CHAPTER 1

Structure of the Nuclear Pore

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The nucleus is a defining hallmark in cells of all the higher organisms: yeast, animals, and plants. As the repository of the genome, it both encloses the chromatin and regulates its accessibility. It is also the site of nucleic acid synthesis, including replication of DNA, transcription and editing of messenger RNA, synthesis of ribosomal RNAs, and assembly of ribosomal subunits. By contrast, the cytoplasm is the site of protein synthesis, where functional ribosomes translate mRNA into polypeptides. The nuclear envelope defines the border between these two distinct biochemical worlds. The nuclear pores (or nuclear pore complexes, NPCs) serve as guardians of this border, acting as the gateway for molecular exchange between the two major cellular compartments. They are deeply integrated to the physiological function of every cellular pathway involving communication between enzymatic, signaling, or regulatory activities on one hand, and gene expression on the other. The nuclear pore complex is also a fascinating molecular machine, facilitating the passage of specific macromolecules in one direction while ferrying others in the opposite sense.

The nuclear envelope (NE) defines the boundary between nucleus and cytoplasm. It is formed by two juxtaposed lipid bilayer membranes, the outer one of which is contiguous with the endoplasmic reticulum. The outer and inner lipid bilayers are also connected continuously through the nuclear pores themselves, though their protein compositions differ. A matrix of filaments underlies the inner nuclear membrane, providing mechanical support and anchoring sites for the enclosed chromatin. In animal cells these filaments are composed largely of lamins, similar in structure to intermediate filaments. Aside from a few known exceptions associated with viral infection, all molecular exchange across the nuclear envelope takes place via the nuclear pores, whose number ranges from many tens to several thousand per nucleus. Thus RNAs and ribosomal subunits are exported to the cytoplasm, while proteins needed in the nucleus must be imported, and often reexported when their task there is done. Each pore is a large multi-protein complex, consisting of 30 or more distinct protein components in multiple copies. Its total molecular weight has been measured at 125 MDa for vertebrate cells, and about 60 MDa for yeast. Individual nuclear pores are thought to mediate traffic in both directions.

The functional task of the nuclear pore is to regulate entry to, and exit from, the nucleus. Specific pathways are discussed at greater depth in other chapters of this book. A degree of consensus has emerged in describing nuclear transport as a receptor-mediated translocation process.[1] Molecular cargo is marked for import (or export) by the presence of peptide signals,[2-4] which are then recognized by specific receptors that serve to usher the cargo across the pore.[5-7] Models of translocation can been categorized into those that anticipate some form of micromechanical movement (for example iris-like closures) of the pore itself on one hand,[8-11] or entirely biochemical sieves on the other.[12-15] While deep modulation of calcium levels has a

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pronounced effect on nuclear pore structure in vitro,[11,16] calcium depletion does not appear to be coupled to nuclear transport regulation in intact cells.[17,18] The lack of intrinsic ATPase activity in the nuclear pore supports the second, nonmechanical class of models.

A rather minimalistic model for nucleocytoplasmic transport describes the nuclear pore and its associated soluble biochemistry as an affinity-regulated chemical pump.[14,19-22] Two apparently distinct modes of transport are identified: small molecules including water, ions, metabolites, and even small proteins (up to ~40 kDa molecular weight) can pass by simple diffusion so that their concentrations in solution equilibrate on the two sides of the NE; larger proteins and protein complexes are transported by an "active" mechanism that is able to pump the molecular cargo against a gradient in concentration, and so to accumulate it on one or the other side of the NE. In the latter case, proteins bearing nuclear localization signal (NLS) peptides associate with receptors of the importin/karyopherin family in the cytoplasm, and dissociate from them inside the nucleus. The canonical import receptor is importin β,[23] also known as karyopherin β,[24,25] or as p97.[26] This receptor interacts with NLS via an importin α (karyopherin α) adapter protein, so that a single cargo molecule enters the nucleus as a heterotrimer with the receptors. Their dissociation is governed by a competitive interaction with the small GTPase Ran,[27,28] which in its GTP form binds the importin β and releases the α molecule and the NLS-cargo.[5,12,29-32] A differential concentration of RanGTP across the nuclear envelope is maintained by the localization of the associated GTP exchange factor RanGEF (independently known as the chromatin condensation factor RCC1) loosely bound to chromatin within the nucleus, and the GTPase activating protein RanGAP associated with the peripheral cytoplasmic structures of the NPC.[33-38] Thus Ran is primarily in the GTP form within the nucleus, and in the GDP form in the cytoplasm.[39] Computer simulations support the assertion that receptor selectivity at the pore is sufficient for its function as a molecular pump, in combination with the Ran cycle; specific transport directionality is not required.[40,41] In some cases the directionality of transport could be inverted by artificially inverting the RanGTP gradient.[42] The same paradigm operates for export, except that the association of the cargo and RanGTP to the export receptor is synergistic rather than competitive.[43-50] Transfer RNAs make use of a specific receptor for export,[51,52] while export of other RNAs is thought to be governed by signals on associated proteins. In the case of large substrates a restructuring of the cargo itself may also be involved. A beautiful example was observed by electron microscopy for Balbiani ring mRNA export in *Chironomus* salivary glands. A series of snapshots shows the spiral ring unwinding and feeding progressively through the pore.[53]

The major role of the fixed structure of the NPC in such a model is to provide a selective translocation barrier, limiting passage to a rather short list of proteins. Those which are able to associate with signal-bearing molecular cargo, most notably importin β, are recognized as nucleocytoplasmic transport receptors, effectively opening the barrier to pass the complex where the cargo alone would be excluded. (It should not be overlooked that the transport receptors may have other roles in the cell as well.[54-56]) Within this picture the "active" transport is achieved primarily by the Ran switch, whose role is primarily to recycle the components of the chemical pump. No "moving parts" are required in the pore itself. A number of other proteins on the NPC's recognition list, particularly those involved in signal transduction such as β-catenin,[57,58] are able to pass pore autonomously. Their directionality and temporal accumulation are governed primarily by retention on nuclear or cytoplasmic structures, rather than by restriction of the reverse passage through the pore (reviewed in ref. 59). Perhaps the essential structural question is how the NPC can be so selective, passing relatively large cargo and complexes while blocking the passage of smaller ones. High selectivity normally implies a high and specific equilibrium affinity, but in the case of transport strong binding would of course be antithetical to translocation.