Understanding Cellular Nanomachines: A minimalist approach to studying nuclear pore complex function by de novo design

by

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Abstract:

The intrigue surrounding the nuclear pore complex (NPC) lies in its ability to restrict or promote cargo translocation between the cytoplasm and nucleus i.e. selective gating. To come to understand the modus operandi of the selective gating mechanism necessitates a detailed knowledge of both the biochemical ingredients and the corresponding biophysical responses of the NPC machinery. In particular, these are comprised of a group of nuclear pore proteins (i.e. nucleoporins or Nups) containing natively unfolded (unstructured) domains that are rich in phenylalanine-glycine (FG)-repeat motifs.

In our laboratory, we have developed a heuristic, interdisciplinary approach to elucidate how the FG-domains and the different transport factors (e.g. transport receptors, RanGTP/GDP) contribute to the selective gating mechanism. Specifically, FG-domains are tethered to gold nanostructures designed to mimic the dimensions of the NPC and topography. Atomic force microscope (AFM) force measurements reveal that the FG-domains are polymer brush–like and serve as an entropic barrier (i.e. steric repulsion) to would-be entrants to the NPC. Privileged access to the NPC is provided by transport receptors (e.g. importin-β), which negotiate the entropic barrier by causing a collapse of the FG-molecules via receptor-FG binding interactions. This striking effect is only reversed by RanGTP, which sequesters importin-β and inhibits FG-binding. Immunogold-labelling electron microscopy (EM) complement these findings by showing that the FG-domains of Nup153 are reversibly collapsed in vivo by importin-β and RanGTP. We anticipate that the receptor-driven collapse of the FG-domains defines the physical aspects of selective gating, and propose that the flux of collapsing and distending FG-domains serves to promote the translocation of receptor-cargo complexes while simultaneously maintaining the entropic barrier.

In closing, we will demonstrate how the aforementioned principles of selective gating can be applied to the construction of a de novo designed “minimalist” NPC. By being able to mimic the nucleocytoplasmic transport process, such an artificial NPC will have the potential to investigate, for example, the manner in which certain viruses hijack the nuclear trafficking machinery to infiltrate the nucleus via the NPC. On a separate note, the principle of selective gating may have potential applications in the development of future NPC-inspired nanopores for use in medical diagnostics and drug discovery technologies.

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Venue: PAP Meeting Room (SBS B3n-19)

Hosted by Prof. Alfred Huan