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
1990	Habilitation in Biochemistry - University of Regensburg, Germany
since 1995	Full Professor - BZH
2003 - 2005	Director - BZH

Ed Hurt

Deciphering nuclear pore complex and and ribosome biogenesis

Goal

We want to find out how the nuclear pore complexes and ribosomal subunits are assembled in the cell. To unravel the underlying mechanisms, we perform biochemical, structural and genetic studies in the model organisms *Saccharomyces cerevisiae* and *Chaetomium thermophilum*, which gave mechanistic insight in how these huge macromolecular assemblies are built up from smaller subcomplexes and how these modules are functionally linked together.

Background

Nucleo-cytoplasmic transport occurs through the nuclear pore complexes (NPCs), which are huge assemblies embedded into the nuclear envelope. Each NPC, whose size is ca. 60 MDa in yeast and 120 MDa in human, is built up by multiple copies (8, 16, 32) of ~30 different nucleoporins (also called Nups), which in most cases are conserved during evolution. The overall NPC structure shows eightfold symmetry with a number of distinct substructures, which were later on also identified as biochemical entities. Prominent structural building blocks are the inner ring complex, which anchors the nuclear and cytoplasmic ring, from which peripheral elements, the nuclear basket and cytoplasmic filaments, emanate. Within the center of the NPC is the transport channel, which is filled by a network of phenylalanine-glycine (FG) rich repeat sequences found in a number of the nucleoporins that constitute the permeability barrier of the NPC. FG repeat domains can transiently interact with shuttling transport receptors, which enables them to pass through the NPC either alone or with bound cargoes. Most nucleoporins are organized in stable subcomplexes, the building blocks of the NPC. For many years, my lab has been studying these NPC modules in yeast, with a focus on the conserved Y-shaped Nup84 complex, the Nsp1-Nup49-Nup57 channel complex and the Nup82-Nup159-Nsp1 complex at the cytoplasmic filaments.

The second major interest of the lab concerns the synthesis of the ribosomal 60S and 40S subunits, which are composed of four ribosomal RNAs (18S, 25S, 5.8S and 5S) and about 80 ribosomal proteins. Eukaryotic ribosome biogenesis is embedded into a complicated pathway of processing, modification and maturation steps, in which a myriad of non-ribosomal factors (ca. 200) form transient interactions with the pre-ribosomal particles, thereby driving ribosome synthesis. The earliest intermediate identified in this path is the nucleolar 90S pre-ribosome, which later gives

raise to pre-60S and pre-40S particles. Pre-60S particles require extensive nuclear maturation prior to export to the cytoplasm, whilst in contrast the simpler pre-40S particles are rapidly exported to the cytoplasm, where maturation is completed.

Research Highlights

In the past years, we have performed structure-function analyses of the NPC in *Saccharomyces cerevisiae* and *Chaetomium thermophilum* by combining, respectively, the excellent genetic and thermophile properties of these two model organisms. With the help of *Chaetomium thermophilum*, we were able to reconstitute and characterize the inner ring complex (IRC) composed of Nup192-Nic96-Nup170-Nup53 and show that another nucleoporin Nup145N can serve as a linker nucleoporin to connect it to other NPC modules. Specifically, the mostly unstructured Nup145N contains several distinct short linear motifs that enable this protein to bind specifically to the two large structural Nups of the IRC, Nup192 and Nup170. At the same time, the Nup145N C-terminal domain can interact with the Nup82-Nup159-Nsp1 complex, thus connecting the IRC with the Nup82 complex at the cytoplasmic face of the NPC (Figure 1).

Furthermore, we could clarify how the IRC can be physically joined to the Nsp1-Nup49-Nup57 channel complex via a short linear motif in Nic96 (Fischer et al., 2015). Finally, we were able to reconstitute an extensive supercomplex of the NPC consisting of three major modules – IRC, Nsp1 channel complex and Nup82 complex - containing a total of eleven Nups. In summary, all these studies gave insight into how distinct subcomplexes of the NPC are interconnected to form higher-order assemblies, which shed new light on how the NPC can be built up from smaller building blocks, flexibly joined by short linear motifs provided by linker Nups.

In order to obtain protein complexes of increased stability isolated directly from *Chaetomium thermophilum*, we developed a transformation procedure including an endogenous resistance marker system suitable for the thermophile (Kellner et al., 2016). Using this method, we were able to generate stable transformants expressing various nuclear pore complex proteins or ribosome biogenesis factors fused to a ProtA-TEV-Flag tandem affinity-tag for the purification of native thermostable nucleoporin supercomplexes or pre-ribosomal particles (see below).

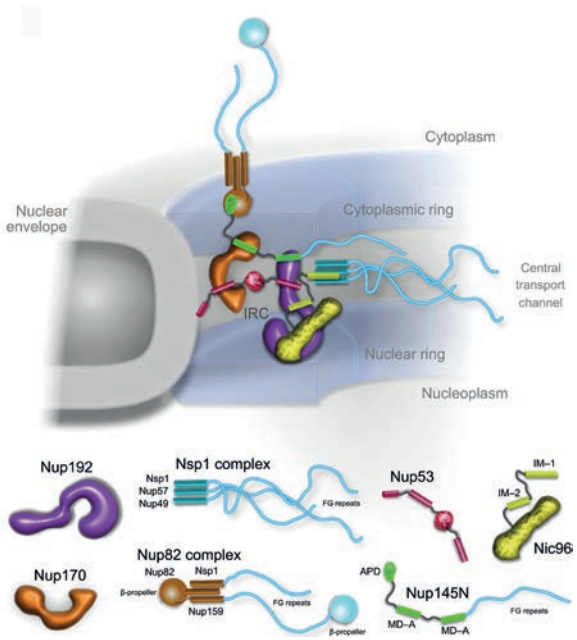

Figure 1: Reconstitution of the inner pore ring module (IRC) with outer ring Nup82 complex and channel Nsp1 complex.

In our research investigating the mechanism of ribosome assembly, we were able to demonstrate how the huge dynein-like ATPase Rea1 and its cofactors Rsa4 and Ytm1 are essential for the assembly for the large subunit. A combination of genetic and biochemical methods allowed us to identify Nsa2 as direct binding partner of Rsa4. Structural approaches including cryo-EM of the Arx1 and Rix1-Rea1 particles (in collaboration with the Beckmann lab at the Gene Center in Munich), crystallisation of Rsa4 and the Rsa4-Nsa2 complex (in collaboration with I. Sinning at the BZH) and NMR analysis of Nsa2 domains (in collaboration with E. Barbar, Oregon State University, Corvallis) enabled us to understand the molecular details of the Rsa4 - Nsa2

interaction (Baßler et al., 2014). Altogether, these data revealed how the Rea1 ATPase creates a mechano-chemical force that can be transmitted via Rsa4 and Nsa2 towards the nascent rRNA of the PTC, inducing rRNA relocation, as recently revealed by cryo-EM analysis of the Rix1-Rea1 pre-60S particle (Barrio-Garcia et al., 2016). By performing cryo-EM analysis on two similar particles that were mutated on either Rix1 or Rea1, a mechanistic model for the transition between these two pre-60S intermediates could be suggested.

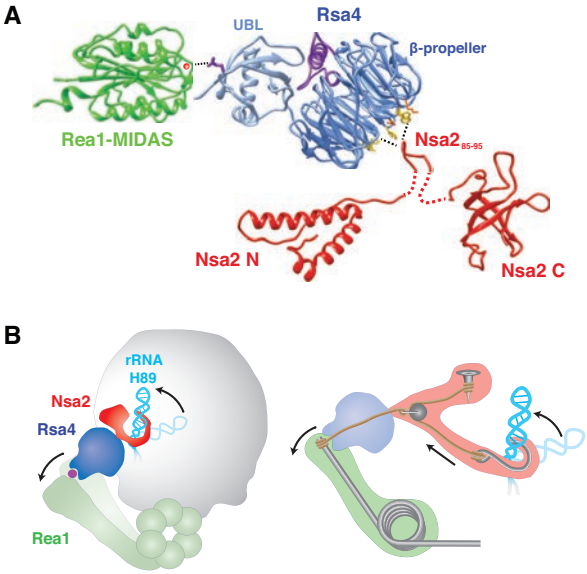

Figure 2. Ribosome assembly factor network revealing how the Rea1 AAA ATPase can remodel the pre-60S subunit during ribosome assembly *(adapted from Baßler et al., 2014).*

The transport of the pre-ribosomal subunits from the nucleus to the cytoplasm is a critical step during eukaryotic ribosome biogenesis, making this cascade-like process as a whole apparently irreversible. To migrate through the hydrophobic FG repeat network of the central transport channel of the nuclear pore complex, the huge hydrophilic pre-60S subunit requires several export factors on its surface. In the past year, we could clarify the mechanism how the RNA export receptor Mex67-Mtr2 can be timely recruited to the pre-60S subunit at the ribosome P0 stalk (Sarkar et al., 2016), which on the mature 60S subunit constitutes a landing platform for the translational GTPases. Initially, a structural homologue of P0 called Mrt4 binds to the nascent ribosome stalk inside the nucleus, thereby blocking not only P0 but also Mex67-Mtr2 binding at this site. Later on Yvh1 releases Mrt4 from the pre-ribosome, creating a time window for Mex67-Mtr2 association at this temporary site, which in the cytoplasm eventually becomes occupied by the ribosomal P0 stalk protein. Thus, a spatio-temporal mark on the ribosomal P stalk accurately regulates timed recruitment of nuclear export receptor to the nascent 60S subunit.

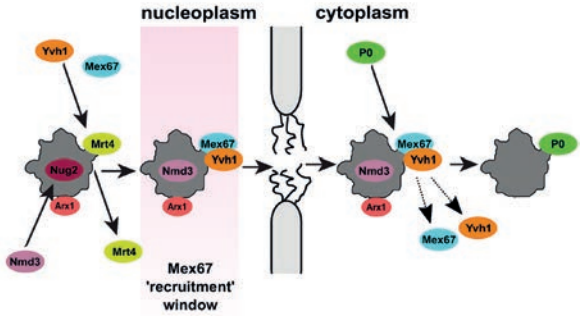

Figure 3. Model of the Mex67-Mtr2 recruitment ot the pre-60S particle *(adapted from Sarkar et al., 2016).*

Despite the advances in our compositional understanding of pre-ribosomal particles, only little structural data were available for the earliest pre-ribosomes. The 90S pre-ribosome composed of ~70 assembly factors and several snoRNAs is the earliest intermediate formed during eukaryotic ribosome synthesis. In collaboration with the Beckmann lab, we could solve the cryo-EM structure of the *Chaetomium thermophilum* 90S pre-ribosome at subnanometer resolution, which revealed how the network of biogenesis factors surrounds the nascent pre-rRNA. One of these key modules, the U3 snoRNP, is strategically positioned at the center of the particle to orchestrate pre-rRNA folding and processing (Kornprobst et al., 2016). Thus, by exploiting *Chaetomium thermophilum* we could gain unforeseen structural insight into how the 90S pre-ribosome facilitates co-transcriptional folding of the nascent pre-rRNA, which occurs in a mold-like scaffold, reminiscent of how polypeptides use chaperone chambers for their protein folding.

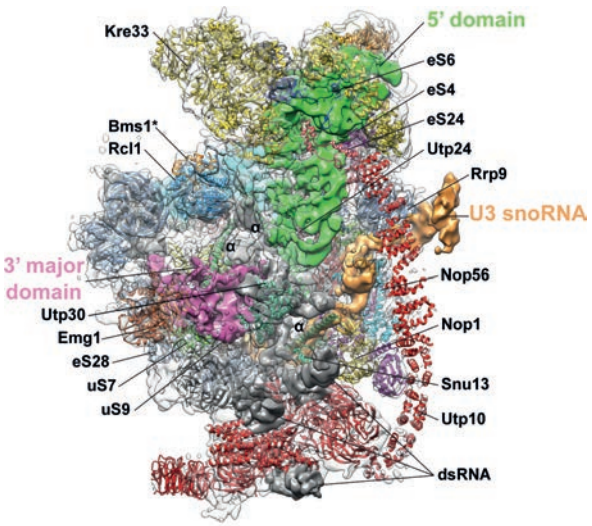

Figure 4. Cryo-EM structure of the 90S pre-ribosome *(adapted from Kornprobst et al., 2016).*

Selected Publications 2014 - 2016

Yoshitaka Matsuo, Sander Granneman, Matthias Thoms, Rizos-Georgios Manikas, David Tollervey and Ed Hurt: Coupled GTPase and remodeling ATPase activities form a checkpoint for ribosome export
Nature 505, 112-116 (2014).

Jochen Baßler, Helge Paternoga, Iris Holdermann, Matthias Thoms, Sander Granneman, Clara Barrio Garcia, Afua Nyarko, Gunther Stier, Sarah A. Clark, Daniel Schraivogel, Martina Kallas, Roland Beckmann, David Tollervey, Elisar Barbar, Irmi Sinning and Ed Hurt: A network of assembly factors involved is involved in remodeling rRNA elements during pre-ribosome maturation
J. Cell Biol. 207, 481-98 (2014)

Monika Gaik, Dirk Flemming, Alexander von Appen, Panagiotis Kastritis, Philipp Stelter, Khanh Huy Bui, Alessandro Ori, Jochen Bassler, Jessica Fischer, Elisar Barbar, Martin Beck and Ed Hurt: Structural basis for assembly and function Nup82 complex in the nuclear pore scaffold
J. Cell Biol. 208, 283–297 (2015)

Matthias Thoms, Emma Thomson Jochen Baßler, Marén Gnädig, Sabine Griesel and Ed Hurt: The exosome is recruited to RNA substrates through specific adapter proteins
Cell 162, 1029-1038 (2015).

Philipp Stelter, Ferdinand Huber, Ruth Kunze, Dirk Flemming, André Hoelz and Ed Hurt: Coordinated ribosomal L4 protein assembly into the pre-ribosome is regulated by its eukaryote-specific extension
Mol. Cell 58, 854-862 (2015).

Lisa Gasse, Dirk Flemming and Ed Hurt: Coordinated ITS2 rRNA processing by the Las1 complex integrating endonuclease, polynucleotide kinase and exonuclease activities
Mol. Cell 860, 808-815 (2015).

Jessica Fischer, Roman Teimer, Stefan Amlacher, Ruth Kunze and Ed Hurt: Linker Nups connect the nuclear pore complex inner ring with outer ring and transport channel
Nature Struct. Mol. Biol. 22, 774-81 (2015).

Clara Barrio-Garcia, Matthias Thoms, Dirk Flemming, Lukas Kater, Jochen Bassler, Roland Beckmann and Ed Hurt: Architecture of a ribosome assembly bound to a combined remodeling-checkpoint machinery
Nature Struct. Mol. Biol. 23, 37-44 (2016).

Matthias Thoms, Yasar Luqman Ahmed, Karthik Maddi, Ed Hurt and Irmgard Sinning: Concerted removal of the Erb1-Ytm1 complex in ribosome biogenesis relies on an elaborate interface
Nucl Acid Res. 44, 926-39 (2016).

Nikola Kellner, Johannes Schwarz, Miriam Sturm, Javier Fernandez-Martinez, Sabine Griesel, Wenzhu Zhang, Brian Chait, Michael P. Rout, Ulrich Kück and Ed Hurt: Developing genetic tools to exploit Chaetomium thermophilum for biochemical analyses of eukaryotic macromolecular assemblies
Sci. Rep. 6, 20937. (2016).

Markus Kornprobst, Martin Turk, Nikola Kellner, Jing-Dong Cheng, Dirk Flemming, Martin Koš, Isabelle Koš, Matthias Thoms, Otto Berninghausen, Roland Beckmann and Ed Hurt: Architecture of the 90S pre-ribosome: a structural view on the birth of the eukaryotic ribosome
Cell 166, 380–393 (2016).

Anshuk Sarkar, Markus Pech, Matthias Thoms, Dirk Flemming, Roland Beckmann and Ed Hurt: Coupling ribosome stalk biogenesis with nuclear export factor recruitment to the nascent 60S subunit
Nat. Struct. Mol. Biol. 10.1038/nsmb.3312 (2016).

Awards and Honors

2015	Eduard Buchner Prize
2011	Reinhart Koselleck Project
2007	Feldberg Prize
2001	Gottfried Wilhelm Leibniz Prize
2007	Member of ACADEMIA EUROPAEA
2005	Member of LEOPOLDINA
1994	Member of EMBO

Ed Hurt
Phone: +49 (0)6221-54 4173
E-mail: ed.hurt@bzh.uni-heidelberg.de