1983	Ph.D. - University of Regensburg, Germany
1984 - 1986	PostDoc - Biocenter, Basel, Switzerland (Prof. G. Schatz)
1986 - 1994	Group Leader - European Molecular Biology Laboratory (EMBL) Heidelberg, Germany, Cell Biology
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Ed Hurt

The mechanism of nuclear pore complex and ribosome subunit assembly

Goal

We aim to understand how complicated macromolecular assemblies - the nuclear pore complex and the ribosomal subunits - are formed in the eukaryotic cell. To reach these goals we apply *in vivo* **cell biology and genetic approaches and** *in vitro* **biochemical and structural reconstitution.**

Background

Nuclear pore complexes (NPCs) are the gateways facilitating nucleocytoplasmic transport across the nuclear envelope. Although the size of the NPC varies between species, the overall structure is evolutionary conserved from yeast to human. Multiple copies (8, 16, 32) of roughly 30 distinct proteins (nucleoporins, or Nups) build up the NPC, which exhibits an eight-fold rotational symmetry and conspicuous substructures such as the spoke-ring complex, cytoplasmic pore filaments and the nuclear basket visualized by different EM techniques. In the past, several model organisms from lower and higher eukaryotes served to study the huge NPC. Although nucleoporins from diverse species differ markedly in their amino acid sequence, their fold-types and domain organization as well as the composition and modularity of the NPC are conserved. The majority of nucleoporins is organized in biochemically stable subcomplexes (e.g. the Y-shaped Nup84 complex), but the mechanism how these modules interact with each other to form the 60-120 MDa NPC assembly is not known. Thus, it is an important goal in our research to reconstitute the entire nuclear pore complex from its individual nucleoporins or derived subcomplexes, and to understand the mechanism of nucleocytoplasmic transport through the active transport channel.

Besides the nuclear pore complexes, ribosomal subunits, which translate mRNAs to synthesize the proteins, are highly sophisticated macromolecular machines with an intricate assembly pathway. During eukaryotic ribosome biogenesis, four ribosomal RNA species and about 80 ribosomal proteins are assembled to form the large 60S and small 40S subunit. This process starts with RNA polymerase I driven transcription of a 35S pre-rRNA species in yeast, which is the precursor to the mature 18S, 5.8S and 25S rRNA. During the subsequent maturation steps, this pre-rRNA is modified, processed by endo- and exonucleases, folded and assembled with the ribosomal proteins and 5S rRNA. Ribosome biogenesis is driven by small nucleolar RNAs (snoRNA) and ca. 150-200 non-ribosomal biogenesis factors. These assembly factors were largely identified by isolation of

pre-ribosomal particles followed by proteomic analysis. Insight into the mechanism of ribosomal assembly came from genetic and biochemical studies in yeast, but recently also structural studies were initiated to characterize nascent ribosomal 60S and 40S subunits by both classical negative stain and cryo-EM. In future studies, the mechanisms of eukaryotic ribosome biogenesis will be unraveled by a combination of different *in vitro* and *in vivo* assays.

Research Highlights

We aim for the in-depth structure-function analysis of the NPC in *Saccharomyces cerevisiae* and *Chaetomium thermophilum (ct)* by combining, respectively, the excellent genetic and thermophile properties of these two model organisms. In the yeast system, we could develop a non-radioactive pulse-chase method combined with affinity-purification to study the temporal and spatial events during NPC assembly. The principle of this method is to induce a protein-tag of a desired biochemical property on a protein of interest in a very fast responding manner. We took advantage of a specialized orthogonal bacterial tRNA/tRNA-synthetase pair expressed in yeast cells, which incorporates an unnatural amino acid, O-methyl-tyrosine, into proteins in response to a nonsense TAG codon engineered into the ORF. Adding O-methyl-tyrosine to the culture medium will suppress the amber stop codon resulting in an in-frame fusion of the protein-tag to the protein of interest. This method allowed us to pulse/chase label nucleoporins of interest, but also ribosome biogenesis factors (see below) in the 1-15 min range followed by subsequent affinity-purification of the labeled Nup and its associated binding partners. Moreover, in the past three years, we could develop *C. thermophilum* as a source for nucleoporins with improved properties to perform EM, crystallization and *in vitro* reconstitution. Specifically, we have assembled several *ct*Nup modules, the *ct*Nup84, *ct*Nup82 and *ct*Nic96 complexes, and tested for an interaction with the large structural Nups and assembly in higher order structures. The reconstituted complexes were analyzed by negative stain EM. One important breakthrough was the reconstitution of the inner pore ring module with thermophile nucleoporins, consisting of Nup192-Nup170-Nic96-

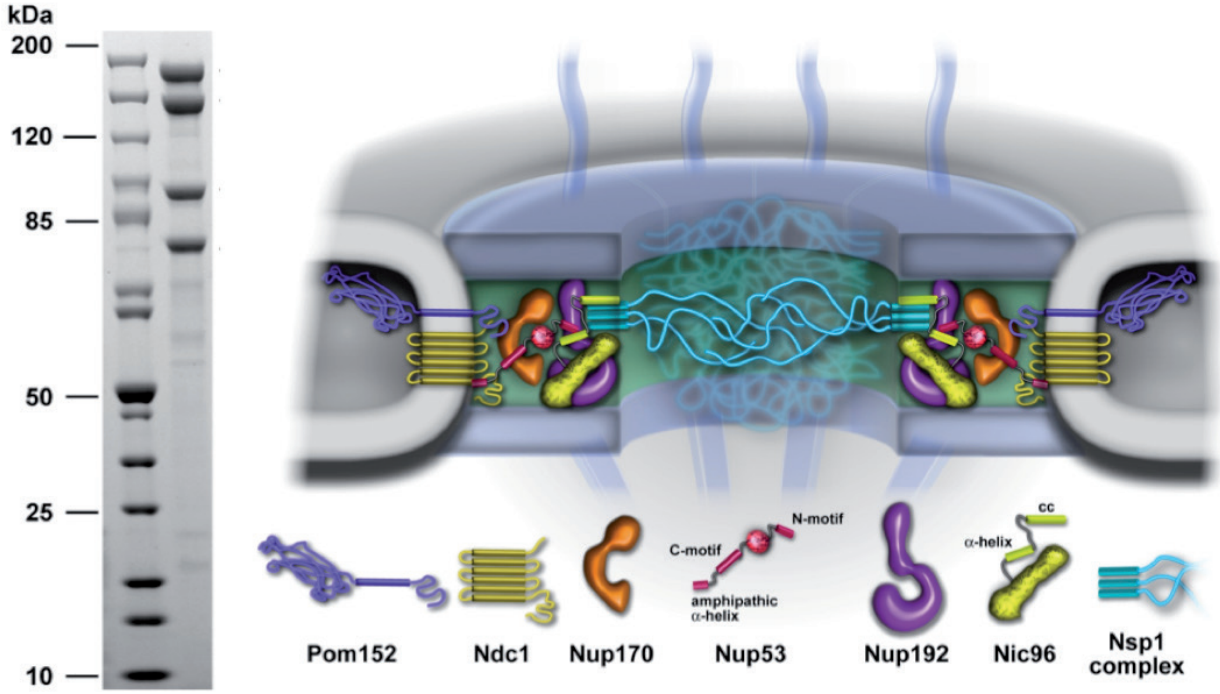

Fig. 1: Reconstitution of the inner pore ring module from a thermophilic eukaryote. *a, SDS-PAGE of the reconstituted Nup192-Nup170-Nic96-Nup53 complex. b, Model of the innner pore ring complex spanning form the nuclear transport channel to the nuclear pore membrane.*

Nup53 (Figure 1). This module will serve as seed to further dock it to other NPC subcomplexes and eventually reconstitute the entire NPC. Moreover, we are currently testing in collaboration with the Hoelz lab (California Institute of Technology, Pasadena, USA) the large structural *ct*Nups and their individual domains for crystallization. From

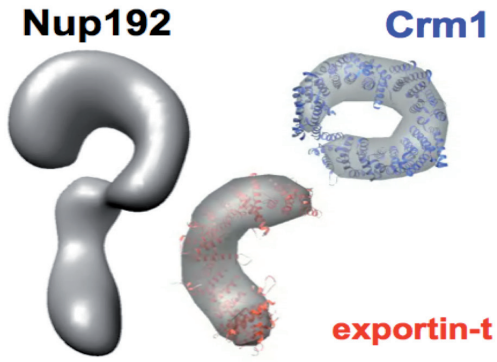

Fig. 2: Comparison of the structure of ctNup192 with shuttling karyopherin transport receptors *(Crm1, exportin-t).*

all these studies, we could get new insight into the structure and assembly of the conserved nuclear pore complex.

When comparing the unusual "S"-like morphology of the α-helically predicted Nup192 molecule to known structures, we noticed that each of the two half circles of Nup192 resembles, in both shape and curvature, the karyopherin transport receptors (e.g. exportin-t or Crm1) that share a superhelical architecture of tandem HEAT repeats (Figure 2). Accordingly, Nups and karyopherins may have a common evolutionary origin. Hence, we speculate that an ancestral Nup (e.g. Nup192) may have lost its ability to get stably incorporated into the NPC, but instead gained binding pockets on the solenoid surface for recruiting export or import cargoes that carry short and exposed targeting signals (e.g. NLS, NES). These evolutionary considerations are supported by findings from a collaborative lab (Ficner group at the University of Göttingen) revealing the x-ray structure of the nuclear export receptor Crm1 from *Chaetomium thermophilum*.

In our research investigating the mechanism of ribosome assembly, we have observed an unforeseen link between nuclear protein import and ribosome assembly. We have discovered a novel import adaptor (Syo1) that synchronously binds two related ribosomal proteins, Rpl5 and Rpl11, which eventually become neighbors on the 60S subunit through their adjacent binding to 5S rRNA. A stoichiometric Syo1-Rpl5-Rpl11 complex was reconstituted *in vitro* that recruited the import receptor Kapβ2 via a PY-NLS at the N-terminus of Syo1, and was imported into the nucleus (Figure 3). The crystal structure of the Syo1-Rpl5 complex (obtained in collaboration with the Sinning lab, BZH) revealed the binding

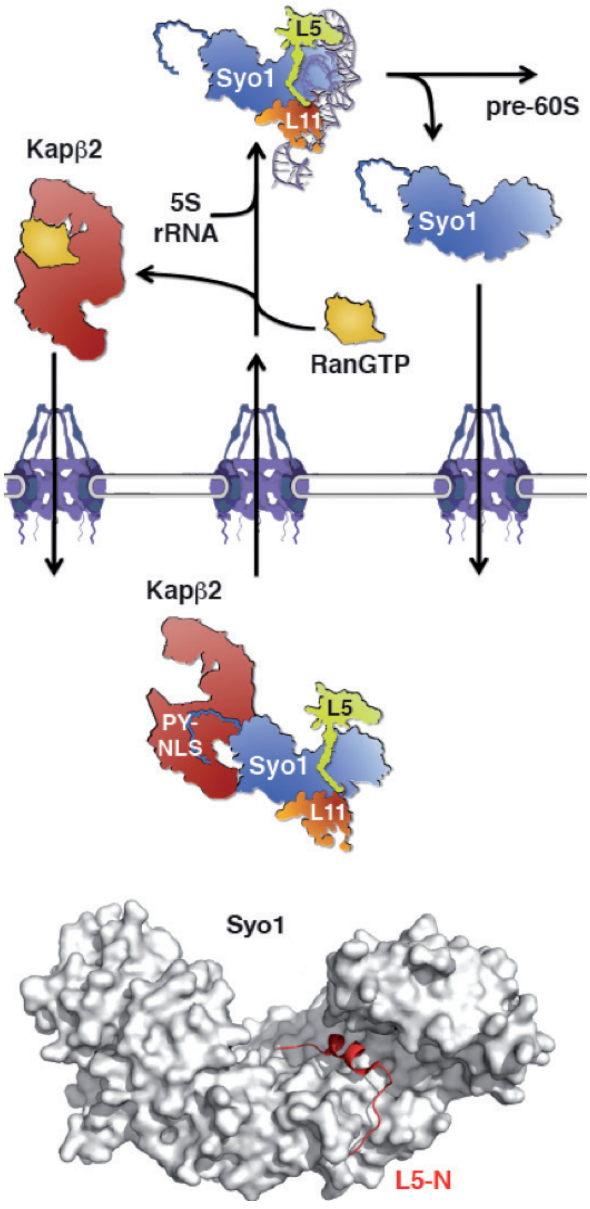

Fig. 3: Model of the Syo1-Rpl5-Rpl11 import into the nucleus *(adapted from Commun Integr Biol. 6:e24792. doi: 10.4161/cib.24792; 2013).*

of the Rpl5 N-terminal peptide motif to a groove on the α-solenoid surface of Syo1. Altogether, the genetic, structural and *in vitro* reconstitution studies have indicated that co-import of Rpl5-Rpl11 coupled with *en bloc* transfer from its import adaptor Syo1 to 5S rRNA could ensure a coordinated and stoichiometric assembly of the 5S RNP onto the evolving pre-60S subunit.

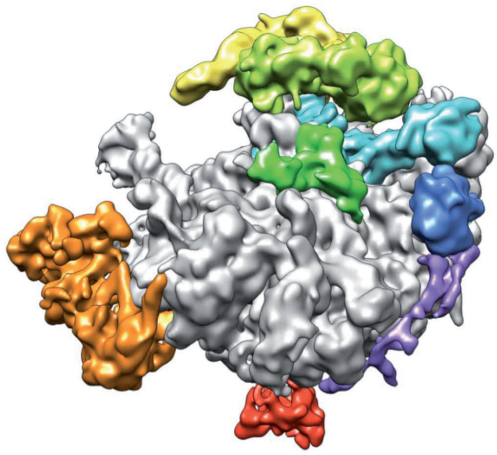

Fig. 4: Cryo-EM structure of the pre-60S ribosomal subunit *(picture provided by C. Leidig and R. Beckmann, Gene Center, University of Munich).*

Despite the advances in our compositional understanding of pre-ribosomal particles, only little structural data is available for nascent ribosomes. Hence, we purified the pre-60S particle and could obtain the first cryo-EM structure of a native pre-60S subunit in association with the assembly factor Arx1 at 9 Å resolution (in collaboration with the Beckmann lab, Gene Center of the University of Munich). This study revealed major structural differences between pre-60S particles and the mature 60S subunit (Figure 4) demonstrating the importance of comprehensive structural investigations of such native pre-ribosomal particles. Our ultimate goal is to come to pseudo-atomic models of pre-ribosomal particles, which will not only be snapshots of the evolving nascent ribosome, but give also insight into the mechanism of ribosome assembly in space and time.

Selected Publications 2011 - 2013

Stefan Amlacher, Phillip Sarges, Dirk Flemming, Vera van Noort, Ruth Kunze, Damien Devos, Manimozhiyan Arumugam, Peer Bork and Ed Hurt: Insight into structure and assembly of the nuclear pore complex by utilizing the genome of a eukaryotic thermophile
Cell 146, 277-289 (2011).

Philipp Stelter, Ruth Kunze, Jessica Fischer and Ed Hurt: Probing the FG repeat network of nucleoporins defines structural and functional features of the nuclear pore complex
J. Cell Biol. 195, 183-192 (2011).

Dirk Flemming, Damien Devos, Johannes Schwarz, Stefan Amlacher, Malik Lutzmann and Ed Hurt: Analysis of the yeast nucleoporin Nup188 reveals a conserved S-like structure with similarity to karyopherins
J. Struct. Biol. 177, 99-105 (2012).

Philipp Stelter, Ruth Kunze, Monika Radwan, Emma Thomson, Karsten Thierbach, Matthias Thoms and Ed Hurt: Monitoring spatiotemporal biogenesis of macromolecular assemblies by pulse-chase epitope labeling
Mol. Cell 47, 788-796 (2012).

Dieter Kressler, Gert Bange, Yutaka Ogawa, Dagmar Pratte, Bettina Bradatsch, Daniela Roser, Jun Katahira, Irmgard Sinning, and Ed Hurt: Coordinated nuclear transport and assembly of functionally related ribosomal proteins onto nascent ribosomes by a common import adaptor
Science 338, 666-671 (2012).

Sébastien Ferreira-Cerca, Vatsala Sagar, Thorsten Schäfer, Anne-Maria Wesseling, Eileen Chai, Ed Hurt and Nicole LaRonde-LeBlanc: Structural basis for kinase-activity dependent placeholder role of Rio2 on the evolving pre-40S subunit
Nat. Struct. Mol. Biol. 19, 1316-1323 (2012).

Jochen Baßler, Isabella Klein, Claudia Schmidt, Emma Thomson, Martina Kallas, Maria Anna Wagner, Gerald Rechberger, Heimo Strohmaier, Ed Hurt, Helmut Bergler: The conserved Bud20 zinc finger protein is a new component of the ribosomal 60S subunit export machinery
Mol. Cell Biol. 32, 4898-912 (2012).

Bettina Bradatsch, Christoph Leidig, Sander Granneman, Marén Gnädig, Bettina Böttcher, David Tollervey, Roland Beckman and Ed Hurt: Structural insight into the late pre-60S ribosomal subunits with bound nuclear export factor Arx1
Nat. Struct. Mol. Biol. 19, 1234-1241 (2012).

Karsten Thierbach, Alexander von Appen, Matthias Thoms, Martin Beck, Dirk Flemming and Ed Hurt: Protein interfaces of the Conserved Nup84 Complex from *Chaetomium thermophilum* revealed by cross-linking mass spectrometry and electron microscopy
Structure 21, 1672-1682 (2013).

Maria Hondele, Tobias Stuwe, Markus Hassler, Felix Halbach, Bianca Nijmeijer, Vladimir Rybin, Stefan Amlacher, Ed Hurt and Andreas Ladurner: Structural basis of histone H2A-H2B recognition by the essential chaperone FACT
Nature 499, 111-114 (2013).

Yoshihira Matsuo, Sander Granneman, Matthias Thoms, Rizos-Georgios Manikas, David Tollervey and Ed Hurt: Quality control of 60S pre-ribosome export involves coupling of a conformation-sensing GTPase to a remodeling AAA-ATPase
Nature, doi: 10.1038/nature12731. Epub 2013 Nov 17.

Awards and Honors

2007	Feldberg Prize
2001	Gottfried Wilhelm Leibniz Prize
Since 2010	Editorial Board of EMBO Journal
Since 2007	Member of ACADEMIA EUROPAEA
Since 2005	Member of LEOPOLDINA
Since 1994	Member of EMBO

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