Kinetic Analysis of Nucleocytoplasmic Transport

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Short Abstract — **Nucleocytoplasmic transport occurs through the nuclear pore complex (NPC), which in yeast is a highly symmetric ~50 MDa complex. Macromolecules larger than ~40kDa such as proteins, mRNAs, and ribosomal subunits must be aided across the NPC by shuttle proteins (karyopherins, or Kaps). Kap-mediated transport involves a family of FG-rich NPC proteins (termed FG-Nups). We aim to determine the binding steps and mechanisms of Kap-mediated transport across the NPC, using biochemical, biophysical, and cell biological approaches. Yeast karyopherins and full-length FG-nups are purified from bacteria, and their affinities are studied quantitatively using bead binding assays and far western assays.**

Keywords **— Nuclear Pore Complex, FG-Nups, Karyopherins, Nucleocytoplasmic Transport**

THE defining characteristic of eukaryotic cells is the compartmentalization of nucleic acid synthesis in a membrane-bound nucleus. The physical barrier provides protection for the nuclear content, but also presents a problem – selected molecules must be able to cross between the nucleus and the cytoplasm. Nucleocytoplasmic transport occurs through the nuclear pore complex (NPC), which in yeast is a highly symmetric ~50 MDa complex consisting of approximately 30 different proteins [1-3]. Small molecules such as water and ions can freely exchange through the NPC, but macromolecules larger than ~40kDa such as proteins, mRNAs, and ribosomal subunits must be aided across the NPC by shuttle proteins, such as Kaps (reviewed in [4]). While much has been learned about the mechanism of nucleocytoplasmic transport [5], many details are still unknown; perhaps among the most important missing details are the binding kinetics of most of the transport-relevant interactions, due to significant technical challenges. The aim of this work is to determine the binding steps and mechanisms of Kap-mediated transport across the NPC, using biochemical, biophysical, and cell biological approaches. Yeast Kaps and full-length FG-nups are purified from bacteria, and their affinities are studied quantitatively using bead binding assays and far western assays. We report a range of affinities between different Kap/Nup pairs. The effect of other proteins such as cargo and non-binding competitor proteins on Kap/Nup binding and selectivity is examined, and Kap/Nup interactions are further probed using protease laddering. The relevance of the Kap/Nup binding results will be confirmed *in vivo* by a nucleocytoplasmic import assay that allows quantitative measurements of import to be made.

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