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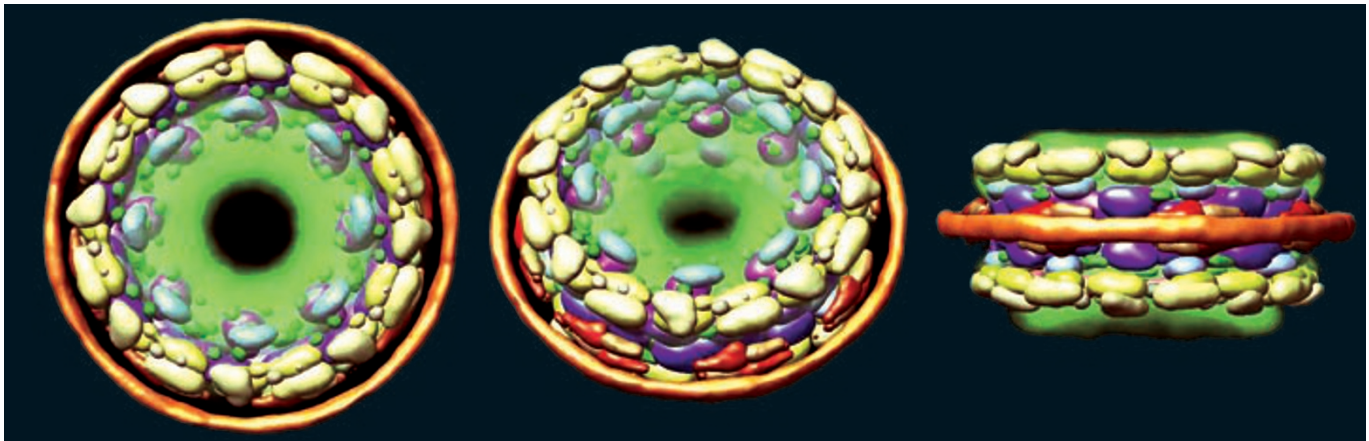

PROTEOMICS

Solving a 3D jigsaw puzzle

Most ten-year-olds can probably manage a jigsaw puzzle with 456 pieces. But, faced with the infinitely more difficult challenge of solving a jigsaw puzzle with as many pieces in three dimensions, the groups of Sali, Rout and Chait developed and applied a proteomics approach combined with computational modelling to determine the architecture of the nuclear pore complex (NPC) from budding yeast.

The authors' approach to structure determination consisted of four main steps. First, they used various techniques to obtain different and synergistic structural information. The overall shape and symmetry of the NPC were known from electron microscopy (EM) and cryo-EM data. Having previously determined the composition of the NPC and the stoichiometry of the components, the team then estimated the shape and size of the NPC components by ultracentrifugation. An immunolocalization map of each component was generated by immuno-EM, and one-on-one interactions between components and composites of co-isolating proteins were determined by various affinity purification experiments.

In the second step, the experimental data were translated into spatial restraints on the configuration of the proteins comprising the NPC. The third step involved optimization methods that used the restraints to generate a total of ~1,000 structures that were consistent with the data, in an approach similar to the determination of protein structures by NMR spectroscopy. Here, atoms were replaced by proteins, and their positions and relative proximities were restrained on the basis of the proteomic and biophysical information the team had gathered.

Last, the fourth step involved analysis of the ensemble of 1,000 structures that satisfy all restraints — in terms of protein positions, contacts and configurations — to produce the final structure, which was described in an accompanying paper.

The general architecture reassuringly agrees with previous data on the NPC structure. The NPC consists of eight spokes arranged radially around a central channel. The spokes, which can be divided into two almost identical nucleoplasmic and cytoplasmic halves, form several co-axial rings: the membrane rings, along with two outer rings and two adjacent inner rings, which form a core scaffold.

> “…a proteomics approach combined with computational modelling to determine the architecture of the nuclear pore complex…”

Interestingly, a class of nucleoporins that are known to mediate nucleocytoplasmic transport are mostly situated on the surface of the core scaffold that faces the central channel. The unstructured regions of these so-called ‘FG nucleoporins’ — comprised of Phe-Gly (FG) repeats — fill the central channel and extend into the nucleoplasm and cytoplasm, which fits with previous models that proposed that the unstructured ‘cloud’ of FG nucleoporins acts as a selective barrier for nucleocytoplasmic transport.

Three views of the nuclear pore complex map, showing the position of each of its structural components (shown in various colours) and the disordered region of FG nucleoporins (green) that act as the selective barrier mediating nuclear transport. Images courtesy of M. Rout, The Rockefeller University, New York, USA.

Remarkably, many nucleoporins are composed of either α-solenoid or β-propeller domains, or both, as in clathrin-like proteins that coat transport vesicles. Similar to vesicle-coating complexes, the core scaffold of the NPC is composed of these clathrin-like nucleoporins and coats the curved membrane of the nuclear pore completely. Both observations further support previous suggestions from this team that NPCs and vesicle-coating complexes may have a common evolutionary origin.

Furthermore, each spoke can be divided into two parallel ‘columns’ in which almost every nucleoporin contains a counterpart of similar size and fold structure in a similar position in the adjacent column. This pattern may represent an ancient duplication event, which has been observed for other coating complexes. The evolutionary origin of the NPC may also suggest an assembly process that is similar to that of coated vesicles. Higher-resolution information might allow this idea to be tested in the future. It will also be interesting to see whether this integrative proteomics approach can be applied to other macromolecular assemblies.

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