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# *Abstract*

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| **Grant Number:** | 1R01GM062427-01 |
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**Abstract:** Nuclear pore complexes (NPCs) are the sole mediators of nucleocytoplasmic exchange. They therefore define the contents of the nucleus. The pivotal role of the NPC in controlling communication between the genetic material and the rest of the cell is reflected in the many oncogenic and developmental defects directly associated with alterations in nucleocytoplasmic transport. A full understanding of how the NPC mediates transport is needed to discern the nature of these defects. In order to gain this understanding, we require a comprehensive inventory of the molecular components of the NPC, a knowledge of how each component contributes to the overall structure of this large molecular translocation machine, and information on the interactions its proteins make with components of the soluble phase. We have therefore taken a comprehensive approach to defining the functional architecture of the NPC in the model eukaryote Saccharomyces (yeast), in which we have produced a low resolution map of the yeast NPC ultrastructure and determined its protein composition. To preserve and study functionally relevant interactions, we are now developing new techniques for the subcellular fractionation and immunopurification of protein complexes from both the stationary and soluble phases of transport. The components of these complexes, and the molecular nature of their interactions, will be characterized using current and emerging mass spectrometric techniques. First, all the NPC components will be purified and the interactions each makes with other proteins will be thoroughly catalogued. We will then determine the position of the NPC proteins and their subcomplexes within our improved maps of the NPC molecular architecture. Members of the mobile phase will be studied in a similar fashion. We will follow the movement of particular transport factors across the NPC, determining the order and sites of interactions that the transport factors make with NPC proteins during nuclear transport. The rules governing transport will be investigated by comparing the data gained from the different transport factors. Our eventual aim is to integrate our ultrastructural and biochemical studies to understand the molecular basis of the translocation of different transport factors across the NPC. These studies should enable us to reconstitute key reactions of nucleocytoplasmic transport in vitro, and test possible mechanistic models in vivo.

**Thesaurus Terms:**
cell nucleus, intracellular transport, membrane activity, membrane transport protein, nuclear membrane, structural biology
G protein, binding protein, biological model, biophysics, cell component structure /function, cytoplasm, intermolecular interaction, molecular assembly /self assembly, molecular asymmetry, molecular site, protein protein interaction, transport protein
affinity chromatography, affinity labeling, mass spectrometry, protein purification, reporter gene, yeast

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