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Evolution & Behaviour

Organizing DNA sets the tempo of gene activation

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If you had to fit two meters of a thread into a ball smaller than the width of a single strand of hair, how would you do it? How would you organize it so that important parts of the thread are close together? This is precisely the way our DNA fits inside our cell nucleus, and the way we do it has direct implications on whether genes are activated at the right time and place.

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A full copy of our DNA is very long – about two meters. Yet, it fits inside the nucleus of our cells; a space of around 6 micrometers – less than the width of a human hair. The DNA fits in this tiny space because it is curled up into a tight shape like a ball of yarn. The way DNA is curled up determines which genes are active and which are not. Understanding this structure of DNA is therefore important.

Our DNA is organized on different levels. If we take a close look at the DNA thread, we see it can form stable loops that temporarily link two or more sequences which otherwise would be very distant along the thread (picture a loop in your shoelaces). At a larger scale, loops are grouped in regions, called TADs, that mainly interact among themselves. These regions are curled along the chromosome like a ball of wool. The way distant DNA sequences are grouped together has a big effect on how different genes and 'enhancers' are activated. Enhances are stretches of DNA that, although they will not translate their information into a protein (as genes do), are necessary to switch-on (activate) genes in specific cells or at a given time. This fine-tuning of gene activation is fundamental during embryogenesis, when organs are being generated and cells undergo multiple identity transitions.

During the last decade many efforts have been directed to disentangle how the three-dimensional organization of our DNA impacts gene activation. However, so far most of the studies were based on removing the proteins that are thought to affect DNA interactions. The consequences of removing these proteins are often difficult to investigate since the effects are all over the place and often lethal to the cell. Alternatively, genome-editing techniques like CRISPR/Cas9 allow cutting out specific DNA

sequences, which gives researchers a more precise tool to figure out the functionality of these sequences. A group of scientists at Princeton used this technique to study how DNA organization affects genetic expression over time during the development of the fruit fly. They generated several fly mutants by deleting stretches of DNA around genes that are known to be responsible for the positioning of body structures like legs or antennae.

To directly visualize gene activation in a living developing embryo under a microscope, they used a technique that tags the RNA molecules with fluorescent labels as they come out from an active gene. They specifically monitored how much these genes were activated during the first hours of fly embryogenesis (after egg laying) and they compared this between the mutants. The researchers generated high-resolution maps showing DNA interaction and classified the DNA regions that shape the 3D genome into two categories: 'insulator' and 'tethering' elements. Insulator DNA elements can separate TADs (the 'balls of wool' in the 3D DNA structure). The insulators form boundaries that separate genes from regulatory sequences (e.g., enhancers) that are meant for different genes. In a way, these are the pieces that lay between two independent balls of wool. On the other hand, tethering elements work as anchors and may help to stabilize the DNA loops, favoring interactions. The researchers altered these insulator and tethering elements near the same regions of DNA and studied the effect of these elements on genetic expression in both time and space. When deleting a specific tether, the place in the body where the neighboring gene is activated was not affected, but the gene did get activated later. On the other hand, the effect of removing insulator elements is more complicated. Deleting insulators led to the fusion of neighboring TADs, which meant decreasing the output of some genes, while others became active when they should not be. All these genetic alterations were mirrored by changes in the number of sex combs, i.e., bristly body structures on the first leg pair of the male fruit fly that help on grabbing the female for mating. These results effectively prove that the 3D shape of the genome orchestrates rapid gene activity. Timing of gene activation proves fundamental during embryonic development, when some genes are only needed during very specific time windows, for example when organs appear.

Identifying and classifying distant DNA enhancers, spatial tethers, TADs and insulators is elucidating the relationship between space and time during embryonic development. In humans, some birth malformations have been linked to alterations of genome organization. Evidence shows that our genome follows a similar hierarchical spatial organization as in flies, albeit we may expect some more layers of complexity, given the bigger size of our genomes and our longer embryonic development. Nevertheless, we are excited about the potential impact this research has on our understanding of the genetics of embryonic development in humans as well.