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Biochemical machines of intracellular transport

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Spatial organization of the cell is required for its operation as a coordinated, living factory. Various types of transport machinery operate to maintain and adapt this organization, and these are in large part responsible for the distinction between the living cell and the sum of its biochemical pathways. At the same time, they represent a convergence of biochemistry with macromolecular mechanics and physics. From single molecule motors to tension sensors involved in chromosome separation or cell adhesion, the generation and management of physical forces by cellular components inspire a broad, interdisciplinary approach to their study, and often elicit a review of fundamental physical concepts as they apply to molecular systems.

Our lab has been involved in researching several such cellular transport machines, including microtubule-associated intracellular movement and force-generation related to actin organization and cell motility. Currently our main focus is on nuclear transport, and especially on the passage of long oligonucleotides through the nuclear pores.

Nuclear transport

Exchange of macromolecules between the nucleus and cytoplasm takes place through 'nuclear pore complexes' (NPCs) embedded in the nuclear envelope. Along with a number of soluble transport receptors, the NPCs are responsible for regulating access to the genome, and for export of mRNA codes to be translated to proteins. At the same time, their operation represents a thermodynamic pump that is selective to its cargo, and yet lacks any obvious force-generating element. These apparently miraculous properties prompt biophysical studies using quantitative fluorescence microscopy and fluorescence correlation spectroscopy, scanning electron microscopy, and, in collaboration with the group of Dr. Ziv Reich, atomic force microscopy.

Of particular interest in the area of nuclear transport is the process by which DNA may enter the nucleus. Though not apparently physiological, nuclear uptake of DNA is a basic step in many types of viral infection, as well as in proposed strategies for genetic therapy. Using cell-free nuclei reconstituted in vitro from extracts of *Xenopus* eggs, which afford direct access to the NPCs, we have observed DNA uptake directly, and have applied the method of optical tweezers to follow the uptake of individual DNA molecules in real time. The results indicate a passive mechanism by which an extended DNA molecule threads through the nuclear pore, while irreversible retention within the nucleus, perhaps due to chromatinization, prevents its outward return.

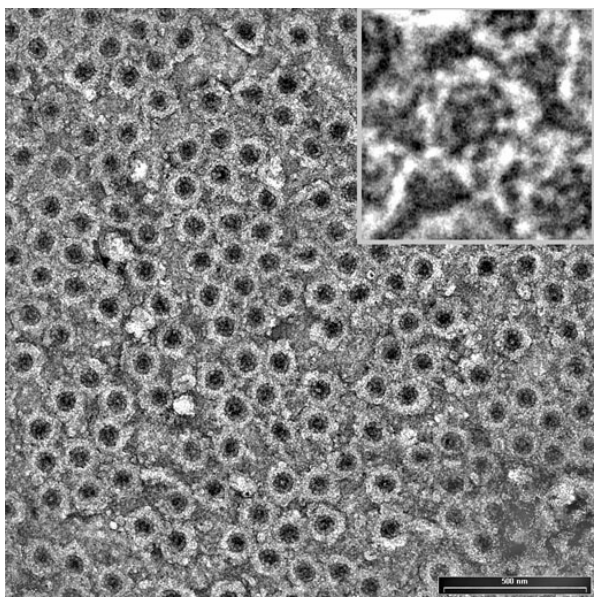

Fig. 1 *Nuclear pores appear at high density on the germinal vesicle of Xenopus laevis oocytes, shown in negative stain by transmission electron microscopy (scale bar=500 nm). The inset shows a single pore at high magnification, where the eight-fold symmetry is evident.*

Currently we focus our efforts on visualization of DNA uptake by electron microscopy, and turn our attention to *Agrobacterium tumefaciens*, a bacterium that infects plant cells by transfer of DNA carrying parasitic genes. This is the only known case of DNA transfer across living kingdoms, and recalls viral mechanisms in several aspects. The single-stranded transfer-DNA is packaged by bacterial virulence proteins (VirE2 and a single copy of VirD2) that structure the flexible polymer into a hollow, helical cylinder of ~15 nm outer diameter. The

virulence proteins also interact with plant factors to co-opt the host cell's nuclear import biochemistry. We apply advanced image processing methods to reconstruct the three-dimensional structure of the VirE2-ssDNA complex, so as to understand its interaction with and passage through the nuclear pore.

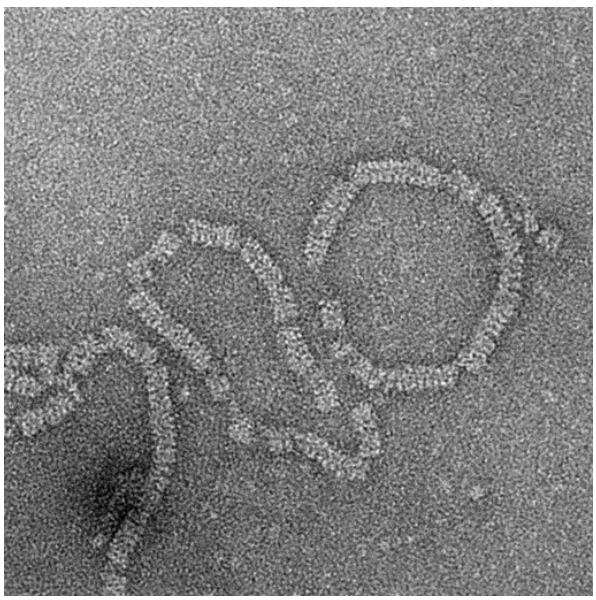

Fig. 2 *VirE2 protein of Agrobacterium tumefaciens forms a helical complex with single-stranded DNA, shown here by transmission electron microscopy. Image processing and reconstruction techniques are used to generate a three-dimensional model.*

Motion and diffusion in microtubule networks

Microtubules are a major component of the cytoskeleton. In conjunction with motor proteins of the kinesin and dynein families, they are closely associated with organelle, vesicle, and molecular transport within the cytoplasm. Their elastic properties as semi-flexible polymers create a peculiar environment in which this transport takes place. Using beads engulfed into living cells as 'model organelles,' we have traced their microtubule-dependent movements with nanometric particle-tracking from video recordings. Where in order to move along microtubules the bead must push other microtubules out of the way, the viscosity of the environment becomes time-dependent and the famous 'random-walker' takes on strongly anomalous behavior. This physics is described in the context of a Generalized Einstein Relation.

Stratification of the cytoplasm

Beads coated by adhesion ligands can be brought specifically to the surface of culture cells using optical tweezers in the microscope. We have found, contrary to expectations, that beads may be engulfed into the cytoplasm rather than moving along the plasma membrane. Bead adhesion and engulfment reveal a stratification in the cytoplasm and membrane. Toward the cell peripheries, engulfed beads move centripetally due to an internal retrograde flow of actin. This flow is not sensitive to inhibition of myosin II. Beads placed on the plasma membrane are not engulfed, but instead adhere to a stationary submembranous cortex, most likely of actin. Such an actin cortex has been implicated previously in cell motility, but was thought to be moving. These observations require a reevaluation of models for cell motility, pointing to the importance of internal cytoskeletal dynamics.

Selected Publications

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