CELL AND DEVELOPMENTAL BIOLOGY

# ANALYSIS OF INTEGRAL MEMBRANE PROTEIN POM34P IN NUCLEAR PORE COMPLEX STRUCTURE AND FUNCTION

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Dissertation under the direction of Professor Susan R. Wente

In eukaryotes, all nucleocytoplasmic transport occurs through nuclear pore complexes (NPCs). These giant proteinaceous structures are embedded in the nuclear envelope where the outer nuclear membrane and inner nuclear membrane join to form a pore. A functional NPC is composed of approximately 30 proteins, termed nucleoporins (Nups). However, the precise mechanism of how the NPC assembles is still unknown. Based on previous studies, we hypothesized that integral membrane proteins play a crucial role in NPC biogenesis and function. To test this hypothesis, I analyzed Pom34p and Pom152p, two integral membrane Nups in *S. cerevisiae*. The first part of my studies characterized Pom34p membrane orientation and its role in NPC structure organization. The results indicated that *POM34* encodes a double pass transmembrane protein with two cytoplasmic domains. It has broad genetic interactions with other *NUP*s, including *NUP170*, *NUP188*, *NUP59*, *GLE2*, *NUP159* and *NUP82*. Lack of the Pom34p N-terminal domain in a *nup188* null *(*Δ) background leads to mislocalization of a subset of Nups and nucleocytoplasmic transport defect. These data indicated that Pom34p is important for the maintenance of NPC structure.

The second part of my studies utilized genetic approaches to identify potential NPC assembly factors. To this end, I conducted a synthetic lethal screen with a *pom34*Δ *pom152*Δ double mutant, which shows no apparent defects in growth, NPC structure and nuclear transport. The screen revealed several mutants allelic to *NUP188*, *NUP170*, and *NUP192*, whose gene products comprise the core framework of the NPC. The synthetic lethal phenotype further illustrates the close relationship between pore membrane proteins (Poms) and structural Nups. Particularly, *NUP192* is an essential gene encoding the largest Nup. This is the first report to link the function of Nup192p to Poms. In addition, I performed a split ubiquitin yeast two hybrid screen aimed at identifying potential interaction partners of Pom152p. The combination of these genetic studies shed light into the understanding of NPC structure organization, and the functional defects associated with structure perturbations.

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