

WORKSHOP I: NUCLEAR STRUCTURE – CHROMATIN I

doi: <http://dx.doi.org/10.7124/bc.0009A3>

Nuclear pore assembles via structurally and molecularly distinct mechanisms after mitosis and during interphase

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The nuclear pore complex (NPC) is the largest non-polymeric protein complex in eukaryotic cells and spans the double membrane of the nucleus (nuclear envelope; NE) to mediate nucleocytoplasmic transport. In mammalian cells, NPCs are assembled in two cell cycle stages, during nuclear assembly after mitosis and nuclear growth in interphase. How the NPC and the double nuclear membrane reassemble concomitantly in late mitosis, and how the NPC newly assembles in the closed NE in interphase, has been unclear. By correlating live imaging with three-dimensional electron microscopy, we have recently revealed that

nuclear pores assemble via structurally distinct mechanisms in mitosis and interphase; during mitotic exit, pore assembly proceeds by radial dilation of small membrane openings, while in interphase, assembly induces a novel asymmetric inside-out fusion of the inner with the outer nuclear membrane. To understand the molecular maturation processes of these two distinct NPC assembly pathways, we created genome-edited GFP knock-in cells for nucleoporins of all major NPC substructures, i.e. the cytoplasmic filaments, the cytoplasmic/nucleoplasmic rings, the inner rings, and the nuclear basket. By FCS-calibrated three-dimensional live cell imaging, we monitored the concentration changes of these GFP-tagged nucleoporins in different regions of the NE where postmitotic and interphase assembly can be spatially distinguished for the first hour after mitotic exit. Quantitative kinetic analysis of the concentration changes showed that the molecular assembly order and maturation kinetics are distinct for postmitotic and interphase assembly, demonstrating that NPC assembly is not only a structurally but also molecularly different process between mitosis and interphase.