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Nucleoporin Condensates Drive NPC Assembly in Oocytes

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Abstract

Oocytes stockpile nuclear pore complexes (NPCs) in cytoplasmic membrane sheets called annulate lamellae (AL) in preparation for rapid cell cycles during embryogenesis. Hampoelz *et al.*, (Cell, 2019) report that AL-NPC assembly depends on the coordinated formation, transport and interaction of biomolecular condensates containing distinct sets of nucleoporins.

Keywords

nuclear pore complex; macromolecular assembly; annulate lamellae; oogenesis; nucleoporin liquid-liquid phase separation condensate

The enclosure of genetic information in the nucleus is one of the great hallmarks of evolution, but creates the necessity for portals through which folded proteins and protein/nucleic acid complexes can cross the nuclear envelope (NE) [1]. The nuclear pore complex (NPC), a cylindrical supramolecular structure embedded in circular pores permeating the NE, is the sole gateway for passage through the NE and can accomplish the selective bidirectional transport of macromolecules up to ~40 nm in diameter at a rate of several hundred events per second. NPCs are composed of ~34 evolutionarily conserved proteins termed nucleoporins (nups), which each occur in multiple copies to form a ~1000 protein organelle with a mass of ~110 MDa in vertebrates. Surrounding a central transport channel is a symmetric core that consists of a doughnut-shaped inner ring embedded within NE pores and two outer coat nucleoporin rings protruding from the inner and outer nuclear membrane, respectively. Asymmetric NPC components known as the cytoplasmic filaments and nuclear basket attach to the symmetric core on either side. The NPC's diffusion barrier is formed by hydrophobic interactions between natively-unfolded phenylalanine-glycine (FG) rich nup segments, which create a liquid-liquid phase separated (LLPS) milieu in the central transport channel while providing docking sites for karyopherin transport factors that ferry cargo into and out of the nucleus. Transport directionality is established by the nucleotide state of the small GTPase Ran [1]. The spatial segregation of the Ran guanine nucleotide exchange factor RCC1, and the Ran GTPase-activating protein (RanGAP) in the nucleus and cytoplasm, respectively, ensures that Ran is maintained in its active GTP-bound state in the nucleus, while inactive GDP-bound Ran is encountered primarily in the cytoplasm. Karyopherin transport factors take advantage of this cellular RanGTP gradient

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with karyopherin•cargo nuclear import complexes being disassembled by RanGTP binding, while karyopherin•cargo nuclear export complexes require RanGTP for assembly.

Different biogenesis pathways have been identified for hierarchical NPC assembly during interphase or following open mitosis. For *de novo* NPC assembly during interphase, nup subcomplexes are transported into the nucleus through existing NPCs and anchored at the inner NE by association with the basket nup Nup153 [2]. Maturing NPCs are then extruded through the NE from the nuclear face outwards, before incorporation of the inner ring and asymmetric nups [3]. Post-mitotic NPC assembly takes place inside radial NE openings as the endoplasmic reticulum (ER) engulfs naked chromatin during anaphase. The coat nup complex is anchored to exposed chromatin via its component Elys, followed by stepwise association of the inner ring and the cytoplasmic filaments [4,5]. Hampoelz *et al.* (Cell, 2019) now describe a third NPC biogenesis pathway, which operates during the stockpiling of NPC-like structures in cytoplasmic membrane sheets called annulate lamellae (AL) in oocytes [6].

The authors had previously shown that pre-assembled NPC scaffolds residing in AL are inserted into fenestrated NEs during early embryogenesis, thus enabling rapid mitotic divisions in the *Drosophila melanogaster* syncytial blastoderm [7]. Using an integrated cell biology approach combining selective nup fluorescent labeling with time-resolved fluorescence microscopy and correlative light and electron microscopy (CLEM), the authors have now obtained snapshots of the AL-NPC assembly pathway during oogenesis. *D. melanogaster* oogenesis takes place in so-called egg chambers, where several nurse cells support the maturation of a single oocyte by supplying nutrients and other materials through cytoplasmic bridges called ring canals (Figure 1). Although AL-NPCs are only present in the oocyte, the authors find that their assembly depends on distinct processes in both cell types. The obligate first step towards AL-NPC biogenesis is the condensation of selected nups into granules, which in nurse cells consist mostly of Nup358. Nup358 is a metazoan-specific, essential cytoplasmic filament nup, which contains binding sites for Ran and RanGAP, and which unlike most other asymmetric nups is also present in AL-NPCs. On the other hand, oocyte-specific condensates are formed by the coat component Nup107 and/or FG-nups. Both types of condensates are transported along the microtubule network in a motor-dependent manner to sites in the ooplasm, where they interact with each other and membrane sheets for AL-NPC assembly. Using gene silencing of Nup358 or its microtubule transport adapter, the authors demonstrate that AL-NPC assembly depends not merely on the presence of Nup358, but on its biophysical state. Condensated but not soluble Nup358 can initiate AL-NPC assembly, perhaps by marking the assembly site analogously to the proposed roles of Nup153 and Elys during interphase and post-mitotic NPC assembly, respectively.

Canonical NPC assembly has been proposed to involve Ran-controlled reversible chaperoning of soluble nups by karyopherin transport factors [8,9]. To investigate the role of Ran in AL-NPC assembly, Hampoelz *et al.* monitored the levels of RanGAP and RCC1 in the egg chamber. In nurse cells, high levels of RanGAP were associated with the cytoplasmic face of the NE and Nup358 granules as expected from known Nup358 properties. Conversely, RCC1 was highly enriched in the oocyte nucleus and even detectable

in the ooplasm, thus creating a RanGTP gradient between oocyte and nurse cells, which could conceivably control the differential formation of Nup358- and oocyte-specific condensates and the de-condensation of Nup358 at ooplasmic AL-NPC assembly sites. The roles of karyopherin transport factors in AL-NPC assembly appear to be more complex. Although the authors did not obtain clear results upon manipulation of a nuclear import karyopherin, possibly due to redundancy among various import factors, gene silencing of the export karyopherin CRM1 dramatically affected nup LLPS granule formation, favoring the condensation of Nup358 in the oocyte and of FG-nups in nurse cells rather than vice versa. The mechanisms by which CRM1 differentially modulates the condensation behavior of different kinds of nup LLPS granules are not immediately obvious and will require further study. More generally, though, spontaneous condensation into LLPS granules has been observed upon biochemical reconstitution of the fungal coat nup complex [10] and it is conceivable that karyopherins could modulate such behavior through differential binding to LLPS-triggering sequences in different nups.

By employing a new experimental system to track and interrogate individual nups, Hampoelz *et al.* have provided first glimpses into the AL-NPC assembly pathway, which provide a rich foundation for future studies and raise exciting questions on the possible role of nup condensation for NPC assembly in general. Considering the many cellular factors required for the stepwise assembly of other complex macromolecular machineries, such as the ribosome, it seems likely that Ran-controlled karyopherins and/or still to be discovered assembly factors play important roles in NPC biogenesis. Ultimately, the addition of an *in vitro* NPC assembly system from recombinant purified proteins to the experimental toolbox is expected to enable such mechanistic insight.

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References

[1]. Lin DH and Hoelz A (2019) ‘The Structure of the Nuclear Pore Complex (An Update)’, Annu Rev Biochem, 88, pp. 725–783. [PubMed: 30883195]

[2]. Vollmer B, Lorenz M, Moreno-Andrés D, Bodenhöfer M, De Magistris P, Astrinidis SA, Schooley A, Flötenmeyer M, Leptihn S and Antonin W (2015) ‘Nup153 Recruits the Nup107–160 Complex to the Inner Nuclear Membrane for Interphasic Nuclear Pore Complex Assembly’, Dev Cell, 33(6), pp. 717–28. [PubMed: 26051542]

[3]. Otsuka S, Bui KH, Schorb M, Hossain MJ, Politi AZ, Koch B, Eltsov M, Beck M and Ellenberg J (2016) ‘Nuclear pore assembly proceeds by an inside-out extrusion of the nuclear envelope’, Elife, 5.

[4]. Franz C, Walczak R, Yavuz S, Santarella R, Gentzel M, Askjaer P, Galy V, Hetzer M, Mattaj IW and Antonin W (2007) ‘MEL-28/ELYS is required for the recruitment of nucleoporins to chromatin and postmitotic nuclear pore complex assembly’, EMBO Rep, 8(2), pp. 165–72. [PubMed: 17235358]

[5]. Dultz E, Zanin E, Wurzenberger C, Braun M, Rabut G, Sironi L and Ellenberg J (2008) ‘Systematic kinetic analysis of mitotic dis- and reassembly of the nuclear pore in living cells’, J Cell Biol, 180(5), pp. 857–65. [PubMed: 18316408]

[6]. Hampoelz B, Schwarz A, Ronchi P, Bragulat-Teixidor H, Tischer C, Gaspar I, Ephrussi A, Schwab Y and Beck M (2019) 'Nuclear Pores Assemble from Nucleoporin Condensates During Oogenesis', Cell, 179(3), pp. 671–686. [PubMed: 31626769]

[7]. Hampoelz B, Mackmull MT, Machado P, Ronchi P, Bui KH, Schieber N, Santarella-Mellwig R, Necakov A, Andrés-Pons A, Philippe JM, Lecuit T, Schwab Y and Beck M (2016) 'Pre-assembled Nuclear Pores Insert into the Nuclear Envelope during Early Development', Cell, 166(3), pp. 664–678. [PubMed: 27397507]

[8]. Walther TC, Askjaer P, Gentzel M, Habermann A, Griffiths G, Wilm M, Mattaj IW and Hetzer M (2003) 'RanGTP mediates nuclear pore complex assembly', Nature, 424(6949), pp. 689–94. [PubMed: 12894213]

[9]. Jäkel S, Mingot JM, Schwarzmaier P, Hartmann E and Görlich D (2002) 'Importins fulfil a dual function as nuclear import receptors and cytoplasmic chaperones for exposed basic domains', EMBO J, 21(3), pp. 377–86. [PubMed: 11823430]

[10]. Lin DH, Stuwe T, Schilbach S, Rundlet EJ, Perriches T, Mobbs G, Fan Y, Thierbach K, Huber FM, Collins LN, Davenport AM, Jeon YE and Hoelz A (2016) 'Architecture of the symmetric core of the nuclear pore', Science, 352(6283), pp. aaf1015. [PubMed: 27081075]

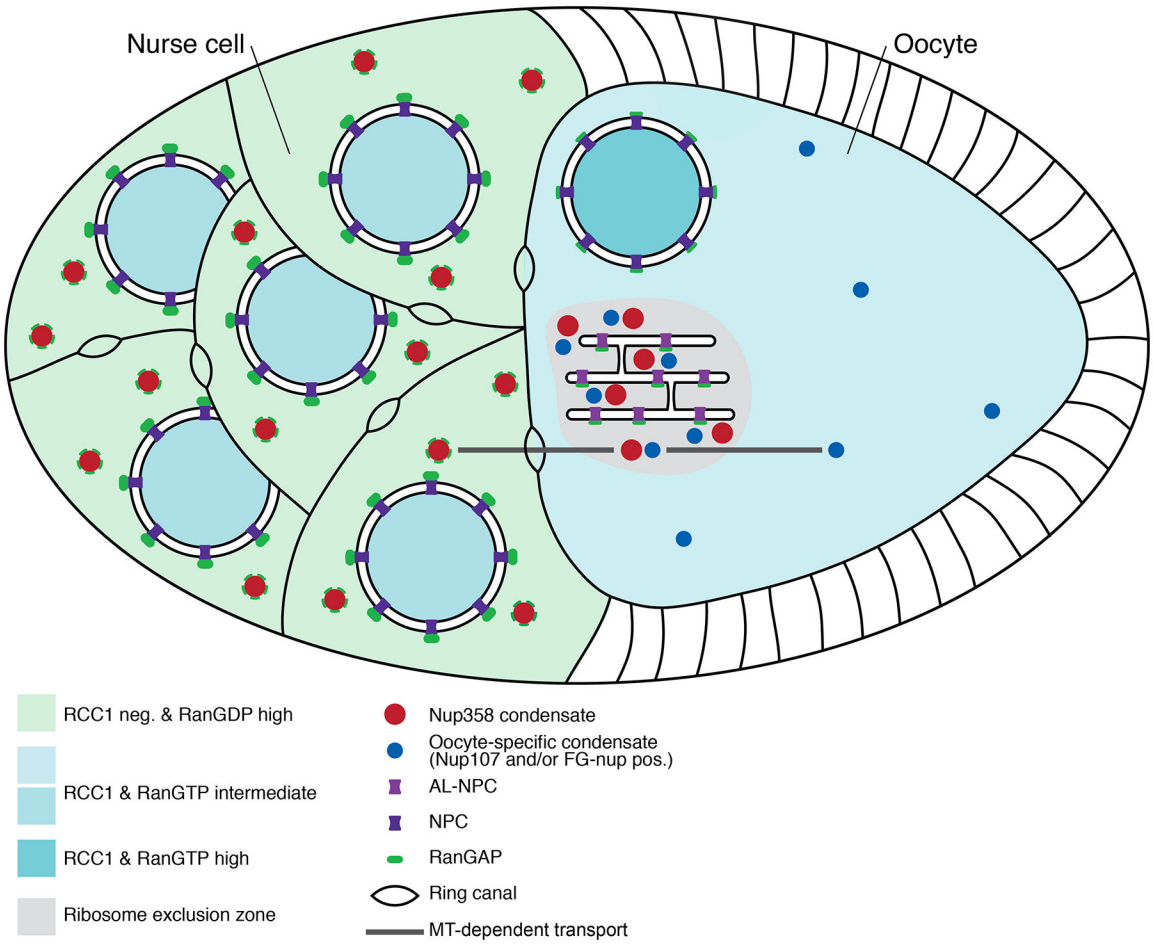

Figure 1. Distinct nucleoporin condensates mediate AL-NPC assembly during oogenesis.

Schematic representation of a *Drosophila melanogaster* mid-stage egg chamber. During embryogenesis the oocyte is nourished by nurse cells, which supply both proteins and RNA through connective ring canals. Nup358 containing condensates form in the nurse cell, where high levels of RanGDP are maintained by the Ran GTPase-activating protein RanGAP located on both the cytoplasmic face of the NPC and within cytosolic Nup358 granules. In the oocyte, upregulation of the Ran guanine nucleotide exchange factor RCC1 leads to a cytoplasmic reservoir, elevating local RanGTP levels, which favors formation of different nup granules containing scaffold and/or FG-nups. Motor-dependent transport of both Nup358- and oocyte-specific granules along microtubules drives contact between these nup condensates at ribosome exclusion zones in the ooplasm, where the granule components are assembled into AL-NPCs on membrane sheets called annulate lamellae for storage. Upon fertilization membrane sheets containing multiple AL-NPC are then inserted into the NE.