Tracking Kinetochores Throughout Meiosis

Cell Biology

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Chromosomes must establish stable biorientation before anaphase to ensure that both daughter cells share the same genetic information. Researchers led by Jan Ellenberg of the European Molecular Biology Laboratory in Heidelberg, Germany are using Imaris 3D/4D image visualization and analysis software to reveal new information about how the process of biorientation occurs during meiosis.

One reason that scientists want to know more about biorientation is because when something goes wrong with the process in female oocytes, the resulting egg may have added or missing chromosomes. Fertilization of such aneuploid eggs is a leading cause of pregnancy loss and can also lead to developmental disabilities.

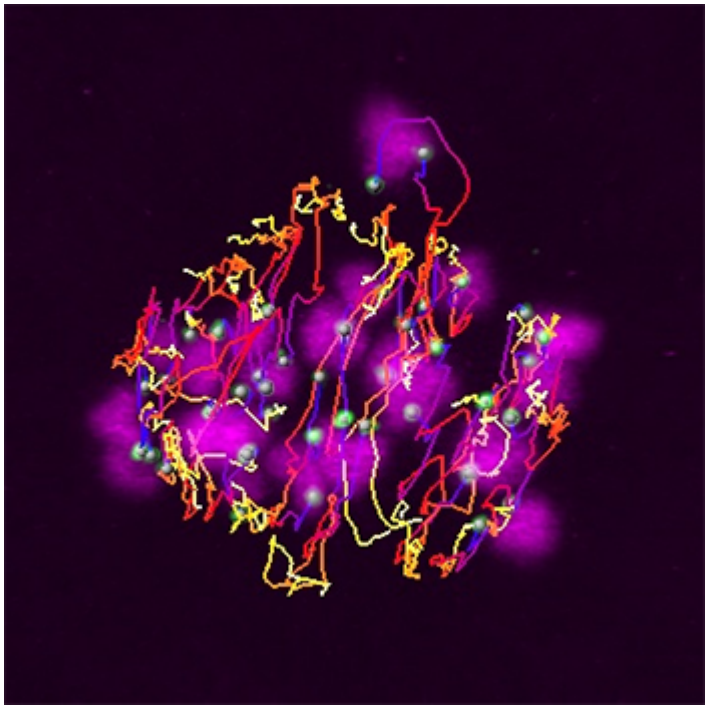
During biorientation in somatic mitosis, microtubules arising from two centrosomes, which predefine spindle poles, attach to kinetochores of sister chromatids so that the sister chromatids move to opposite poles of the spindle during cell division. In contrast, oocytes do not have centrosomes. The researchers have previously shown that during biorientation in mouse oocytes an acentrosomal spindle is assembled through self-organization of many microtubule-organizing centers (MTOCs). However, it is still not clear how homologous chromosome biorientation occurs during the long and complex process of spindle self-assembly.

Kinetochore Tracking

To find out, the researchers used Imaris, the most powerful and versatile 3D and 4D image analysis software solution on the market and 3D confocal fluorescence microscopy at high spatial and temporal resolution. Both tools made it possible to track all homologous kinetochores during the approximately eight hours that elapse from nuclear envelope breakdown to the beginning of chromosome segregation in anaphase. “We wanted to systematically and quantitatively analyze chromosome dynamics in mouse oocyte meiosis,” said Dr. Tomoya Kitajima, who is part of the research team at EMBL. “So we established the imaging and tracking pipeline that can track all kinetochores and chromosomes throughout meiosis using Imaris. This provided the first complete 4D map of kinetochores in any cell division.”

The group analyzed kinetochores and chromosomes dynamics during the first meiotic division of live mouse oocytes by performing time-lapse imaging with a confocal microscope at 90 second intervals for 9 hours after maturation was induced. The kinetochores were fluorescently labeled with EGFP-CENP-C, chromosomes with Histone 2B (H2B)-mCherry, and microtubules with EGFP-MAP4. “We used Imaris for reconstruction of our images into 3D, detection of kinetochore positions by automatic spot detection, and tracking of the kinetochores by automatic 3D spot tracking,” said Dr. Kitajima.

To correct for global cellular movements, the researchers registered the kinetochore positions over time to the centroid of all kinetochores before using Imaris 3D spot tracking to follow the kinetochores. They evaluated the tracks and manually corrected rare track errors in Imaris. “The automatic spot detection function in Imaris worked efficiently to detect kinetochore signals in our images. We just needed some manual corrections after the automatic detection, but thanks to the Imaris' user-friendly interface it was easy,” said Dr. Kitajima. The researchers also measured spindle elongation by performing 3D surface rendering based on the spindle fluorescent signal and fitting those with an ellipsoid in Imaris.



In this image of prometaphase the fluorescent signals are chromosomes (purple) and kinetochores (green) of the first meiotic division in a mouse oocyte. Imaris was used to track the kinetochore signals over time (color code is based on track’s time statistics: blue – earlier time points to yellow - time points closer to track’s end.)

The data gained from Imaris allowed the researchers to systematically and quantitatively analyze kinetochore and chromosome dynamics during the first meiotic division. The group discovered that i) chromosome congression precedes biorientation, ii) that two-thirds of all biorientation attempts are erroneous, and iii) that 86% of all homologous chromosomes go through error correction process of their kinetochore-microtubule attachments before establishing stable biorientation. The finding that homologous chromosome biorientation is such an error-prone process in the oocytes the researchers studied could help explain what are some of the underlying causes of relatively frequent aneuploidy in mammalian and human eggs.

Research Paper: Complete Kinetochore Tracking Reveals Error-Prone Homologous Chromosome Biorientation in Mammalian Oocytes, Cell, Volume 146, Issue 4, 568-581.