Chk2, the Rad53 ortholog, is highly mobile and spreads over the entire cell nucleus shortly after genotoxic stress (Lukas et al., 2003). This raises the question of whether checkpoint signaling modifies nuclear pores locally, at the specific stalled replication fork where torsional stress is encountered, or whether it detaches tethered genes throughout the nucleus.

The conclusions arising from the work by Bermejo and colleagues provide a framework to mechanistically decipher all of these issues and expand our knowledge of cellular responses to replication stress. Oncogenic deregulation of replication and transcription are intimately tied to replication stress (Halazonetis et al., 2008). Going forward, it will be important to consider the possibility that nuclear pore components, particularly those involved in tethering chromatin to the nuclear periphery, may be a source of replication stress in human diseases arising from the loss of genomic integrity, such as cancer.

ACKNOWLEDGMENTS

The work in the authors' laboratories is supported by the Danish Cancer Society, the Danish National Research Foundation, the Lundbeck Foundation, and the European Commission (projects: DDResponse, Biomedreg, and Infla-Care).

REFERENCES

Bermejo, R., Capra, T., Jossen, R., Colosio, A., Frattini, C., Carotenuto, W., Cocito, A., Doksani, Y., Klein, H., Gómez-González, B., et al. (2011). Cell *146*, this issue, 233–246.

Grinthal, A., Adamovic, I., Weiner, B., Karplus, M., and Kleckner, N. (2010). Proc. Natl. Acad. Sci. USA *107*, 2467–2472.

Halazonetis, T.D., Gorgoulis, V.G., and Bartek, J. (2008). Science *319*, 1352–1355.

Khadaroo, B., Teixeira, M.T., Luciano, P., Eckert-Boulet, N., Germann, S.M., Simon, M.N., Gallina, I., Abdallah, P., Gilson, E., Géli, V., and Lisby, M. (2009). Nat. Cell Biol. *11*, 980–987.

Kitamura, E., Blow, J.J., and Tanaka, T.U. (2006). Cell *125*, 1297–1308.

Köhler, A., and Hurt, E. (2010). Mol. Cell *38*, 6–15.

Lukas, C., Falck, J., Bartkova, J., Bartek, J., and Lukas, J. (2003). Nat. Cell Biol. *5*, 255–260.

Nagai, S., Dubrana, K., Tsai-Pflugfelder, M., Davidson, M.B., Roberts, T.M., Brown, G.W., Varela, E., Hediger, F., Gasser, S.M., and Krogan, N.J. (2008). Science *322*, 597–602.

Osborne, C.S., Chakalova, L., Brown, K.E., Carter, D., Horton, A., Debrand, E., Goyenechea, B., Mitchell, J.A., Lopes, S., Reik, W., and Fraser, P. (2004). Nat. Genet. *36*, 1065–1071.

Paulsen, R.D., Soni, D.V., Wollman, R., Hahn, A.T., Yee, M.C., Guan, A., Hesley, J.A., Miller, S.C., Cromwell, E.F., Solow-Cordero, D.E., et al. (2009). Mol. Cell *35*, 228–239.

Nuclear Pore Structure: Warming up the Core

Amnon Harel[1,*] and Yosef Gruenbaum[2]

[1]Department of Biology, Technion - Israel Institute of Technology, Haifa 32000, Israel

[2]Department of Genetics, Institute of Life Sciences, Hebrew University of Jerusalem, Jerusalem 91904, Israel

*Correspondence: amharel@tx.technion.ac.il

DOI 10.1016/j.cell.2011.06.045

Structural determination of the nuclear pore complex has been limited by the complexity and size of this cellular megalith. By taking advantage of exceptionally stable nucleoporins from the thermophilic fungus *Chaetomium thermophilum*, Amlacher et al. (2011) provide new insight into a core element of the nuclear pore scaffold.

Nuclear pore complexes (NPCs) are intricate biological machines that mediate all traffic between the nucleus and the cytoplasm in eukaryotic cells. NPCs are embedded in fusion pores between the inner and outer nuclear membranes and are composed of multiple copies of ~30 different proteins, termed nucleoporins (Nups) (Hetzer and Wente, 2009). NPC structure is likely conserved in all eukaryotes and exhibits an eight-fold rotational symmetry with additional filamentous extensions protruding from the nuclear and cytoplasmic facades (Figure 1). As one of the largest and most complex macromolecular assemblies, with an estimated mass of 40–60 MDa and ~500 individual polypeptide chains, the NPC has been a tough nut to crack. In this issue, the groups of Ed Hurt and Peer Bork reveal exciting new data on a central core element of the pore, using proteins from an unexpected thermophilic accomplice, the fungus *Chaetomium thermophilum* (Amlacher et al., 2011). Additionally, by reporting the full genome of this eukaryote the authors establish a new model organism for the structural analysis of large protein complexes.

Recent progress in the structural determination of the NPC has relied on the recognition of the modular nature of its building blocks: nucleoporins and their subcomplexes. The three broad classes of Nups include a small group of membrane-anchored proteins, a large group

of barrier Nups containing phenylalanine-glycine (FG) repeats, and another large group of scaffold Nups, which form the stable architectural framework of the NPC (Brohawn et al., 2009; Onischenko and Weis, 2011). The scaffold Nups are generally conserved at the structural level and are mostly composed of β-propellers, α-helical domains, or a tandem combination of these motifs. Although increasingly large structures of scaffold Nups have been solved by crystallography (Brohawn et al., 2009; Hoelz et al., 2011), it is still challenging to place these modules within the three-dimensional framework of the fully assembled NPC (Maimon and Medalia, 2010). Moreover, the contacts and interfaces between nucleoporin subcomplexes remain unknown. Previous studies, in yeast and in vertebrates, have pointed to a potential network of interactions between Nups localized to the inner pore ring region. However, it remained unclear whether these scaffold Nups form a stable module spanning the distance between the curved pore membrane and the central transport channel.

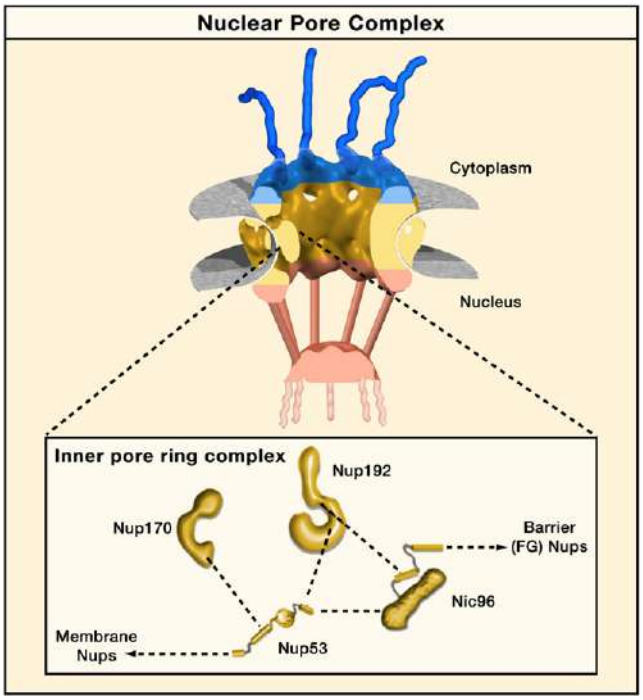

Figure 1. The Inner Pore Ring Module within the Nuclear Pore Complex

A cross-section through a model of the three-dimensional architecture of the nuclear pore complex (NPC) is shown at the top (image kindly provided by Ueli Aebi). The structural framework of the NPC has an eight-fold rotational symmetry around the central transport channel and consists of the cytoplasmic ring moiety with attached cytoplasmic filaments (blue), the central spoke-ring assembly (yellow), and the nuclear basket (pink). The central core region, containing the inner pore ring (also called the spoke ring), is enlarged. Work by Amlacher et al. (2011) with thermostable *Chaetomium thermophilum* Nups suggests that this part of the NPC scaffold is composed of two large Nups, which are flexibly bridged by short linear motifs protruding from Nup53 and Nic96. Terminal binding motifs connect this module to components of the pore membrane on one side and to the FG Nups of the transport channel on the other side.

In the current study, Amlacher et al. first performed two-hybrid analysis and in vitro binding assays with yeast scaffold Nups. These experiments verified some of the previously reported interactions, identified distinct interaction motifs of yNic96 with the largest scaffold Nups, but soon hit an "invisible wall." The main difficulty stems from the low stability of these large structural Nups and from apparently labile interactions with their nearest neighbors within the NPC structure. To overcome this challenge, the authors sought thermostable orthologs of the scaffold Nups from the filamentous fungus *Chaetomium thermophilum* (*ct*). This fungus thrives at temperatures of up to 60°C, undergoes a closed mitosis, and exhibits all the typical intracellular hallmarks of eukaryotes, including a nuclear envelope perforated by NPCs. The authors present the entire genome of this thermophile and identify most of the known Nups conserved in other eukaryotes. Indeed, these *ct*Nups prove to be incredibly stable compared to their yeast counterparts. The authors heterologously express in yeast *ct*Nups with affinity tags and purify some of the largest structural Nups as stable monomeric proteins, which remain soluble up to 57°C. Specific interactions between scaffold Nups were then reconstituted in vitro, beginning with pairwise interactions and leading up to a hetero-tetrameric complex of the presumed inner pore ring components. Thus, the formation of a stoichiometric complex between *ct*Nup192, *ct*Nup170, *ct*Nic96, and *ct*Nup53 could be accomplished by the immobilization of one partner by its affinity tag. Surprisingly, the largest scaffold Nups do not directly interact with each other but are bridged by short linear motifs protruding from the flexible domains of other Nups.

The superior properties of the *ct*Nups enabled the authors to perform single-particle analysis by negative-staining electron microscopy. This analysis reveals distinct and mostly homogeneous structures for each of the single proteins analyzed and more variable shapes for the higher-order assemblies. Two of the largest scaffold Nups, *ct*Nup192 and *ct*Nup188, exhibit a twisted S-shaped morphology that resembles the open-state shape of some karyopherins. Karyopherins are shuttling nuclear transport receptors that mediate selective passage through the NPC via interactions with the FG repeat Nups lining the central pore channel. The authors propose that the similar molecular shape and curvature may reflect the possibility that karyopherins and Nups share the same evolutionary origin (Devos et al., 2006).

Combining the observations from biochemical assays and electron microscopy, Amlacher et al. propose a model for the inner pore ring complex of the NPC. In this model, the large Nup192 and Nup170 are bridged by short flexible motifs extending from their partners, Nup53 and Nic96, which are dubbed "linker Nups" (Figure 1). Based on previous reports, it is suggested that this module interacts directly with the anchoring pore membrane proteins on one side and with barrier (FG repeat) Nups on the other side. Thus, the authors envision a central core element that spans the distance from the membrane to the central channel and suggest that the surprising flexibility within this module may allow the NPC to adjust to cargoes

of different sizes. The current study demonstrates a mutually exclusive interaction of Nic96 with either Nup192 or Nup188, confirming recent observations on the vertebrate orthologs of these scaffold Nups (Theerthagiri et al., 2010). It remains unclear whether there are in fact two alternative forms of the inner ring module, in which Nup188 replaces Nup192, or if a more complicated arrangement exists. This should also be viewed in light of the redundancies observed between other NPC components (Stavru et al., 2006).

The current findings are sure to stimulate additional structural studies. The superior biophysical properties of the *ct*Nups make them ideal candidates for crystallography. This should provide atomic resolution details on specific Nups and in particular on the unusual contacts between the members of the inner ring module. It will be important to compare at least parts of the structure between organisms and to determine how the inner ring module connects to other elements of the NPC scaffold. The impressive success of this strategy raises the hope that the genome and proteome of *Chaetomium thermophilum*, presented in this work, will prove useful tools for deciphering the structure of other cellular machines (Chiu et al., 2006).

ACKNOWLEDGMENTS

Research in our laboratories is supported by grants from the Israel Science Foundation (1072/10 to A.H. and 1828/08 to Y.G.).

REFERENCES

Amlacher, S., Sarges, P., Flemming, D., van Noort, V., Kunze, R., Devos, D.P., Arumugam, M., Bork, P., and Hurt, E. (2011). Cell *146*, this issue, 277–289.

Brohawn, S.G., Partridge, J.R., Whittle, J.R., and Schwartz, T.U. (2009). Structure *17*, 1156–1168.

Chiu, W., Baker, M.L., and Almo, S.C. (2006). Trends Cell Biol. *16*, 144–150.

Devos, D., Dokudovskaya, S., Williams, R., Alber, F., Eswar, N., Chait, B.T., Rout, M.P., and Sali, A. (2006). Proc. Natl. Acad. Sci. USA *103*, 2172–2177.

Hetzer, M.W., and Wente, S.R. (2009). Dev. Cell *17*, 606–616.

Hoelz, A., Debler, E.W., and Blobel, G. (2011). Annu. Rev. Biochem. *80*, 613–643.

Maimon, T., and Medalia, O. (2010). Nucleus *1*, 383–386.

Onischenko, E., and Weis, K. (2011). Curr. Opin. Cell Biol. *23*, 293–301.

Stavru, F., Hülsmann, B.B., Spang, A., Hartmann, E., Cordes, V.C., and Görlich, D. (2006). J. Cell Biol. *173*, 509–519.

Theerthagiri, G., Eisenhardt, N., Schwarz, H., and Antonin, W. (2010). J. Cell Biol. *189*, 1129–1142.