3D in SEM, (S)TEM, Ion Imaging, incl. FIB-SEM and SBF-SEM

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High resolution structural analysis of a centromere-specific histone H3 in mitotic plant chromosomes with FIB/FESEM tomography

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Chromosome architecture and the ultrastructure of centromeres have been a central research topic in our group over last two decades. Centromeres have varying morphological characterics, but are generally composed of parallel fibrils and bunched fibrils, or chromomeres, of both chromatin and proteinaceous nature [1]. With high resolution analytical FESEM techniques the distribution of different histones in the centromere were investigated.

Of particular interest is the distribution of the centromere-specific histone H3, CenH3. Combined LM and FESEM techniques including DNA-specific platinum staining and FluoroNanogold labeling allowed quantification and high resolution analysis of 3D distribution of CenH3. The majority of CenH3 localizes to two distinct areas, presumably on each sister chromatid, of the primary constriction [2]. In addition, cummulative results from several studies on histone variants in barley shows that the lateral outermost fibrils of the centromere i) do not stain for DNA, ii) do not label for phosphorylated histone H3 (serine 10), iii) do not label for canonical H3P, iv) do not label for CENH3 [2]. With low voltage SEM analysis, it could be determined that only very few CENH3 markers are located on the centromere surface [3].

In order to determine the 3D distribution of CenH3, barley centromeres were investigated using FIB tomography. Marker quantification, 3D reconstruction and animations of the CENH3 domain were calculated using FIB/FESEM tomographic data. It could be shown that, contrary to consensus models for centromere organization and kinetochore assembly, CENH3 is located in the chromosome interior of both the centromere proper and the pericentric region. 3D tomography confirmed the interior localization of CENH3, also showing distribution of individual markers in their spatial context with the highest resolution achieved to date [3]. In parallel investigations, the same interior localization of CenH3 could be found in pea centromeres [4].

This partially challenges the current trilaminar model of centromere structure, that is, however, based on human chromosomes [5]. This raises classical questions about how resolution limitations may influence interpretation of data, as well as how much influence preparation methods may have on the fidelity of immunolocalization for SEM. There is a possibility that CENH3 distribution may differ between plant and animals, as there exists an early uncontested postulation of a "ball-and-cup" structure for plant kinetochores based on TEM data [6, 7]. For convergence of seemingly contradictory findings it will be necessary to continue combining biochemical, cytological and high resolution structural approaches. Further SEM studies localizing tubulin and other centromere-related proteins such as CENPC will be necessary to determine whether and where residues are detectable on both isolated chromosomes and *in situ* chromosomes, allowing for successful preparation and labeling methods. Since immunolocalization of more than one epitope for SEM investigations is currently only a remote possibility, it is important to maintain a correlative LM-SEM approach, aiming toward super-resolution labeling of multiple antigens in order to achieve the goal of atttaining a high resolution structural kinetochore model.

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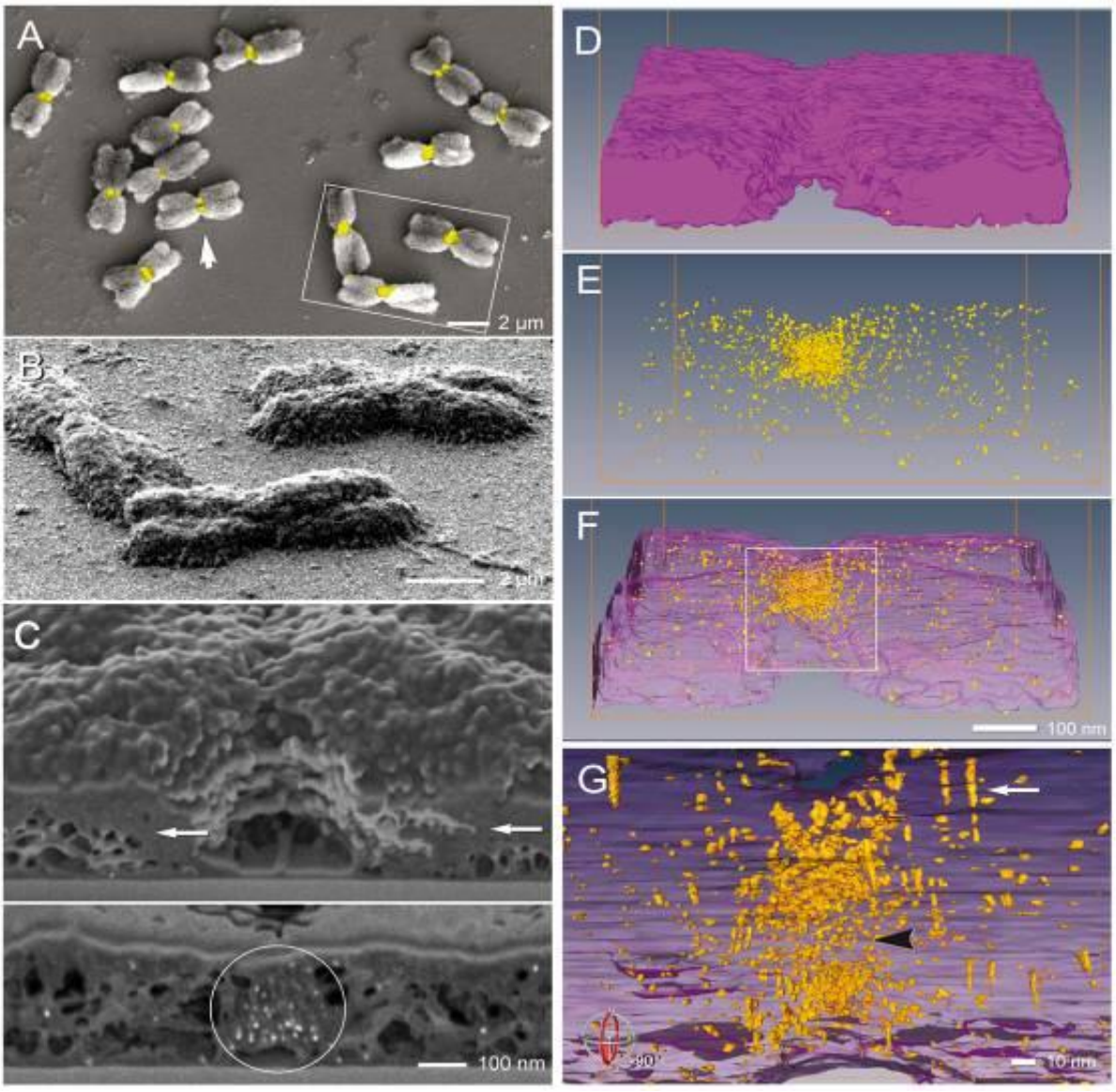

Figure 1. SEM micrographs and 3D reconstructions from FIB/FESEM tomographic series of barley chromosomes that are immunolabeled for the centromere-specific histon H3 variant CenH3. A Orthographic view of one chromosome complement in metaphase (2n=14) with superimposed backscattered electron signal (yellow) showing CenH3 labels. B Chromosomes (frame in A) after tilting for FIB tomography. C Image of milled surface of centromere at onset (arrows indicate cut surface), and after several "sections". CenH3 markers (white signals in circle) are concentrated in the centromere. D-G 3D reconstruction of CenH3 distribution in the centromere and in the pericentric region (CenH3=yellow, chromosome surface=magenta). When the chromosome surface is transparent, it is obvious that the CenH3 signal is not localized on the centromere surface. High resolution animation confirms a CenH3 gap between the chromatids (black arrowhead), and also shows occasional distortion of signals due to FIB "stalling" during series (white arrow) (see Schroeder-Reiter et al. 2012).