughout development." This is an important finding since it helps explain why the TAD organisation of genomes is similar across tissue types and evolutionary conserved regions between species.

However, the scientists could demonstrate that the establishment of TAD boundaries is independent of transcription itself, despite being associated with transcriptionally active regions. "This is of interest since it suggests that the machinery or mechanisms leading to transcription might play a role in TAD boundary establishment", says Hug. The scientists observed

that Zelda, a pioneer transcription factor protein that opens the chromatin so that the transcription machinery can access the DNA, is necessary to establish some TAD boundaries. "We therefore think that Zelda and maybe other proteins with a similar function, in concert with RNA Pol II, create the TAD boundaries and thus are responsible for the 3D chromatin architecture", says Hug.

"When the proteins that determine TAD boundaries - and thus are critical for the chromatin architecture - are disrupted, this can result in distinct developmental disorders and cancer", says Vaquerizas. "Our newly gained insights into how the 3D chromatin architecture is established and maintained will thus have a major impact on further studies looking at its impact on gene expression during development and disease."

More information: Clemens B. Hug, Alexis G. Grimaldi, Kai Kruse and Juan M. Vaquerizas. Chromatin architecture emerges during zygotic genome activation independent of transcription. *Cell* 169: 216-228, April 6th, 2017, DOI: 10.1016/j.cell.2017.03.024

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