Summary

The eukaryotic nucleus requires acute delivery of specialized lipids during expansion, as observed during *Tetrahymena thermophila* conjugation, when the micronucleus undergoes a 10–15-fold enlargement to form the macronucleus. Drp6 plays a critical role in the incorporation of new lipids into the nuclear membrane during macronuclear (MAC) expansion. Although Drp6 interacts with with three phospholipids namely cardiolipin (CL), phosphatidic acid (PA), and phosphatidylserine (PS), its recruitment to the nuclear envelope is specifically mediated by CL. Notably, unlike most eukaryotes, the *Tetrahymena* nuclear envelope contains approximately 3% CL, highlighting its potential functional significance. To elucidate the source of CL and its role in nuclear expansion, we identified a single cardiolipin synthase that localized to three distinct compartments: the mitochondria, nuclear envelope, and plasma membrane. Tracking fluorescently labeledlabelled CL revealed rapid transport to the nuclear envelope within five minutes of incorporation into the plasma membrane — kinetics incompatible with classical retrograde trafficking through the Golgi–ER system, which typically requires hours. Brefeldin A treatment did not impede CL delivery, arguing against ER–Golgi retrograde involvement. Live-cell imaging identified Rab7-positive late endosomes as the principal vesicular carriers; expression of dominant-negative Rab7-T23N disrupted CL transport. Super-resolution microscopy showed that late endosomes form pocket-like appositions with the nuclear envelope without full fusion, consistent with a nucleoplasmic-reticulum–associated late endosomal (NR-LE) pathway and suggesting lipid transfer via membrane contact sites rather than membrane fusion. The plasma membrane therefore serves as a critical CL reservoir: inhibition of plasma-membrane CL synthase markedly impaired macronuclear expansion.

Drp6 specifically associates with cardiolipin-containing membranes *in vivo*, showing strong colocalization with cardiolipin-rich vesicles. Nuclear envelope recruitment of Drp6 is strictly

cardiolipin-dependent, whereas cardiolipin targeting occurs independently of Drp6, indicating lipid-mediated protein recruitment. Drp6 localizes predominantly to Rab7-positive late endosomes, defining a lipid-directed, Rab7-dependent targeting pathway.

All known dynamin family proteins self-assemble on target membranes; Drp6 likewise self-assembles at the nuclear envelope. We tested whether, in addition to CL binding, self-assembly is required for nuclear recruitment. Drp6 undergoes solution-phase self-assembly independent of membranes: preassembled oligomers are subsequently recruited to the nuclear envelope. Comparative sequence analysis identified a conserved tetrapeptide motif (GKFR, residues 411–414) near Interface 3. Alanine substitution of this motif (Drp6-4A) reduced higher-order oligomers >3-fold and increased monomer abundance >2-fold, demonstrating the motif's importance for oligomerization. Importantly, Drp6-4A retained wild-type CL binding in liposome flotation assays yet failed to accumulate at the nuclear membrane, demonstrating that oligomerization and membrane binding are functionally distinct. By contrast, an earlier study shows that the CL-binding mutant Drp6-I553M retained oligomerization but lost nuclear localization, establishing that both CL binding and higher-order oligomerization are independently required for efficient nuclear recruitment. Ultra-structure expansion microscopy and high-speed super-resolution live-cell imaging showed that *in vivo* dimensions of the self-assembled complexes closely match transmission electron micrographs of *in vitro* oligomers, confirming recruitment of preassembled oligomers to the nuclear envelope.

These findings reveal a sophisticated, multi-layered regulatory system: cardiolipin is synthesized at plasma membrane sites serving as a strategic stockpile; this lipid is selectively internalized via dynamin-dependent endocytosis into Rab7-positive late endosomes that dock at nuclear invaginations for lipid transfer through membrane contact sites; Drp6 is recruited through cooperative interaction of its lipid-binding domain with cardiolipin and pre-assembled oligomeric structures. This multi-component system demonstrates that nuclear expansion is not

simply a matter of local lipid synthesis but rather involves coordinated mobilization of distributed lipid synthetic networks and specialized trafficking machinery. The finding that the plasma membrane functions as a functional lipid reservoir for nuclear expansion challenges traditional compartmentalization models and establishes that eukaryotic cells have evolved distributed biosynthetic networks to support critical developmental processes. The mechanistic diversity revealed by Drp6—particularly its assembly-then-recruitment strategy—highlights the evolutionary plasticity within the dynamin superfamily and suggests that alternative assembly mechanisms may govern the function of other dynamin-related proteins in diverse cellular contexts. This thesis identifies a novel endocytic pathway mediating selective cardiolipin transport from the plasma membrane to the nuclear envelope and establishes how a dynamin-related protein achieves membrane-selective recruitment through cooperative lipid binding and solution-phase self-assembly.

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