Irregular organization in the human chromosomes revealed by X-ray scattering

How is a long strand of DNA organized in the cell? In molecular biology textbooks, we often find a typical figure (Fig. 1(a)). To begin with, DNA is wrapped around histones and forms a "nucleosome" (10-nm fiber) structure. This nucleosome has been assumed to be folded into the regular "30-nm chromatin fiber." In one of the famous models, the "hierarchical helical folding model," it is assumed that the 30-nm chromatin fiber is folded progressively into larger fibers such as ~100-nm and then ~200-nm fibers, to form the final mitotic chromosomes (Fig. 1(b)) or large chromatin fiber (chromonema fiber) in interphase cell nuclei.

What do mitotic chromosomes actually look like in cells? To see chromosomes as intact as possible, we performed cryo-electron microscopy (cryo-EM) [1]. Surprisingly, cryo-EM and subsequent computational image processing did not reveal any 30-nm chromatin fibers in the mitotic chromosomes, but merely a uniform disordered structure, which strongly argues against the "established view" (Figs. 1(a) and 1(b)).

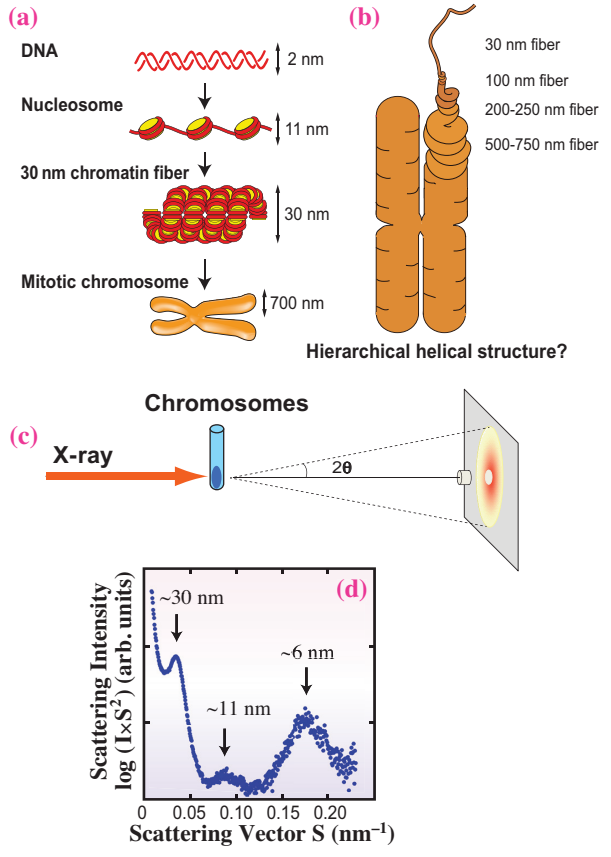

Fig. 1. **(a)** Textbook image of chromatin, **(b)** hierarchical helical folding model, **(c)** experimental setting of small-angle X-ray scattering, and **(d)** a typical scattering pattern of human mitotic chromosomes.

On the other hand, cryo-EM observation can examine only a limited portion of a chromosome, not the whole, because the section thickness is only around 50 nm. It was thus difficult to observe possible hierarchical regular structures in the chromosomes, if any.

To investigate the bulk structure of the mitotic chromosomes in solution, we performed small-angle X-ray scattering (SAXS) [2,3]. Generally, when X-rays are irradiated onto non-crystal materials, scattering at small angles can generally reveal periodic structures within samples (Fig. 1(c)). At beamline **BL45XU**, the synchrotron X-ray beam was irradiated onto isolated human chromosomes at the bottom of a glass capillary, and scattering patterns were recoded (Fig. 1(c)). A typical scattering pattern of mitotic chromosomes showed three peaks: 30-nm, weak 11-nm, and 6-nm peaks (Figs. 1(d) and 2(a)). This result is completely consistent with those of experiments conducted by Langmore & Paulson [4]. While the 11 nm and 6 nm peaks are believed to originate from the spatial arrangement of nucleosomes, but the 30-nm peak was assumed to represent the side-by-side packing of the 30-nm chromatin fibers, and has long been regarded as strong evidence for the existence of these fibers in chromosomes. However, why could we not observe such a 30-nm structure in the mitotic chromosomes by cryo-EM?

To elucidate the nature of the 30-nm peak observed by SAXS, the isolated chromosomes were examined by cryo-EM. Again, no 30-nm chromatin structures were observed inside chromosomes. However, the cryo-EM images unexpectedly showed that the chromosome surface was coated with electron-dense granules of the similar size as ribosomes (Fig. 2(a), bottom). Biochemical analyses confirmed that they are ribosomes contaminating the chromosome surface. The ribosomes were regularly stacked with a ~30 nm spacing. We removed the ribosomes from the chromosome surface by washing with an isotonic buffer, with maintaining the size and shape of chromosomes (Fig. 2(b), bottom). Strikingly, in the chromosomes, no 30-nm peaks were detected by SAXS (Fig. 2(b), top). However, other peaks originating from the internal structure of nucleosomes remained (Figs. 2(a) and 2(b)). We then concluded that regularly folded 30-nm chromatin fibers are essentially not present in human mitotic chromosomes.

Next, we investigated an entire region of mitotic chromosomes at beamline **BL29XUL** using a newly developed apparatus for ultra-small-angle X-ray

scattering (USAXS) (Fig. 2(c)) [2,5]. Again, we found no regular periodic structures, including the 100–150 or 200–250 nm structure, between ~50 and 1000 nm in mitotic chromosomes (Fig. 2(d)), contradicting the hierarchical helical folding model (Fig. 1(b)). The cryo-EM, SAXS, and USAXS data collectively indicate that the irregularly folded nucleosome fibers make up the bulk structure of human mitotic chromosomes.

How is the nucleosome fiber organized into a mitotic chromosome? Since condensin and topoisomerase IIα, which are essential for chromosome condensation, form an axis in the chromosome, we assume that they globally hold the nucleosome fibers around the chromosomal center (Fig. 3(a)) [2]. Locally, nucleosome fiber is folded in an irregular manner toward the chromosome center (Fig. 3(a)). Furthermore, SAXS and USAXS also revealed that there are no periodic structures in the chromatin in the interphase cells [3], suggesting that the bulk interphase chromatin also consists of irregularly folded nucleosome fibers (Fig. 3(b)).

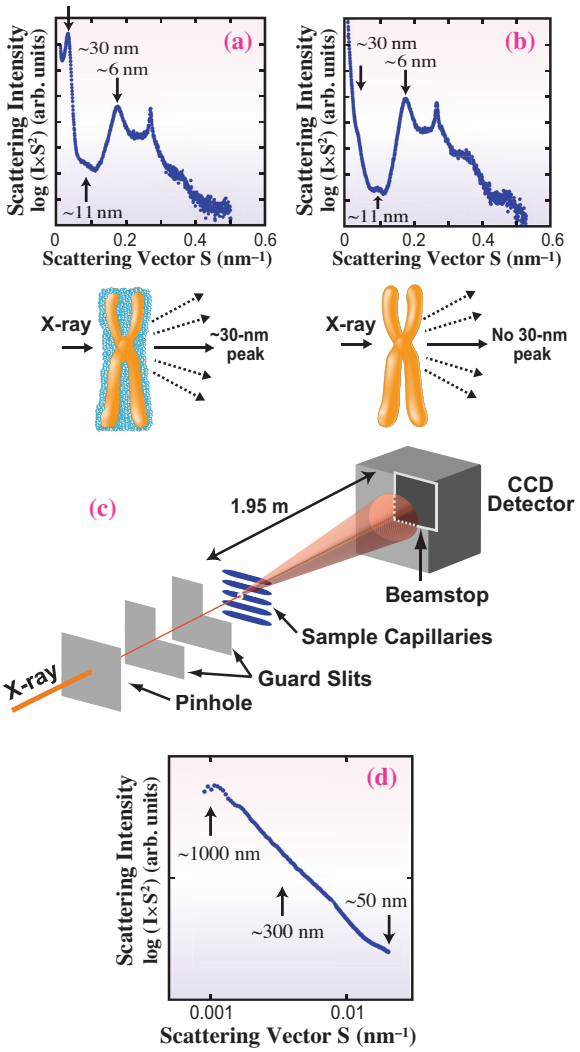

Fig. 2. **(a)** SAXS profile of mitotic chromosome fraction with ribosomes (top) and scheme (bottom), **(b)** SAXS profile without ribosomes (top) and scheme (bottom), **(c)** experimental setting for USAXS [5], and **(d)** USAXS profile of human mitotic chromosomes.

In conclusion, X-ray scattering analysis revealed that interphase chromatin and mitotic chromosomes have similar local organizations: compact, irregular folding of nucleosome fibers without the 30-nm chromatin fiber (Figs. 3(a) and 3(b)). Although the term "irregular" or "disordered" might give the impression that the organizations are likely functionally irrelevant, the irregular folding implies little physical constraint and a highly dynamic property, leading to a high degree of DNA accessibility. The irregular organization may thus have several advantages in template-directed biological processes in interphase nuclei, including RNA transcription and DNA replication and repair/recombination.

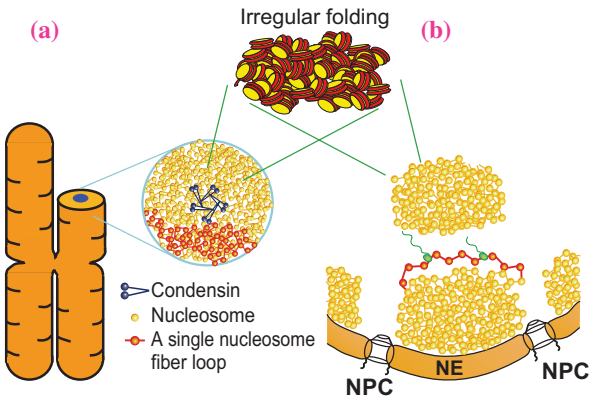

Fig. 3. **(a)** Model structure of mitotic chromosomes and **(b)** interphase chromatin. NE, nuclear envelop; NPC, nuclear pore complex.

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