Protein modules in ribosome biogenesis:
NOP7/ERB1/YTM1 subcomplex

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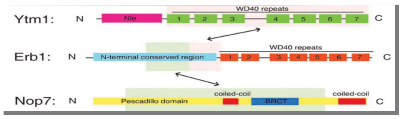
Introduction

Ribosome biogenesis plays a **crucial role** in the development and proliferation of all eukaryotic cells and involves many steps of rRNA processing, which are carried out by association and dissociation of numerous proteins that can act as scaffolds of the nascent ribosome or play role in the tight regulation of this **highly energy-consuming process**. It is of a great interest to better understand the molecular basis of the events that permit the assembly of a fully functional ribosomal particle, specially if we remark its importance in **tumorous cells** that require high protein expression levels for their **fast proliferation.** The enormous complexity of this pathway makes its study a **difficult and challenging task** if a global approach is considered. A modular point of view which takes into consideration only these factors that can be functionally and temporarily linked, thus forming **sub-complexes**, seems to be the best option in order to understand the process.

The mammalian **PeBoW complex** is described to be a heterotrimeric particle **essential** in the 60S subunit synthesis and is involved in the processing of the ITS2 during rRNA maturation. The counterpart of PeBoW in yeast is formed by **NOP7, ERB1** and **YTM1** proteins which were shown to interdependently associate with pre60S although their exact role is unknown. Silencing and knock-down studies showed that each factor is **necessary** for the path to continue and that the complex has to be stripped off from the pre-ribosomal particle in order to **promote** its **normal maturation**. ERB1 is known to interact with both, NOP7 and YTM1, thus holding the complex together. Little is known about the complex from a **structural point of view**. Sequence based predictions identified C-terminal **β-propeller** domains within YTM1 and ERB1 proteins and a well conserved **BRCT** motif was found in NOP7, nevertheless **no previous empirical studies** regarding the complex structure were carried out.

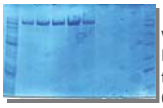


From **Tang et al.** Interactions among Ytm1, Erb1, and Nop7 Required for Assembly of the Nop7-Subcomplex in Yeast Preribosomes

Goals

Our aim is to **solve the structure** of the yeast homolog of PeBoW complex so that we can shed light on its exact function in the ribosome biogenesis. Once we get to know the structural details of the particle we will perform functional assays in *Saccharomyces cerevisiae* in order to **link the domain architecture with its function.**

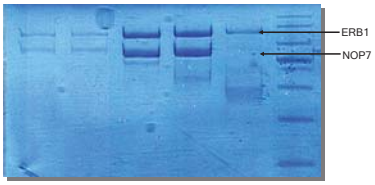
Results



Full length 6xHis-NOP7 protein was expressed in *E. coli* BL21-RIPL 16h at 20ºC by adding IPTG to the final concentration of 0.5mM.

Full length 6xHis-ERB1 was over-expressed in *E. coli* BL21-RIPL strain grown in the ZY media using the autoinduction method described by Studier.

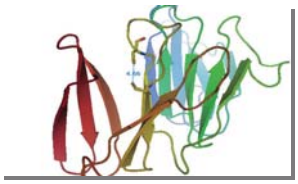
The **interaction** between **NOP7 and ERB1** was confirmed by **gel filtration** as both proteins co-eluted from the Superdex 200 column.



The expression of full-length **6xHis-YTM1 in BL21-RIPL** resulted in inclusion body formation and no soluble protein was obtained. Subsequent changes of tags and strains showed that the protein was solubilised but **aggregated** when fused to the C-terminus of MBP.

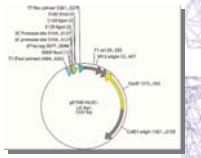
Automated modelling of the β-propeller domain from YTM1 showed two adjacent cysteins that could possibly form a **disulfide bridge** thus making the structure more stable and soluble.

Small scale expression test in **Origami2 (DE3)** strain yielded soluble protein but its aggregation state still must be confirmed.



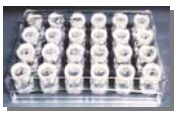
Methodology

PCR & LIC CLONING
pNKI/pMBKI/pGKI
pNIC28-Bsa4



PROTEIN EXPRESSION
E. coli: BL21; BL21-RIPL; Rosetta2; Origami2; (all DE3)

PROTEIN PURIFICATION
Affinity columns, Size exclusion chromatography, ÄKTA System

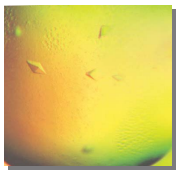


CRYSTALLIZATION
Thermofluor assays;
96-well screens (sitting drop);
24-well optimization (hanging drop)

DIFFRACTION
In-house diffractometer;
ERSF; DLS;

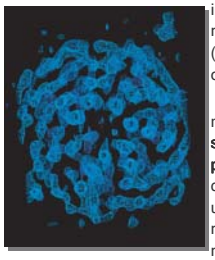
Purified complex of NOP7/ERB1 was used in **crystallization screenings** and small diamond-shaped crystals appeared in the condition B4 of JCSG+:

0.1M Hepes7.5;
10% PEG 8000;
8% ethylene glycol



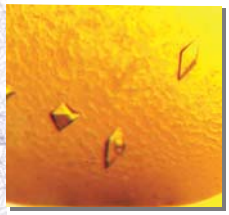
In order to confirm if both proteins were present in the crystal a SDS-PAGE gel was run and only one band could be observed (data not shown). Subsequent MALDI-TOF analysis allowed to identify the band as a **degraded fragment of ERB1**, however the exact boundaries of the crystallized peptide could not be established.

The size and quality of the **original** crystals could not be improved in a set of optimization trials and the first X-ray diffraction experiments resulted in **low-resolution datasets** corresponding to native (max. d: 2.8Å) and derivative crystals (SeMet and HA soaks, max d: 5.1Å) not sufficient for obtaining experimental phases.



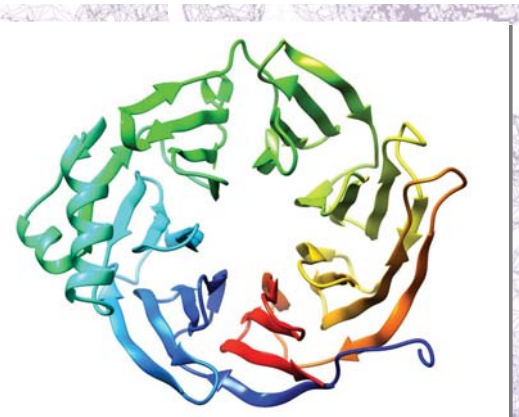
The native dataset was used for molecular replacement trial performed with **Balbes server** and the structure of the **C-terminal β-propeller of ERB1** was initially traced in the density map although it was not possible to unambiguously assign side chains and the final map was strongly contaminated by the model.

With the purpose of getting better resolution and more accurate density map the crystallization trials were repeated using the **original condition plus additives** from the Hampton Additive Screen. A set of conditions yielded **diamond-shaped and trapezoidal** crystals.

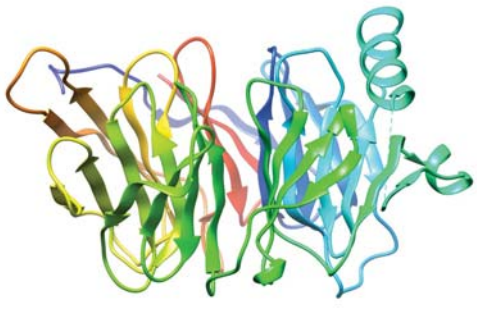


All the crystals were diffracted in the Diamond Light Source (I03 Beamline) and the **maximum resolution achieved was 1.64 Å**.

Additive	Concentration	Resolution (Å)
Taurine	0.1 M	5.4
γ-Butyrolactone	40% v/v	2.5
Ethanol	30% v/v	1.64
Methanol	30% v/v	1.8
1-Butanol	7% v/v	2.04
1,4-Dioxane	30% v/v	2.09
Dichloromethane	0.75% v/v	5.2



The **diffraction** of the crystals obtained with additives was **sufficient for phasing** using molecular replacement module from Phenix. A poly-Ala β-propeller was used for search in order to avoid model bias and after **model-building and refinement** cycles the structure of a complete β-propeller was solved.



The structure shows the typical architecture of a **seven-bladed propeller** closed by the **"Velcro" type 1+3** (first β-sheet from the WD1 + 3 β-sheets from WD7). The protein is highly solvated, specially in the inner tunnel of the propeller.

Between blades 2 and 3 it possesses an **insertion** of 50 aa that cannot be entirely seen in the electron density map probably due to its **flexibility**. The N-terminal helix of the insertion participates in **crystal packing** thus it acquires more rigid position.

Crystal data and data-collection statistics

Results of automated processing from Xia2 pipeline

Space group	P 21 21 21
Unit-cell parameters (Å)	
a	52.02
b	62.43
c	158.22

Resolution range (Å)	62.43 - 1.64 (1.68 - 1.64)
Observed reflections	413389 (30940)
No. of unique reflections	62681 (4547)
Completeness (%)	98.5 (97.4)
Multiplicity	6.6 (6.8)
s/s(I)>	20.8 (2.7)
Rmerge(%)	0.04 (0.659)

Values in parentheses are for the highest resolution shell.

Data refinement statistics

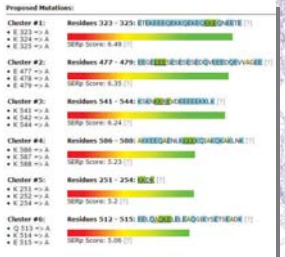
From phenix_refine module

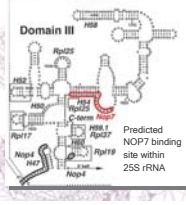
Resolution(Å)	58.97-1.64
Reflections	62639
R work	0.17
R free	0.1822
RMS (bonds)	0.0110
RMS (angels)	1.375

Perspective

In order to elucidate the structure of the **NOP7/ERB1/YTM1** we have been focusing on the full length proteins but since ERB1 undergoes proteolysis we decided to prepare a set of **new constructs** that would make the complex more stable thus preventing the crystallization of the β-propeller domain of ERB1 alone.

A new approach has been considered to solve the structure of **NOP7**. A prediction made by UCLA-MBI SERp Server selected 6 areas suitable for **mutations** that can promote crystallization of the protein.





Since **ERB1 and NOP7** were shown to **bind rRNA** it could be promising to co-crystallize both with nucleic acids.

From **Granneman et al.** A cluster of ribosome synthesis factors regulate pre-rRNA folding and 5.8S rRNA maturation by the Rat1 exonuclease