Electron tomography of cadherin-mediated progenitor cell-cell junctions

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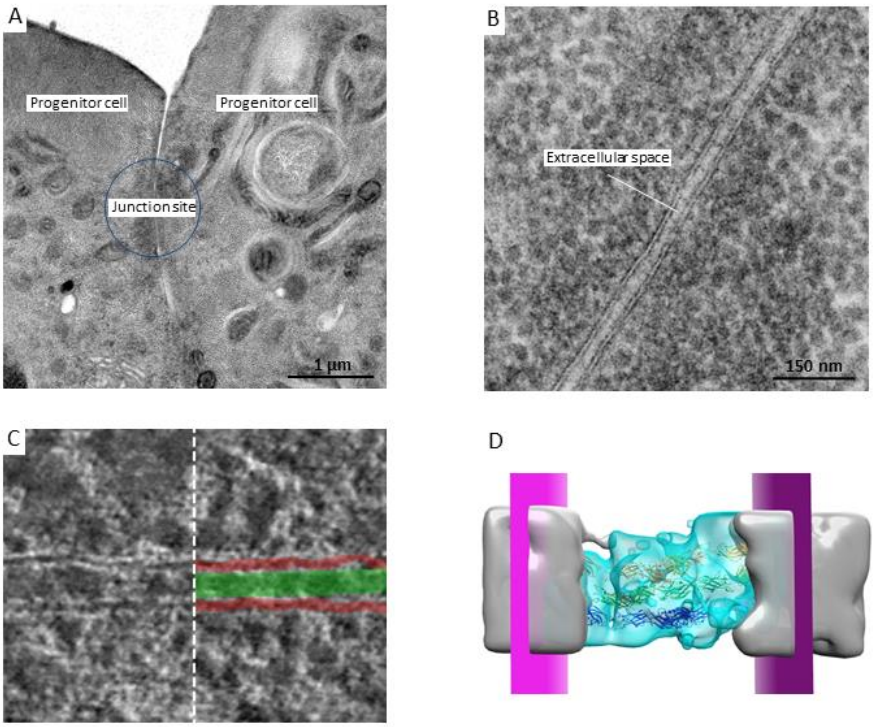
Early stage cadherin-containing cell-cell adhesion sites were studied in primary germ layer progenitor cells from Zebrafish. They form adherens junctions of different strength and size depending on cell type and cortical tension. Previous studies on cultured cells provided evidence that tension at adherens junctions promote cadherin clustering (Engl et al., Nat Cell Biol. 2014, 16:587-94). Here, we aimed to reveal the ultrastructural arrangement of cadherin molecules at cell-cell adhesion sites. We showed that changes in cortex tension affect the packing and topology of cadherin molecules at the junction sites interfaces, and that these clusters fuse upon myosin-II-dependent tension increase induced by lysophosphatidic acid treatment.
Progenitor cells from freshly dissociated dome-stage Zebrafish embryos were first allowed to form spontaneous contacts and adhere to carbon-coated sapphire disks. Samples were high-pressure frozen in a HPM-010 and freeze-substituted in an AFS1 device (both Leica Microsystems). Samples were then Epon embedded, sliced at 70-250 nm thickness and mounted on half-mesh grids. They were observed under a JEM 2800 STEM (Jeol) operated at 200 kV in TEM and STEM bright-field mode, respectively. For recording of image tilt series, STEM Recorder V3 and Magica Controller were employed (both System in Frontier Inc.). Images were collected at 2° intervals between +/-76° of actual single tilt axis. Images were aligned by cross-correlation and 3D structure computed by weighted back-projection. 3D-CTF correction was applied (Turonova et al., J Struct Biol. 2017, 199:187-95).
In order to extract the electron density maps of adherens junctions, progenitor cell plasma membranes were segmented using a fully convolutional neural network (FCNN). A training set of 32x32px patches (n=852) of plasma membrane bilayer and background structures was manually annotated, and the FCNN was optimized with stochastic gradient descent. The trained network was used to predict the location of cell plasma membranes in the entire stack. A three-dimensional quadratic surface was fitted to each of the cell's plasma membranes and the density maps of enclosed regions were extracted, narrowed by the size of the protoplasmic membrane leaflet. Raw electron tomography stacks were denoised by 3D Gaussian filtering and rotated for rough horizontal junction alignment. Resulting density maps were segmented using the "segment map" feature available in the UCSF Chimera software to extract density segments corresponding to assemblies of cadherin dimers. Quantitative content of cadherin for each assembly was then assessed using macromolecular modeling software PyRy3D (www.genesilico.pl/pyry3d). Atomic structure of the protein was fitted inside the density segments in different number of copies in order to find a quality of fit threshold that could help estimate the content of cadherin dimers corresponding to each assembly.

Figure A: STEM image of a contact site between progenitor cells. B: Contact site revealing the structure of adherens junctions. C: Section through the tomographic reconstruction after post-processing indicating the predicted location of cell plasma membranes (red overlay) and enclosed region (green overlay). D: 3D model generated from tomographic data, showing the plasma membranes (magenta), protein transmembrane segments (grey), density from the map (cyan) and fitted cadherin atomic structure (color transition).


A
Progenitor cell
Progenitor cell
Junction site
1 µm
B
Extracellular space
150 nm
C
D