ELCS in Ice: Cryo-electron Microscopy of Nuclear Envelope Limited Chromatin Sheets

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EMBL

1. WHAT ARE ELCS?

Nuclear Envelope-Limited Chromatin Sheets (ELCS) form during excessive interphase nuclear envelope growth in a variety of cells. ELCS appear as extended sheets within the cytoplasm connecting distant nuclear lobes. Cross-section stained images of ELCS, viewed by transmission electron microscopy, resemble a sandwich of apposed nuclear envelopes separated by ~30 nm, containing a layer of ordered chromatin fibers.

2. EM PROCEDURES

The ultrastructure of ELCS was compared by three different methods: 1) aldehyde fixation/dehydration/plastic embedding/sectioning and staining; 2) high-pressure freezing/freeze substitution into plastic/sectioning and staining; 3) high-pressure freezing/cryo-sectioning/cryo-electron microscopy. Human leukemic (HL-60/S4) cells were treated with retinoic acid (4 days) to induce granulopoiesis, growth of nuclear envelope membranes, formation of lobulated nuclei and ELCS.

3. COMPARISON OF DIFFERENT PROCEDURES

ALDEHYDE FIXATION

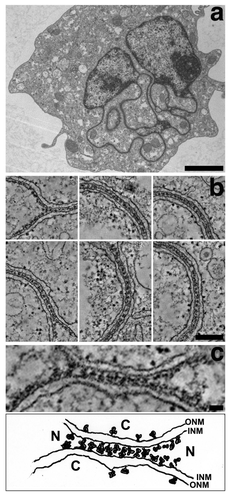

Fig. 1 Thin section transmission electron micrographs of plastic embedded and stained retinoic acid treated HL-60/S4 cells. a, Low magnification view of a single HL-60/S4 granulocytic cell with cross-section views of two large nuclear lobes and extensive ELCS. *Scale bar* 2 µm. b, Cross-section views of ELCS (six panels) taken from tomographic reconstructions. *Scale bar* 200 nm. c, Enlarged tomographic cross-section view of ELCS bridging between two nuclear lobes, accompanied (below) by a schematic representation of the same field. Abbreviations: N, nuclear lobe; C, cytoplasm; ONM, outer nuclear membrane; INM, inner nuclear membrane. Ribosomes are represented associated with the cytoplasmic surface of the ONMs. Putative 30 nm chromatin fibers are represented spanning the space between the apposed INMs. *Scale bar* 30 nm

HIGH PRESSURE FREEZE SUBSTITUTION (FS)

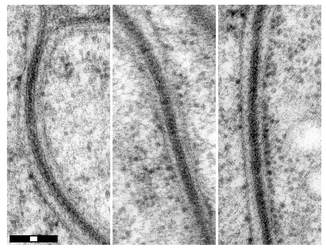

Fig. 2 Freeze-substitution (FS) images of ELCS. High magnification cross-sections following high-pressure freezing/freeze-substitution, sectioning and staining. Putative 30 nm chromatin fibers are not seen. *Scale bar* 200 nm. "30 nm" white bar is inserted into the black Scale bar.

HIGH-PRESSURE FREEZE CRYO-SECTION CRYO-EM (CEMOVIS)

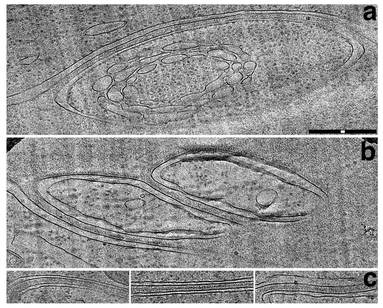

Fig. 3 CEMOVIS images of ELCS. a and b, ELCS with "pockets" enclosing cytoplasmic membranes and ribosomes. c, short linear stretches of ELCS. *Scale bar* 500 nm. "30 nm" white bar is inserted into the black Scale bar.

THICKNESS OF ELCS

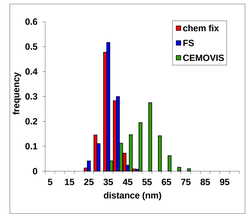

Fig. 4 Histogram of the fraction of measured ELCS with specified distances between apposed inner nuclear membranes, comparing three sample preparations. Y-axis: fraction of total measurements in a single preparative procedure. X-axis: distance (nm) between the apposed membranes.

	N	mean (nm)	σ (nm)
Aldehyde Fix	2221	33.9	4.1
FS	2071	33.5	4.1
CEMOVIS	2042	49.3	8.1
CEMOVIS*	2042	57.4	7.6

*** Extrapolated uncompressed distance**

Table 1 Distance between apposed inner nuclear membranes of ELCS. This table displays the measurement statistics, summarizing the total number of measurements (N), and the mean (nm) and standard deviations (σ) of the distances between the inner nuclear membranes, comparing three sample preparative procedures.

4. TOMOGRAPHY of aldehyde fixed ELCS

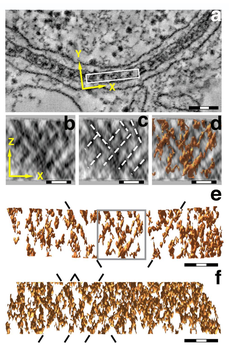

Fig. 5 Visualization of the chromatin fiber arrangements in chemically fixed ELCS. a, Computed slice from an ELCS tomogram with boxed area containing chromatin fiber cross-sections shown in b, c, d and outlined in gray in e. b, Projection along the y-axis of the box outlined in a, revealing locally parallel and tilted stretches of chromatin fibers. c, Superposition of dashed lines designating the fiber paths, on the projection in b. d, Isosurface rendering of the same projection as b. e, Isosurface rendering of ELCS in a, but rotated by 90° to give a tangential view, the volume (grey box) shown in b-d and the entire region shown in a. f, Isosurface rendering of a second region showing extended tangential views of ELCS. The "tics" shown in e and f indicate straight chromatin threads that appear to form X and V patterns. *Scale bars* are 100 nm in a,e,f; 50 nm in b,c,d.

5. CONCLUSIONS

- **ELCS exist in vivo. They are not an artifact of fixation.**
- **ELCS in ice are thicker than after dehydration and embedding in plastic.**
- **EM tomography of aldehyde fixed cells supports that the putative "30 nm fiber" in ELCS are composed of two overlapping layers of parallel "10 nm fibers".**

6. MODEL

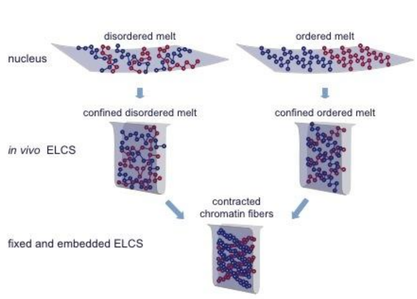

Fig. 6 Modeling chromatin fiber folding during ELCS formation. Schemes demonstrating how nuclear envelope associated ~10 nm chromatin ("ordered melt" or "disordered melt") might generate apparent ~30 nm fibers in aldehyde fixed, dehydrated, embedded and sectioned ELCS, as visualized by electron microscope tomography .

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