Quantifying Chromatin Fractal Dimension through ChromEM Staining

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Traditionally, the study of gene transcription views the nucleus as "molecular machines", and thus primarily focuses on specific gene sequences with a "loci-by-loci" basis. However, the chemical reactions are not taking place in dilute solutions. Quite contrarily, these chemical reactions occur in a high compacted region inside the nucleus. Besides the molecular composition of a gene sequence, the location of this gene on the chromatin also plays a crucial role in the accessibility of this specific gene. It is known that chromatin structure spans across multiple length scales [1], and the regulation of gene expression by chromatin structure not only relies on the sequence binding motifs, but also on the local environment.

More and more evidence has supported that the chromatin structure can be described as a mass fractal mathematically. In specific, the chromatin fractal dimension (D) is one of the most effective measures to quantify the chromatin structure in terms of compaction. In a fractal model, the autocorrelation function of chromatin mass (B_ρ) scales as a power law to the spatial separation (r) following $B_\rho \sim r^{D-3}$ [2]. In molecular dynamics simulations, it has been reported that D inserts a double-folded influence on gene expression [3]. On one hand, as D increases, the total accessible area on the chromatin increases, which will promote the overall gene expression. On the other hand, as D increases, the local variance of mass-density increases, leaving some specific genes in a highly condensed environment with low diffusion rate, thus suppressing the expression of these genes. To obtain D experimentally, Hi-C can be employed to provide indirect measurement (contact map), which can be later converted to D of the ensemble of numerous of cells [4]. However, the D of individual cell and the local distribution of D inside each nucleus is lost via such approach.

To extract D for each nucleus, as well as its spatial distribution throughout the nucleus, an imaging technique at 3 to 5 nm resolution with quantitative contrast is required. Conventionally, the image contrast in TEM of biological sample arises from heavy metal stains. Due to different affinities of biomolecules to heavy metals, e.g. osmium, uranium, the image contrast is hard to interpret and usually appears binary (Figure 1A, cheek cell prepared with osmium staining). Recently, a novel DNA staining method, chromeEM [5], has been introduced to visualize chromatin structure quantitatively with EM. We have successfully adapted the chromEM method on various cell lines, and obtained interpretable electron micrographs of chromatin structure. For example, the chromatin intensity shows continuous variations inside the A549 (*Homo sapiens* pulmonary carcinoma) nucleus stained by ChromEM (Figure 1B). We investigated the compaction difference of euchromatin and heterochromatin in BJ cell (*omo sapiens* foreskin) (Figure 1C). Qualitatively, the euchromatin (Figure 1D) contains more loosely packed chromatin than heterochromatin (Figure 1E) and shows a decrease in the overall chromatin density.

We further quantified the chromatin fractal dimension D from the EM images by analyzing the chromatin autocorrelation function directly. As the fractal model assumes, chromatin is isotropic, and the 3D fractal dimension can be approximated from the fractal dimension of a 2D cross section. In this work, 50 nm

thick sections were used for calculating the fractal dimension. In both cell lines, the autocorrelation function showed a strong power law behavior ranging from 30 nm to 100 nm, confirming that the chromatin can be described by the fractal model in the intermediate length scale. We also employed linear regression on the logarithm of the mass scaling to extract the fractal dimension. We found a significant increase in the fractal dimension D for a cancerous cell line (A549) compared to a non-cancerous cell line (BJ) (Figure 2). The finding could potentially facilitate future cancer detection based on the chromatin nanostructure [6].

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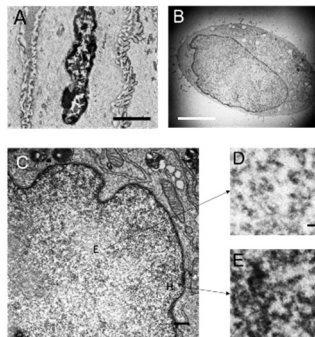
Figure 1. ChromEM images show interpretable chromatin compaction inside the cell nucleus. A. Cheek cell with conventional TEM. B. A549 cell with ChromEM method shows contrast variations inside the nucleus C. BJ cell nucleus consists of euchromatin (D) and heterochromatin (E), and the two shows significantly different packing and overall density. Scale bar: A: 3 µm and B: 5 µm, C: 500nm, D and E: 30nm.
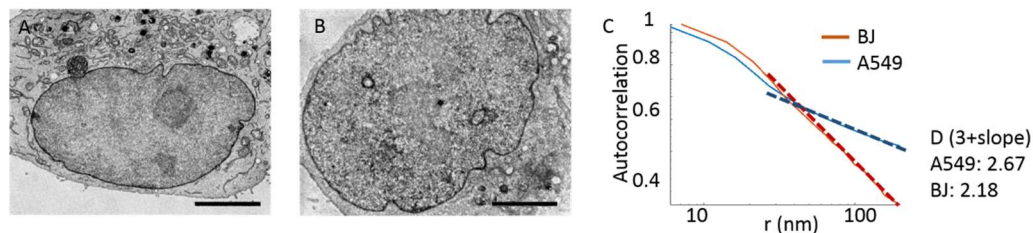

Figure 2. Comparison of chromatin for cancer and healthy cell lines. (A) Pulmonary carcinoma A549 cell and (B) Healthy BJ cell in inversed contrast HAADF. (C) Calculated from autocorrelation function, the fractal dimension of A549 is significantly higher than that of BJ.