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Transport Machinery of the Nuclear Pore Complex

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Macromolecular transport in the cell represents a convergence of biology, biochemistry, and biophysics. We bring a physics-based, interdisciplinary approach to its study. Our efforts currently focus on the nuclear pore complex. This large channel is responsible for molecularly specific, bidirectional transport into and out of the nucleus. It sits in a position to regulate all communication between the cytoplasm, plasma membrane, and organelles with the genome.

Scanning electron microscope images of an array of nuclear pores are shown in Fig. 1. We take a combined structural and kinetic approach to study transport across the nuclear pore. Our particular interest is in nuclear import of DNA substrates, as a model for essential steps in both viral infection and medical gene therapy.

Building blocks of the nuclear pore complex

The nuclear pore is built of approximately 30 distinct proteins (nucleoporins, or Nups), arranged in multiple copies in an eight-fold symmetric channel that penetrates the two lipid membrane bilayers of the nuclear envelope. The Nups are themselves clustered in stable sub-complexes. During the open mitosis of animal and plant cells, the nuclear envelope breaks down and the nuclear pores dissociate into these same sub-complexes. At telophase the nuclear envelope is reformed, and the pore components reassociate to form new nuclear pores.

This cycle of assembly and disassembly allows for reconstitution *in vitro* of nuclei with functional nuclear pores. An especially powerful system is based on extracts from *Xenopus laevis* eggs, whose cell-cycle state can be manipulated biochemically. The extracts themselves can also be manipulated, for example by immunodepletion of particular components or entire Nup sub-complexes. Collaborating with the lab of Prof. Douglass Forbes at UC San Diego, we have shown that the Nup107-160 complex is essential for initial stages of NPC assembly. Lacking this large and central complex, enveloped nuclei form but are entirely devoid of nuclear pores.

DNA transfer complex of *Agrobacterium*

The soil bacterium *Agrobacterium tumefaciens* infects plant cells by transfer of parasitic genes, the protein products of which produce metabolites for the bacterium. In the process, single-stranded DNA from a tumor-inducing plasmid is complexed with two bacterial proteins: VirD2 in a single copy on the 5' end, and VirE2, stoichiometrically along the

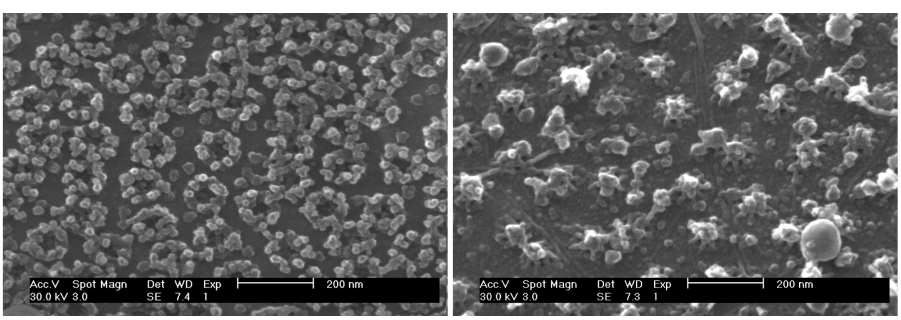

Fig. 1 *Nuclear pore complexes on the germinal vesicle of Xenopus laevis oocytes are seen by scanning electron microscopy. The left panel shows the cytoplasmic face, while the right panel shows the nucleoplasmic side.*

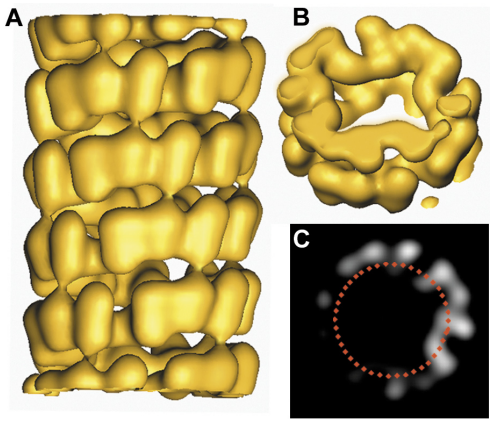

Fig. 2 *Transfer-DNA from Agrobacterium tumefaciens is delivered to the plant host in complex with virulence proteins. A three-dimensional reconstruction of the complex by electron microscopy and computerized image processing yields the helical structure shown in panels A and B. The red ring in panel C shows the estimated location of the ssDNA.*

length. VirE2 wraps the oligonucleotide into a hollow helical structure. With Sharon Wolf of the Electron Microscopy Unit, we performed a three-dimensional reconstruction of the VirE2-ssDNA complex using advanced image processing methods. The structure, seen in Fig. 2, suggests mechanisms of protection from nucleases and interface to the nuclear transport machinery.

We continue to study the structure of this complex in association with host factors required for its nuclear import. We also use the complex as a reagent to study NPC structure and translocation kinetics using a combination of electron and optical microscopy methods.

Nuclear transport and microtubule delivery

Nuclear targeting of VirE2 requires an interaction with the VIP1 protein, a plant-specific host factor. A minor mutation in VirE2 yields a functional nuclear localization signal in animal cells, however. Complexing the proteins with fluorescently-labeled DNA provides a substrate whose motion can be tracked quantitatively in the optical microscope. Using cytoskeletal networks reconstituted as well in *Xenopus* egg extracts, we could detect actively-driven movement along microtubules, specifically of the complex formed from the "animalized" mutant VirE2. Inhibitors to dynein motor activity blocked this active movement, indicating that the nuclear localization signal leads to minus-end directed movement on microtubules. In the intact (animal) cell where microtubules polymerize from a centrosome, this movement would tend toward the nucleus. We now extend our studies to explore possible interaction of the DNA transfer-complex with the cytoskeleton in plants as well.

Selected Publications

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