Published in final edited form as:

Science. 2017 July 28; 357(6349): 354–355. doi:10.1126/science.aao1893.

The genome—seeing it clearly now:

Genome architecture is revealed with electron tomography

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It is a curious fact that in many fields of science, central questions that one might assume to have been answered decades ago remain perennially unresolved. In genome biology, one such cornerstone problem has been how DNA is organized in an intact cell nucleus. This conundrum is an important and intriguing one because the human genome is over 2 m in length and yet it is packed into a cellular compartment that is merely about 10 μ m in diameter. On page 370 of this issue, Ou *et al.* (1) now take a major step toward answering this foundational question.

We know, mostly from biochemical in vitro studies, that DNA forms higher-order structures in the shape of chromatin fibers, which comprise DNA wrapped around architectural histone proteins, together referred to as nucleosomes and often thought of as “beads on a string” (see the figure). Interactions between distal parts of the chromatin fiber result in higher-order folding and compaction (2). But why do we not know what this compacted chromatin polymer looks like in a cell nucleus? Most models suggest that the chromatin fiber is around 5 to 100 nm in diameter. This places chromatin in the “blind spot” of most imaging methods. These dimensions are largely below the resolution limit of light microscopy. Even super-resolution imaging, which increases the resolving power of the light microscope beneath the diffraction limit, is not sufficient to directly visualize these dimensions. Yet, existing electron microscopy (EM) techniques, which can easily resolve objects at this scale, cannot be used to unambiguously discern chromatin fibers in intact cells because native chromatin is not electron dense.

Ou *et al.* have now developed ChromEMT (chromatin electron microscopy tomography), a technique that overcomes these imaging limitations and enables visualization of chromatin fibers in the three-dimensional (3D) space of the mammalian cell nucleus. The method relies on multiple experimental advances in labeling, as well as acquisition and analysis, of EM images. Most importantly, the authors identified a fluorescent dye that binds to DNA and can be photoconverted to produce an electron-dense precipitate that decorates the DNA, making it visible by EM. In addition, tomography is used, in which cell slices are repeatedly tilted in the electron beam and imaged from multiple angles to enable tracking and determination of the fine structure of the chromatin fiber. Application of ChromEMT to the analysis of the genome in human small airway epithelial cells provides unprecedented insight into the organization of chromatin in the nucleus.

The first key finding of Ou *et al.* is that the vast majority of chromatin in the nucleus is predominantly organized as a disordered polymer of 5 to 24 nm in diameter, suggesting

minimal organization beyond the beads-on-a-string primary structure. Little evidence is found for higher-order organization into 30- or 120-nm fibers, as would be expected from the classic textbook models based on in vitro visualization of non-native chromatin. These findings are in line with the inability of several other imaging methods to detect 30-nm fibers in nuclei (3–5). This finding raises the possibility that the higher-order chromatin structures observed in vitro may preferentially form because of a higher propensity of nucleosomes in the fiber to aggregate under conditions far more dilute than the crowded environment of the nucleus. It is important to point out that the reported EM-based findings do not rule out the existence of higher-order fibers in vivo, as they may occur sporadically throughout the genome or they may only be induced under particular physiological conditions. Of note, higher-order chromatin structures in vivo were reported in a recent study based on DNA cleavage mapping followed by sequencing (6). The different conclusions of these studies may be due to the distinct spatial scales that were investigated and the ability of imaging to probe chromatin structures in single cells compared to population-based DNA cleavage mapping.

A second, related, major problem in the field is how different chromatin states are generated in a cell nucleus. Analysis by ChromEMT shows little difference in the higher-order organization of chromatin fibers in different regions of the nucleus, including in euchromatin (which is decondensed, often containing active regions of DNA), in heterochromatin (which is generally condensed and inactive), and even in highly condensed mitotic chromosomes. All these chromatin states predominantly contain fibers of 5 to 24 nm in diameter. The similarity between interphase and mitotic chromosomes is particularly striking because chromosomes undergo vast morphological changes in chromatin compaction during mitosis. These results lead to the provocative conclusion that the genome is generally packaged in the nucleus as disordered polymer chains of 5 to 24 nm in diameter and that higher-order chromatin features, such as euchromatin, heterochromatin, and mitotic chromosomes, are generated by differential packaging density of the same type of fiber rather than by distinct organizational principles, as previously thought (see the figure). In line with this interpretation, recent live-cell observations have reported comparable DNA accessibility and binding patterns of transcription factors on inter-phase and mitotic chromatin (7).

How sure can we be of these results? It can be argued that it is somewhat disconcerting that none of the previously reported, well-characterized higher-order chromatin structures were detected with ChromEMT. In addition, one might be suspicious of the finding that the same 5- to 24-nm structures were found in all types of chromatin analyzed, including mitotic chromosomes, raising the issue of intrinsic measurement bias of the method. In addition, ChromEMT relies on chemical fixation of samples, which could introduce artifacts. However, several observations are reassuring. As a positive control, ChromEMT detects higher-order chromatin structures when they exist, as demonstrated by the visualization of 30-nm-diameter fibers in nuclei from chicken erythrocytes isolated under conditions previously shown by various EM methods to induce higher-order fibers (8). It also seems unlikely that the observed structures are due to fixation artifacts because, if anything, cross-linking fixation as used in ChromEMT would be expected to induce artifactual higher-order structures rather than the smaller fibers observed. However, uncertainties remain. For example, tracing of chromatin fibers over longer distances, and through multiple

tomographic cell slices, is still challenging, even using sophisticated 3D reconstruction approaches, making the precise determination, and particularly the interpretation, of local and global structures difficult.

The ability to visualize the chromatin fiber in the cell nucleus is a landmark achievement that opens the doors to probing chromatin structure in relation to its function. Correlative studies of chromatin structure with overall activity under various physiological conditions and in differentiation, senescence, and disease are the obvious follow-up studies. Moreover, the organization of chromatin in cells from intact tissue may be accessible with this approach. However, the true potential of ChromEMT will be in its combination with protein-detection methods to map proteins onto the chromatin fiber structure to tag specific genome regions, and in the use of experimental systems to tether specific proteins that modulate the chromatin state or chromatin activity and to probe the relationship of genome structure to function. It may also be attractive to attempt to combine ChromEMT with light microscopy, including super-resolution imaging, to investigate chromatin structure in living cells. These are challenges for the future—for now, one of the enduring foundational questions in the field of genome biology has been answered. ■

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Figure. Packaging DNA.

ChromEMT reveals that DNA is packaged into “beads-on-a-string”; fibers, which are assembled at different densities according to function.