

Meeting report

Dynamics of the nucleus

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A report of work on the structure and function of the nuclear envelope and nuclear pores, presented at the 39th annual meeting of the American Society for Cell Biology, Washington DC, December 11-15, 1999.

Much of cytoplasmic transport occurs within and between membrane-bound compartments. By contrast, transport of RNA and proteins between the nucleus and the cytoplasm requires that membrane-free soluble complexes cross the nuclear lamina (the proteinous fibrillar structure that abuts the nucleoplasmic face of the double-bilayer nuclear membrane) and the nuclear membrane, which they do by passing through the nuclear pores. The availability of the *Saccharomyces cerevisiae* genome sequence has led to the recent identification of the full set of nuclear pore proteins (nucleoporins) and the large family of nuclear import factors (importins or karyopherins), and the situation is likely to be at least as complex for nuclear export factors.

Nuclear structure

An important tool for the study of the components of the nuclear envelope has been the electron microscope. High-resolution reconstructions of nuclear pores have been available for some time. The current goal of many workers in this area is to understand the structural and biochemical changes that occur as particles translocate through nuclear pores. In many images of pores, material is present at the center of the pore. Whether this 'plug' is a component of the pore, material in the process of translocation through the pore, or just detritus or a fixation artifact has been debated but never resolved. By imaging the cytoplasmic face of pores with an atomic force microscope at different temperatures, Daniel Stoffler (University of Basel; see Figure 1) suggested that the plug might represent material in the process of transport. He found 45% of unfixed and unstained pores imaged at 4°C had plugs, whereas only 12% of pores imaged at 25°C had plugs.

In addition, plugs were also depleted from pores incubated with ATP. It will be interesting to see how various ATP analogs affect this assay.

The dynamics of pore structure were also amply demonstrated by imaging the nucleoplasmic basket that extends out from the nuclear envelope. When pores were imaged by atomic force microscopy and cryoelectron microscopy in the absence of Ca²⁺, baskets appeared closed. When the same baskets were imaged by atomic force microscopy after incubation in a Ca²⁺-containing buffer, the baskets opened in a manner suggestive of the dilation of an iris. This effect appears to be specific for Ca²⁺; Mg²⁺ does not open baskets. The molecular mechanism underlying the conformational change is not yet known, but Nup153p - a nucleoporin with a number of zinc-finger domains that might be responsible for Ca²⁺ binding - has been localized to the nucleoplasmic basket. How the changes in basket dynamics relate to the import process itself remains to be seen.

As well as forming a barrier between the cytoplasm and the nucleus, and regulating the transport of macromolecules between these two compartments, the nuclear envelope is implicated in a variety of other processes. Most recently, the gene that encodes emerin, a component of the nuclear lamina, has been identified as the site of a number of mutations associated with an X-linked form of muscular dystrophy known as Emery-Dreifuss muscular dystrophy (EDMD). Patients with this disease develop normally until early childhood when defects in the musculature appear. This striking phenotype has suggested a role for the nuclear lamina in myogenesis, possibly through an influence on the transcription of determinants of myogenesis. Is this due to a specific effect of emerin or a more general defect in nuclear envelope function? Point mutations in the single human gene that encodes nuclear lamins A and C cause an autosomal form of EDMD. Could the generation of defects in the nuclear lamina provide an animal model for EDMD? The answer to this question was presented by Teresa Sullivan (National

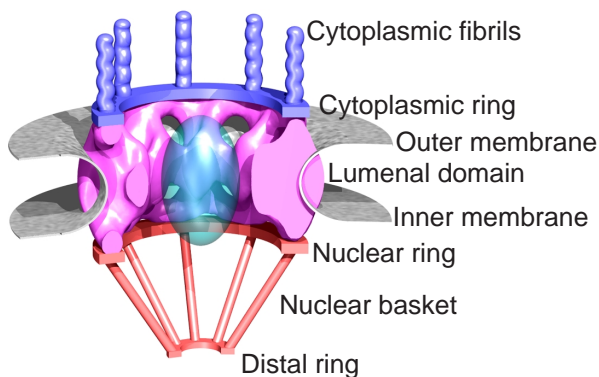


Figure 1

A three dimensional model of the nuclear pore. This stunning model of the nuclear pore machine is based on images from electron and atomic force microscopes and summarises the current state of knowledge of the structure of the nuclear pore. Transport of macromolecules into the nucleus occurs from the top to the bottom of the model. Cytoplasmic fibrils (top, blue) extend from the surface of the pore into the cytoplasm and are thought to function as tracks that guide macromolecular complexes destined for the nucleus to the pore opening. The pore itself is a symmetrical ring (pink) that perforates the double bilayer nuclear membrane (light blue). The ellipsoidal material (transparent blue) in the centre of the pore represents the plug often seen in images of pores. Finally, the nucleoplasmic basket (red) may open and close to regulate the import and export of material. Figure kindly provided by Daniel Stoffer (University of Basel).

Cancer Institute), who has generated a mouse lacking the lamin A/C gene.

Lamins A and C are abundant peripheral membrane proteins that are members of the intermediate filament family. The two proteins are produced by the same gene and generated by differential splicing. The proteins are absent from the nuclei of early vertebrate embryos and only begin to appear once cells have terminally differentiated. Mice lacking the lamin A/C gene are viable at birth, but within two to four weeks begin showing growth deficiencies and general wasting. All mice die within eight weeks of birth from a syndrome that is histologically strikingly similar to human muscular dystrophy. Moreover, these mice lose all their white fat, suggesting a role for the nuclear lamina in adipocyte signaling. Electron microscopic examination of fibroblasts derived from lamin-A/C-deficient animals showed discontinuities in the nuclear envelope. The localization of nuclear envelope components confirmed this result: there were large sections of the nuclear envelope devoid of two envelope markers, LAP2 α and lamin B, and emerin was not localized to the nuclear envelope at all. Transfection of the lamin-A/C-deficient fibroblasts with a full-length lamin A gene restored the structure of the nuclear envelope and the localization of nuclear envelope components. The development of mouse embryos lacking lamin A/C to birth, and the survival of fibroblasts derived from them, argues that lamins A and C are not required for cell proliferation. The similarity

between EDMD and the lamin-A/C-deficient mice, however, suggests a specific role for the nuclear lamina in some aspect of postnatal myogenesis or possibly muscle repair. More generally, as the development of mouse and human embryos is not adversely affected by these mutations, the nuclear lamina may be critical only for transcriptional regulation in terminally differentiated cells. Lamin-A/C-deficient mice should provide a model system for study of human autosomal muscular dystrophies and the function of the nuclear envelope in cell differentiation.

Nuclear transport

Transport of RNA and protein within the nucleus and between the nucleus and the cytoplasm occurs in the form of soluble complexes associated with factors that mediate targeting, passage through the nuclear pore, and perhaps transport to the appropriate final destination. As far as is known, all import and export factors are sensitive to the state of the guanine nucleotide bound to the small GTPase Ran. In the nucleus, Ran is kept in the GTP-bound form by the presence of the guanine nucleotide exchange factor Rcc1. In the cytoplasm, GTP hydrolysis is stimulated by a Ran GTPase-activating protein. In the presence of Ran-GDP, cytoplasmic import factors bind their nuclear-destined cargo and transit to the nucleus where, upon nucleotide exchange, the complex dissociates. Export factors work in the opposite manner. But how is the Ran-GDP:Ran-GTP gradient interpreted?

The original experiments that described nuclear import revealed a nearly binary process - all detectable protein bearing a classic nuclear import signal was transported into the nucleus. But it is now known that import and export of certain proteins is highly regulated. For example, an important determinant of the activity of many transcriptional regulators is localization: when trapped in the cytoplasm, these factors cannot access their DNA-binding sites. An example of this type of regulatory mechanism was presented by Erin O'Shea (University of California San Francisco), whose recent work has focused on the response of budding yeast cells to changes in phosphate concentration in their environment. This response is largely mediated by changes in the activity of a complex of a cyclin-dependent protein kinase, Pho80, and its regulatory cyclin-homolog subunit, Pho85, and the localization and activity of the Pho4 transcription factor. In high phosphate conditions, the Pho80-Pho85 kinase phosphorylates Pho4 at five different sites. This phosphorylation causes the localization of Pho4 to the cytoplasm where it is unable to stimulate the transcription of phosphate-responsive genes. When phosphate levels are low, Pho4 is hypophosphorylated and thus is located in the nucleus where it stimulates transcription. How does phosphorylation affect localization? The phosphorylation of two sites stimulates the binding of Pho4 to Msn5, a nuclear export factor. Phosphorylation at a third site inhibits the binding to Pse1, an importin/karyopherin family member.

There is a further level of regulation - the binding of Pho2, another transcriptional activator necessary for Pho4 function, is regulated by the phosphorylation of a fourth site on Pho4. So, multiple overlapping signals are used to determine partitioning of Pho4 between the nucleus and cytoplasm and ultimately, the activity of Pho4.

Ian Mattaj (European Molecular Biology Laboratory) also presented evidence that nuclear transport signals may be more complicated than originally thought. In yeast, U2 small nuclear RNAs (snRNAs) must bind to the cap-binding complex (CAP), an export factor called Xpo1, and Ran, for export to occur. A fifth factor, p55, is also required for the formation of this complex *in vitro*. Interestingly, p55 must be phosphorylated for complex formation - it is phosphorylated in the nucleus, but dephosphorylated in the cytoplasm. The importance of p55 phosphorylation suggests that whereas the status of Ran-bound nucleotide is the major signal for import and export factors, other signals may be used to assure the fidelity of transport.

Is movement of proteins and RNA through the nuclear pore the whole story of nuclear dynamics? The steady-state distribution of many non-chromatin nuclear components is known, but how newly imported factors arrive at this destination is the focus of work in many groups. Angus Lamond (University of Dundee) presented evidence for a pathway for delivery of small nuclear ribonuclear proteins (snRNPs) to their steady-state site of concentration at nuclear 'speckles', ill-defined structures that can be seen both by light and electron microscopy. By introducing a pulse of snRNPs tagged to green fluorescent protein (GFP), the movement of snRNPs from the cytoplasm into the nucleus was visualized. Newly made snRNPs first move into coiled bodies (another class of nuclear structures of unknown function) and then subsequently into speckles. Lamond suggested that the nucleus might compartmentalize its functions in a similar way to compartmentalization of the cytoplasm. This might explain, in part, the presence of a number of discrete domains within the nucleus. Movement of components between different compartments would require targeting and receptor functions, similar to those involved in cytoplasmic vesicle targeting, as well as a transport system, like cytoplasmic microtubules. Lamond presented time-lapse movies of coiled bodies in living cells moving between nuclear compartments and suggested that these dynamics, like those previously observed for splicing factors, might suggest a transport function - but evidence for a system of targeting, receptors and transport for coiled bodies like that for cytoplasmic particles and organelles remains elusive at present.