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Lab Note

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Visual assessment of global chromatin intranuclear localization and its cellular diversification in mouse cells

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DNA is the core that controls the life activities of cells. In eukaryotic cells, DNA is dispersedly packaged into multiple strands of chromatin to regulate cellular life activities; however, the underlying logic and significance of this configuration remain unclear. In mouse cells, pericentromeric satellite DNA from multiple strands of chromatin can aggregate to form chromocenters [1,2]. Based on microscopic imaging techniques and analysis, we further reveal that there is nonrandom recombination aggregation between chromatin in different mouse nuclei, leading to differentiation of nuclear structure and correlation with cell function. We speculate that the recombination aggregation of chromatin nuclear localization can further enrich limited DNA genetic information, and its induced nuclear structural diversification is crucial for the formation of cellular diversification.

The chromocenters of mouse cells are regions with abundant pericentromeric satellite DNA, which can be stained by DAPI to present a bright spot [3]. Recent studies have revealed that the pericentromeric satellite DNA of mouse cells is involved in the intranuclear anchoring of chromatin [4], so the chromocenters can be used as the anchoring point of the chromatin, and then the chromatin of interphase cells can be observed. In this study, we found that the number of DAPI bright spots in the nucleus was not equal to the number of chromatin strips, and in many cases, it was much fewer than the number of chromatin strips [3,5] (Figure 1A,B). In this regard, we speculate that the convergence of anchor points of different chromatin leads to a decrease in the number of observable chromocenters and part of the chromatin satellite DNA sequence is decompressed, so the highlighted spots cannot be displayed under the condition of DAPI labelling, causing a reduction in the observable chromocenter numbers.

To verify the above speculation, we used satellite DNA *in situ* hybridization and centromere-associated protein (ACA) immunofluorescence to label all chromosomes and identify their intranuclear anchor points. The results showed that both pericentromeric satellite DNA and ACA-related fluorescent spots exist in each chromosome, and both fluorescent signals are located in the chromocenters (Figure 1A,B), which indicated that the convergence of chromatin anchor points may be the main reason for the reduction in chromocenter counts. In addition, if pericentromeric satellite DNA decompression is established, then theoretically loose chromatin requires more nuclear space, and the corresponding nuclear volume will be larger, but *in situ* analysis of mouse liver tissue showed that the cells with fewer chromocenters nuclei instead have smaller volumes (Supplementary Figure S1). The above data showed that the convergence of chromatin anchor points results in the number of chromocenters being less than the number of chromatin strips, and the analysis of chromocenters can reflect the global chromatin nuclear localization to a certain extent.

Previous studies have shown that the number of chromocenters in different types of mouse cells is between 8 and 30 [5]. Here, we separately counted the number of chromocenters in primary macrophages, primary neuronal cells, and in situ hepatocytes, and the results showed that the counts of the three types of cells were significantly different, but the counts were relatively concentrated in the same type of cells (Figure 1C and Supplementary Figure S2). Interestingly, these cells in mice have the same DNA genome and the same amount of chromatin, suggesting that these chromatin aggregates and reorganizes in the nucleus, and they follow certain rules. In fact, the number of chromocenters also fluctuates within the same type of cells, but we believe that in sufficiently subdivided cell subtypes, a relatively consistent phenomenon may appear. Taking vasoactive intestinal peptide-expressing (VIP) interneurons as an example, VIP interneurons are one of the subclasses of ionotropic serotonin receptor 5HT3a (5HT3aR) interneurons [6,7], and their nuclei usually have only one

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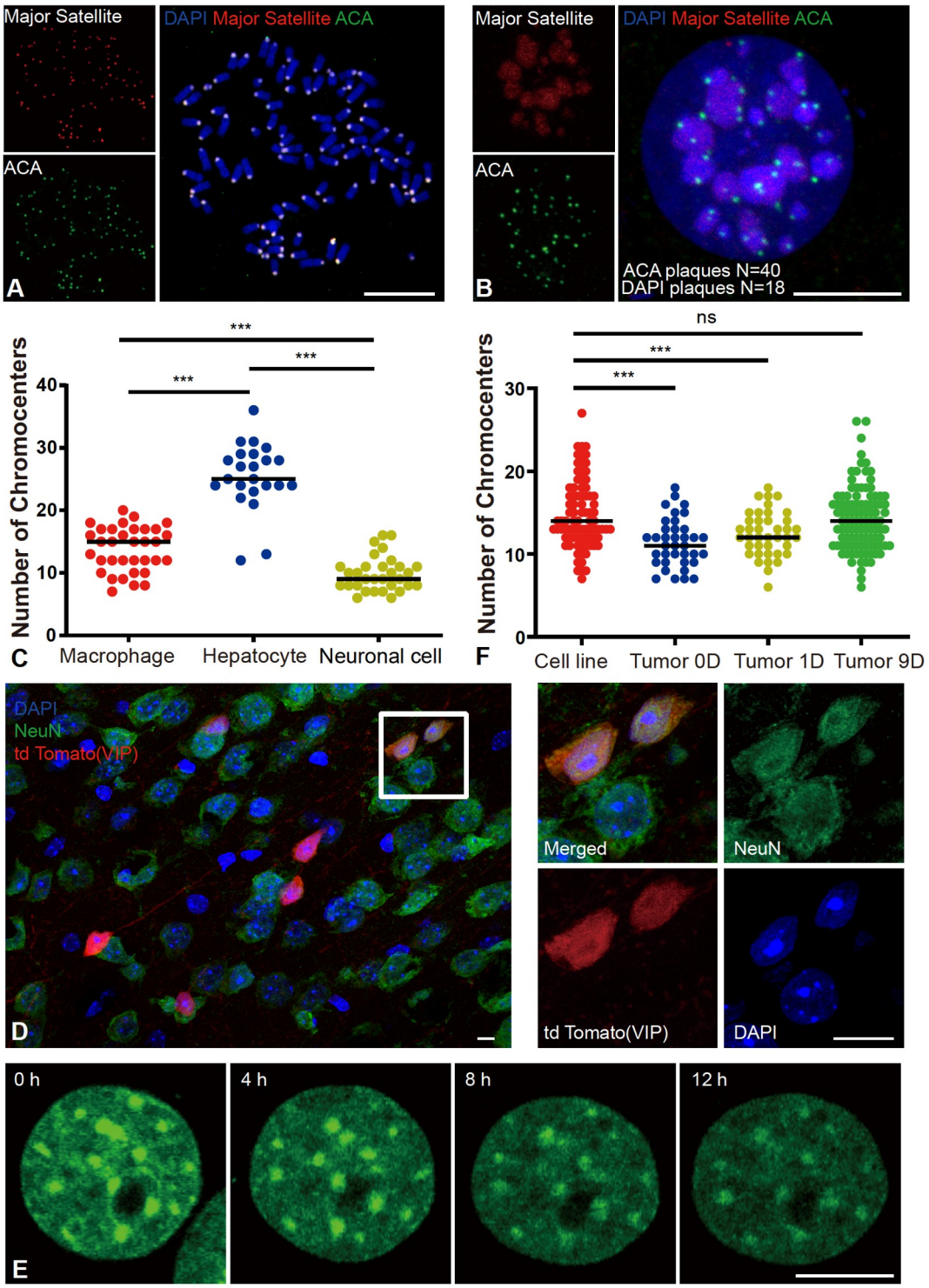


Figure 1. Intranuclear localization and dynamic analysis of global chromatin in mouse cells (A) Satellite DNA *in situ* hybridization and ACA antibody recognition were used to label and image each chromosome, respectively. Scale bar: 10 μm. (B) *In situ* hybridization of satellite DNA and antibody recognition of ACA were used to image the intranuclear anchor points of each chromatin labelled and imaged, respectively, where "N" is used for "number". Scale bar: 10 μm. (C) Statistical analysis of the number of chromocenters in the nucleus of primary macrophages, primary neuronal cells, and *in situ* hepatocytes. (D) Imaging analysis of VIP interneuron chromocenters in mouse brain slices. Among them, NeuN is a broad-spectrum marker for neurons, and td-Tomato is a specific marker for VIP interneurons. Scale bar: 10 μm. (E) Time-series imaging of chromocenters in living cells using GFP-H2B labelling. Scale bar: 10 μm. (F) Counting analysis of chromocenters in 4T1 cells under different survival conditions, where "D" is used for "day". ***$P < 0.001$.

chromocenter, maintaining absolute consistency (Figure 1D). The reason why the nuclear structure of VIP is so stable may be due to the sufficient functional classification of 5HT3aR interneurons. The above data suggest that the global chromatin nuclear distribution is nonrandom and has a certain correlation with cellular function.

In addition, if global chromatin nuclear localization is associated with the performance of cellular functions, theoretically, this structure should also be stable during intercellular phases of the same cell (excluding special external interventions), thereby maintaining its essential cellular functional properties. To this end, we constructed 4T1 cells stably transfected with H2B-GFP and verified the consistency of H2B-GFP and DAPI labelling [8] (Supplementary Figure S3). Following 12 h of follow-up observation under live cell conditions, it was found that the cells migrated dynamically over time under culture dish conditions, and the nuclei also underwent rotational changes and continuously adjusted their posture (Supplementary Video S1). However, through image analysis, we still easily found that the number and relative positions of nuclear H2B-GFP plaques did not change significantly during the whole process, maintaining absolute stability (Figure 1E and Supplementary Figure S4). These results suggest that global chromatin localization in the nucleus is relatively stable and that this stable nuclear structure is necessary for the maintenance of essential cellular properties.

Interestingly, we also found that the nuclear chromocenters vary significantly when cells grow in different microenvironments. We planted green fluorescently labelled 4T1 cells subcutaneously in immunodeficient mice, obtained tissue sections after tumor formation, and compared the chromometer counts with cells under normal culture dish conditions. The results showed that the counts of chromocenters in the sliced cells were significantly lower than those in the culture dish environment. Subcutaneous tumor primary cells were further extracted and cultured for 9 days, and the chromocenter count gradually returned to the level of the culture dish state (Figure 1F and Supplementary Figure S5). In fact, it has recently been described in the literature that mammalian rod cells have distinct global chromatin distribution differences between day and night to adapt to changing environments [9,10]. The above data show that the living environment can induce reversible changes in the global chromatin nuclear distribution of cells, suggesting the possible impact of the living environment on the cells.

In conclusion, the scattered packaging of DNA into multiple strands of chromatin in eukaryotic cells will facilitate further recombination and localization of global chromatin in the nucleus, thus forming a basis for cellular diversification. As the nuclear anchor of chromatin, chromocenters are formed by the nonrandom arrangement of chromatin centromeric-related structures, and their distribution characteristics not only directly show the structure of the nucleus but also have significance for the study of cell function. Our study showed that the number of chromocenters tends to be the same in well-subdivided subcell types, such as VIP cells (Figure 1D). However, in the case of inadequate cell subclassification, the number of chromocenters fluctuated, as shown in Figure 1C for the three primary cells. Moreover, it is clearly plausible that the number of chromocenters between two completely different types of cells could be significantly different. Therefore, the number of chromocenters can be used to evaluate the consistency of cell types and functions to a certain extent. We propose that cells of the same type have similar or even the same number of chromocenters, but the same number of chromocenters cannot infer cell homogeneity. The main reason is that mice have 40 chromatins, and the mathematical algorithm based on permutation and combination with the same number of chromocenters implies that there may be multiple recombination patterns between chromatin. Although our discussion of global chromatin intranuclear localization transitions on the formation of cell diversification is still insufficient, related studies can provide important clues to reveal the laws of multicellular life. In the future, if specific labelling of different chromatin anchor points can be realized, the differences in global chromatin nuclear localization between cells can be more clearly distinguished, which will provide a new way to study biological problems such as cell differentiation, function-specific formation, development and cell carcinogenesis.

Supplementary Data

Supplementary data is available at *Acta Biochimica et Biophysica Sinica* online.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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