STRUCTURAL BIOLOGY

Zooming in on nuclear logistics

Cryo-electron tomography, single-particle analysis and cross-linking mass spectroscopy join forces to solve the nuclear pore scaffold puzzle.

'Truly nut-like', as their name origin suggests, eukaryotic cells divide and conquer the intricacies of cellular physiology. A fine example is the spatial segregation of transcription and translation, with DNA kept snugly in the nucleus and RNA and proteins shuffling in and out of it to safeguard the readout of genetic mandates. This comes at the price of elaborate logistics, where nuclear pore complexes (NPCs) act as the sole molecular bouncers at the nuclear gates. With each NPC containing multiple copies of ~30 nucleoporins that total nearly 1,000 subunits, the question is: how do you study such a massive structure?

"X-ray crystallographers have provided an almost complete catalog of high-resolution structures that build the NPC," says Martin Beck from the European Molecular Biology Laboratory in Heidelberg. "However, the nuclear pore is so huge that obviously you cannot crystallize it entirely, so you need to combine different methods that integrate the different resolution levels in a meaningful way."

To fit the available parts list in a bigger picture, Beck and colleagues resorted to a combination of state-of-the-art electron tomography (ET) and cross-linking mass spectrometry (XL-MS). Starting with human tissue culture cells, they successfully isolated a ten-protein NPC scaffold subcomplex, called hNup107, and subjected it to single-particle analysis by ET. The real challenges were to then assign the resulting low-resolution model to its exact location in the NPC and to fill in the resolved electron density with available higher-resolution structures.

Although cryo-ET has been the method of choice for studying the global NPC architecture, previous studies have been limited to resolutions coarser than 60 Å and thus have not permitted modeling of individual components. And as the high copy number of subcomplexes allows for both inter- and intrasubcomplex contacts, orthogonal techniques such as interaction screening methods have been themselves plagued by ambiguities. As a result, at least three

GENOMICS

LOCATING THE KISS OF DEATH

Chromatin immunoprecipitation of transcription factors tagged for degradation reveals how protein turnover regulates transcription.

How do stem cells change their fate, going from pluripotent mother cell to daughters that are committed to a particular lineage? This basic question, at the heart of many research efforts in the stem cell community, is also one André Catic, a member of David Scadden's laboratory at Harvard University, is asking. He has approached it from a fresh perspective. "The proteome represents the current state of a cell," says Catic. "To change its state, won't it be necessary to reset the cell and erase the proteome?"

Catic was inspired by work done in yeast that showed an association of the protein-degradation machinery with DNA as well as the short-lived nature of some transcription factors after activation. These data gave him and his colleagues confidence that transcription factors are likely destroyed on the DNA soon after they are activated. "This makes the cell nimble enough to turn on genes, then turn them off; it gives the cell an opportunity to clear the zone, if you will," explains Scadden.

Catic decided to use the earliest signal of degradation, the attachment of polyubiquitin chains, appropriately named 'the kiss of death,' to pinpoint the location of degradation. He transduced mouse embryonic fibroblasts with epitope-tagged ubiquitin and carried out immunoprecipitation of the ubiquitin followed by high-throughput sequencing of the bound DNA fragments. "A big problem," says Catic, "is that ubiquitination is incredibly nonspecific." To avoid background from ubiquitinated histones and other nondegraded substrates, the researchers used proteasome inhibitors to accumulate the ubiquitin chains on the short-lived transcription factors and then compared enrichment of ubiquitin peaks in cells with and without inhibition. Looking at sequences that were enriched only in cells with inhibited proteasome activity, the team discovered binding motifs for about 80 transcription factors, mostly in expressed genes. They saw

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Model of the NPC scaffold integrating different resolutions. Image adapted from data courtesy of M. Beck.

starkly different architectural models for the NPC scaffold have been proposed.

What typically kill cryo-ET resolution are both the limited throughput and the very low signal-to-noise ratio of the data. With the latest generation of microscopes, detectors and automated data acquisition, however, "the data sets become larger; thus, the resolution goes up," explains Beck. Nevertheless, he adds that although secondary-structure cryo-ET resolution is now possible in principle, high intrinsic dynamics of the NPC add an extra layer of complexity.

Working on isolated nuclear envelopes with cutting-edge instrumentation and a sophisticated set of analytic tools, Beck and colleagues managed to get a hold of NPC dynamics and to solve the NPC scaffold structure at 32-Å resolution. This allowed them not only to unambiguously assign the location of the predominant hNup107 subcomplex in the electron density map, but also to pinpoint the location of additional subcomplexes in the scaffold. The researchers used XL-MS to validate the resolved structure. To discriminate between inter- and intrasubcomplex contacts, they analyzed the isolated hNup107 complex in parallel to whole NPCs. Combining the resulting distance restraints with high-resolution structures offered an almost complete interpretation of the observed electron density for the hNup107 scaffold. Careful analysis of subcomplex arrangement and structure dynamics led to an architectural model that offers both a consensus to previous studies and insight into cargo transport mechanics.

"There are still other subcomplexes—which need to be looked at, [not only] in isolation but also in their exact position in the NPC," says Beck. We are looking forward to a sequel.

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RESEARCH PAPERS

Bui, K.H. *et al.* Integrated structural analysis of the human nuclear pore complex scaffold. *Cell* **155**, 1233–1243 (2013).

the highest degradation close to mitochondrial genes targeted by CREB (cAMP response element–binding) protein. Notably, the inhibition of degradation led to an increase in expression of these CREB-target genes. Because CREB is known to activate transcription, this result seemed puzzling at first, until the researchers discovered that it is not CREB itself that is being degraded but rather a repressor that colocalizes with CREB on promoters. Elimination of the co-repressor leaves CREB free to enhance the expression of its target genes.

Catic is quick to point out that this derepression by degradation is unlikely to be a general mechanism—the way in which degradation influences a given transcription factor will need to be examined anew in each case. The researchers found that degradation was not exclusive to proteins bound to gene promoters but was even more prevalent at enhancers. The challenge of following up on these results is that target genes for enhancers are often not known.

With the method established, Catic is now ready to apply it to his original question: how protein turnover regulates cell-fate transitions. "We were looking at a very boring state of these fibroblasts," he says, "standard conditions, plenty of nutrients." He plans to shake the cells out of their complacency, stress them with DNA damage and differentiate them to fat cells to see the role degradation plays in these processes.

In principle, the method can be applied to any cell type of choice; Catic only cautions that one needs to be able to introduce the epitope tag and that cells need to withstand several hours of proteasome inhibition to allow for sufficient accumulation of ubiquitinated substrate.

For now the team will work with clonal cell populations, but Catic thinks an *in vivo* application is also conceivable given that proteasome inhibition is well tolerated in mice.

Nicole Rusk

RESEARCH PAPERS

Catic, A. *et al.* Genome-wide map of nuclear protein degradation shows NCoR1 turnover as a key to mitochondrial gene regulation. *Cell* **155**, 1380–1395 (2013).