NUCLEAR ORGANIZATION

Capturing genome folds in single sensory neurons

Chromatin organization in the nucleus plays an important role in cell-type-specific gene expression. A new study reports reconstruction of the 3D genome in single sensory neurons and provides insights into the regulation of genes encoding odorant receptors.

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Neuronal diversity is critical for the complex functions performed by the nervous system. The establishment of distinct neuronal identities is driven at least in part by the organization of the genome in nuclear space. To understand this connection, Tan et al.[1] compared the 3D genome organization in nuclei of rod cells with that of olfactory sensory neurons, two types of highly specialized sensory neurons that show extremely diverse cellular morphologies and functions.

Hi-C methods[2] allow the identification of DNA contacts across the entire genome, but they generally require analysis of a large number of cells and therefore provide a population average of genome interactions. Cell-to-cell variability may not be captured in analyses of bulk samples. To address this issue, Tan et al.[1] used a modified version of a single-cell Hi-C method[3,4], called 'Dip-C', which enables reconstruction of the 3D genomes in single diploid cells[5].

Previous studies have shown that the nuclear distributions of heterochromatin and euchromatin in olfactory sensory neurons and rod cells are strikingly different from those in most other cell types[6–8]. Typically, heterochromatin localizes at the nuclear periphery and euchromatin is situated toward the nuclear interior. In rod cells from nocturnal animals, heterochromatin localizes in the nuclear center, whereas euchromatin localizes in the nuclear periphery[8]. In a similar but less striking fashion, heterochromatin in mature olfactory neurons aggregates centrally in the nucleus as well; however, the olfactory nuclei also show a rim of heterochromatin in the nuclear periphery, which is absent in rod cells[8–10]. Tan et al. were able to reproduce these unusual nuclear architectures in their single-cell 3D genome reconstructions, in which the authors used the CpG frequency of all adjacent loci as a proxy to identify the two types of chromatin compartments[1] (Fig. 1a).

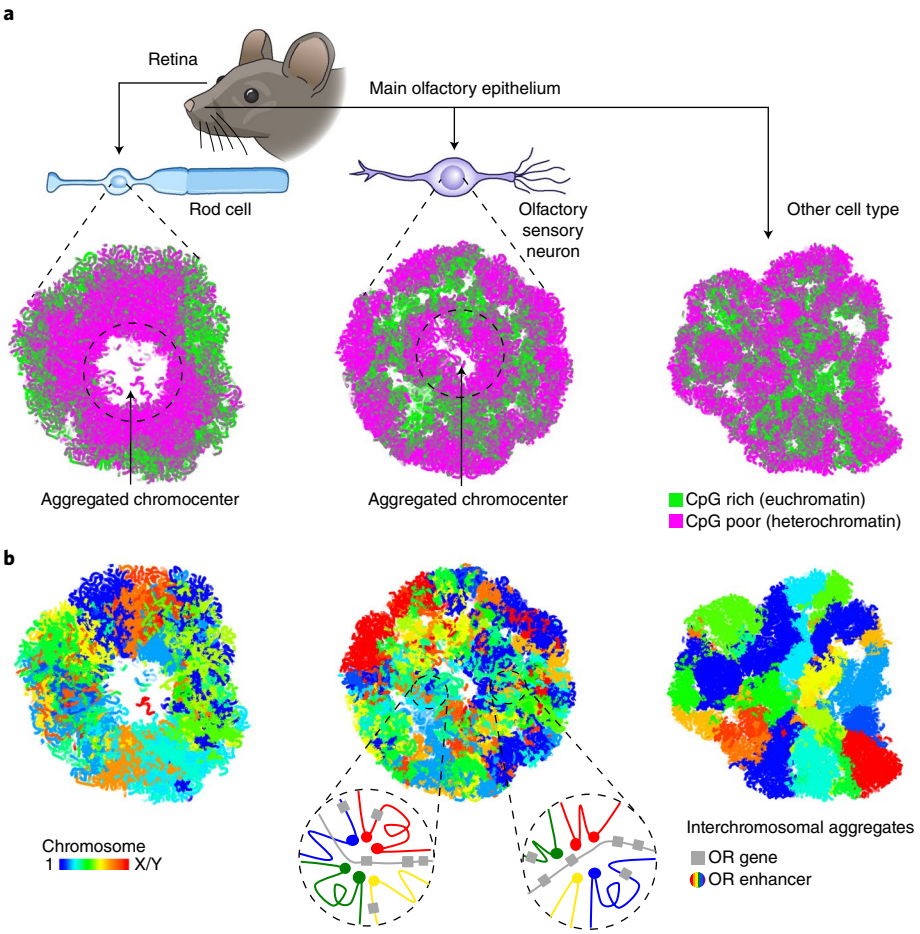

Fig. 1 | 3D genomic organization in single sensory neurons. Tan et al. reconstructed DNA interactions in single nuclei from mouse rod cells and olfactory sensory neurons[1]. **a**, Cells isolated from the mouse retina and from the olfactory epithelium were subjected to Dip-C. The average CpG frequency of all adjacent loci was used to define euchromatic compartments (high CpG frequency; green) and heterochromatic compartments (low CpG frequency; pink) in these nuclei. Aggregated chromocenters (dense heterochromatic foci consisting mainly of satellite repeat DNA sequences) are located in the central region of the nuclei of rod cells and olfactory neurons (dashed circles) but not in that of the nuclei of other cell types. **b**, Reconstituted nuclei of rod cells and olfactory neurons show greater chromosomal intermingling than do other cell types. Dashed circles represent aggregates of OR genes (gray squares) and OR gene enhancers (colored circles) from different chromosomes. These specific aggregates do not occur in the nucleus of rod cells or other cell types. Presumably, only one of the OR gene–enhancer aggregates is active in each mature olfactory sensory neuron. Nuclear images are adapted from ref. [1].

Although olfactory neurons display nuclear features similar to those of rod cells, they are unique in their expression of genes encoding odorant receptors ('OR genes'). Each olfactory neuron expresses only 1 of ~1,000 different OR genes that are dispersed over most chromosomes[11,12]. Moreover, only one of the two homologous alleles of the gene, maternal or paternal, is expressed per neuron[11]. 3D DNA-FISH experiments have shown that despite being located at distinct genomic positions, OR genes are aggregated in nuclear space and are associated with the central repressive heterochromatin compartment[6,7]. How can this nuclear organization lead to the expression of one single OR gene? A large number of enhancer sequences have been previously identified that can act in cis to regulate OR genes located in their proximity[13,14]. However, they can also act in trans to regulate the expression of OR genes that are located on different chromosomes[15]. Such observations led to a model in which OR gene enhancers, which, like the OR genes, are located on different chromosomes, come together in the nucleus to interact and activate one single OR gene, accounting for monogenic expression[16].

Individual chromosomes usually occupy distinct territories in the nucleus, and therefore interactions in trans between chromosomes are rarer than interactions between loci located on the same chromosome[17]. 3D reconstruction of diploid cells allowed Tan et al. to visualize the spatial distribution of chromosome territories in the nucleus[1]. This analysis revealed that there is greater intermingling of the chromosomes in both rod cells and olfactory neurons than in other cell types (Fig. 1b). The rod cells and olfactory neurons show higher frequencies of interchromosomal contacts (over 40% of the total Hi-C contacts) than do their respective progenitors or other cell types. This might favor the occurrence of trans interactions in rod cells and olfactory neurons. In cells that exhibit more delimited chromosome territories, such contacts would be less frequent.

The authors further found that on average, in nuclei from mature olfactory neurons there are frequent interchromosomal contacts between OR genes and their enhancers. This was not observed in nuclei from rod cells or rod and olfactory progenitor cells. These findings indicate that these trans interactions have a specific gene-regulatory function in this cell type and are in agreement with the model of monogenic OR expression driven by a multi-enhancer aggregate composed of a large fraction of the existing OR gene enhancers[16]. The composition and distribution of these aggregates in single olfactory nuclei had not been analyzed thus far, however.

Taking advantage of the single-cell 3D genome reconstructions, Tan et al. were able to analyze the interactions between the complete set of OR genes and their enhancers[1]. Use of the Dip-C method allowed the authors to discriminate between the paternal OR genes and enhancers and their maternal counterparts (~2,000 OR genes and 126 enhancers). They found that in each neuron, OR gene loci from different chromosomes were located toward the nuclear interior, with many of them residing near the central chromocenter aggregates, consistent with previous DNA-FISH data[6,7]. One might expect to find one major multi-enhancer aggregate per nucleus that would be interacting with the one active OR gene. However, the single-cell analysis revealed that only ~30% of the OR gene enhancers were near OR gene enhancers from other chromosomes. In the reconstituted nuclei, the OR gene enhancers usually formed multiple small aggregates per nucleus, with each aggregate containing on average a random subset of about seven enhancers from about four chromosomes (Fig. 1b). The authors conclude that only a small set of all OR gene enhancers are likely to be required for OR gene transcription in a given neuron. These results fit well with previous findings that developing olfactory neurons express multiple OR genes at low levels before they finally express a single OR gene at high levels[18,19] and indicate that additional mechanisms must ensure expression of only a single OR gene.

There are some limitations to the work by Tan et al.[1] that should be taken into account. Single-cell Hi-C methods usually result in lower genome coverage than does bulk Hi-C[20]. In addition, since unsorted neurons were used in the experiments, the authors were not able to determine which OR gene was active in each one of the analyzed neurons.

Notwithstanding the limitations, the results of Tan et al.[1] raise a number of interesting questions. For example, what determines which enhancer aggregate in the nucleus will be active? How are the remaining enhancer aggregates inactivated? Do the nuclei of immature olfactory neurons have more enhancer aggregates than the nuclei of mature neurons have? How are the maternal and paternal enhancer alleles organized during monoallelic expresion of the OR genes? Are they completely segregated in the nucleus, or can they interact within the same multi-enhancer aggregate?

Overall, the work by Tan et al. demonstrates that 3D genome reconstruction of single olfactory neurons constitutes a promising approach for investigating the mechanisms through which monogenic and monoallelic OR gene expression is established[1]. In addition, the approach should also help in analysis of the complexity of genome organization in rod cells and other neuronal types, which will ultimately help to delineate the mechanisms that lead to neuronal diversity. ❐

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Competing interests

The authors declare no competing interests.