Proposed Plan of Research

Title: Identifying mechanisms of specificity for transport across the nuclear pore complex
Key Words: nuclear pore complex, FG nucleoporins, karyopherin, nucleus, transport
Background: A hallmark of eukaryotic cells is the presence of specialized, membrane-bound organelles. With the evolution of these organelles, selective transportation across the membrane necessarily co-evolved. Of particular importance is nucleocytoplasmic transport, as both RNA and protein molecules must be transported across the nuclear membrane.

The nuclear pore complex (NPC) provides the selective and efficient gateway of nucleocytoplasmic transport[1]. Selectivity is provided by a class of nucleoporin proteins known as FG Nups, which contain domains enriched in phenylalanine (F) and glycine (G) residues. FG domains are composed of multiple, tandem repeats of different FG sequences: GLFG, and FXFG, etc. (L, leucine; X, any amino acid). Whereas the C terminal regions of FG Nups are anchored at the NPC periphery, the FG domain of the proteins is unstructured and extends into the NPC center, generating a transport barrier[2]. Indeed, molecules larger than 40kDa require binding to a nuclear transport receptor to cross the NPC[1]. These transport receptors recognize nuclear localization signals (NLS) for import, and nuclear export signals (NES) for export, and receptors are specific for their cargo. Proteins are transported by karyopherins (Kaps), such as Kap121, while mRNA-protein complexes are transported by Mex67-Mtr2. These transport receptors function by binding FG Nups in addition to their cargo. Although FG Nups are essential for selective transport across the NPC, identifying the precise mechanism of transport has been difficult.

Several models for transport across the NPC have been suggested. These models largely propose that all FG Nups contribute to the formation of a barrier, and transport receptors overcome this barrier by stochastic binding to FG domains[1,2]. These models, however, fail to address possible differences in the binding of distinct transport receptors to different FGs, as previously suggested[3,4]. Importantly, half of the mass of FGs can be deleted with no growth defects, suggesting no major transport perturbance[5]. These results conflict with models suggesting the requirement for a large concentration of FGs to maintain the barrier[2].

To probe the role of FG Nups in the NPC barrier, the ZZZ lab has generated a novel collection of *S. cerevisiae* FG deletions. Using this innovative approach, work done in this lab has greatly influenced the field of nucleocytoplasmic transport. Specifically, using this mutant collection, it was unexpectedly found that different combinations of FG Nup deletions have differing effects on specific Kap transport[3]. For example, deleting the FG domain of Nup Nsp1 prevents Kap121-mediated protein transport but has no effect on mRNA transport. In contrast, deleting the FG domain of another Nup, Nup57, results in a defect in Mex67-Mtr2 mRNA transport but not protein transport. These results demonstrate that there are functional differences between Nup57 and Nsp1, and this may be true for all Nups. However, the question remains: *How are FG Nups selective for different transport receptors during* in vivo *transport?* Two non-mutually-exclusive explanations for this specificity exist. **First**, this selectivity might be due to specific FG sequences (e.g. GLFG, FXFG, etc) or due to the spacer sequences between these repeats. **Second**, the spatial positioning of the given FG Nup in the pore might be important in providing the correct docking sites for the FG binding domains of the transport receptors. **The goal of this proposal is to utilize multiple disciplines to test these possibilities.**

Hypothesis: FG Nups play an active role in transport by providing spatial location of their selective FG domains, yielding a specific pathway of binding sites for transport receptors.

Experimental Plan: As a genetic approach, I propose to assay steady state levels of Kap121 and Mex67 cargo using FG domain "swap" constructs built from the ZZZ lab FG deletion strain. Specifically, I will assay transport in strains with either wild type (as a control), FG domain

deletion (ΔFG), or in-frame substitutions with different FG domains (swaps). The FG Nups I plan to assay first are Nsp1, Nup57, and Nup49. These proteins are of prime interest because they have selective effects on Kap121- and Mex67-mediated transport (see above)[3], the sequence of their FG domains differ, and though they comprise a complex, the location of their FG domains within the pore differ[6]. To assay for transport, I will use techniques well-established in the ZZZ lab[3,5]. Specifically, a live-cell GFP-tagged NLS reporter will assay Kap121 transport and *in situ* hybridization using an oligo d(T) probe will assay mRNA localization.

By using this system, I will determine if the specific FG sequence of Nups and/or their spatial localization are necessary for efficient cargo transport. As an example of the experimental workflow, consider that a Nup57 FG domain deletion mutant has an mRNA export defect. If replacing this Nup57 FG domain (GLFG) with the Nsp1 FG domain (FXFG) rescues mRNA transport, then it is the location of the FG domain in Nup57 and not its specific identity that is important for transport. If this replacement does not rescue mRNA export, then the specific FG domain is important for transport. To address the role of location within the NPC, the GLFG domain of another Nup not required for mRNA transport will replace the GLFG domain of Nup57. It this suppresses the mRNA export defect, then the type of FG domain determines export. Additionally, different FG domain swaps will provide further insight into the mechanism of transport specificity as other FG deletion mutants show differential defects in transport.

As a biochemical assay, I will analyze the differential affinities of transport receptors for FG domains *in vitro* using the Bead Halo assay (REF. 7). This biochemical test is a qualitative assay for low affinity (as is true of Kap-FG binding) or high affinity binding, and has previously been used to assay FG-FG binding[7]. For this assay, purified GST-tagged FG-domain will be immobilized on glutathione-sepharose resin and probed for binding to a purified GFP-tagged transport receptor in solution. Positive interaction results in accumulation of a GFP "halo" around the glutathione bead, and fluorescence levels indicate binding strength.

Anticipated Results: I hypothesize that the spatial positioning of the specific FG domain determines the selectivity of nucleocytoplasmic transport. Thus, I anticipate only partial rescue of the transport defects of ΔFG strains when the FG domain is either swapped for another domain or swapped into another location. Additionally, I expect to observe different binding affinities for FG domains among different transport receptors. My results will build into an improved model of the transport mechanism and selectivity of the NPC.

Intellectual Merit: My extensive lab experience in cell biology appropriately positions me to tackle this important and far-reaching question in nuclear cell biology. Importantly, I have chosen to pursue training in the strong mentoring environment of Dr. ZZZ's lab.

Broader Impact: This study will provide further insight into specific transport across the NPC, which has broad implications in the regulation cellular function for all eukaryotes. I will use this project to mentor undergraduate and graduate students, and I will communicate the results of this project extensively through publications and presentations at regional and national conferences.

Originality Statement: AAA has written this original proposal.

1. Ref 1
2. Ref 2
3. Ref 3
4. Ref 4
5. Ref 5
6. Ref 6
7. Rev 7