

Block course organization: Methods in Cellular Biochemistry

Refresh Teaching

Ivo Zemp

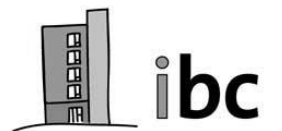
Senior Scientist (Lab of Prof. U. Kutay)

Institute of Biochemistry, D-BIOL

Feb 15, 2022

ETH

Eidgenössische Technische Hochschule Zürich
Swiss Federal Institute of Technology Zurich



Block courses at D-BIOL

- What do D-BIOL block courses look like?
 - Courses of 3.5 weeks, 3.5 days a week, for 3rd year bachelor students
 - Practicals in small groups on a selected topic
 - Seminars and literature discussions in addition
- Goals:
 - Students gain first insights into research topics and working methods
 - Students learn to design, carry out and assess experiments
 - More course-specific goals: e.g focus on methodologies in our block course, etc.
 - ‘get to know each other’

Block courses at D-BIOL

- Block courses can have many different themes
 - Focusing on a topic: e.g. Molecular Mechanisms of Cell Dynamics, Membrane Biology, Causes and Consequences of Unstable Genomes
 - Focusing on methods: NMR spectroscopy, RNA biology, Methods in Cellular Biochemistry
- Setting for the block course can be quite different:
 - small groups in many labs
 - more classroom-like

Block course basics: practical work and lectures

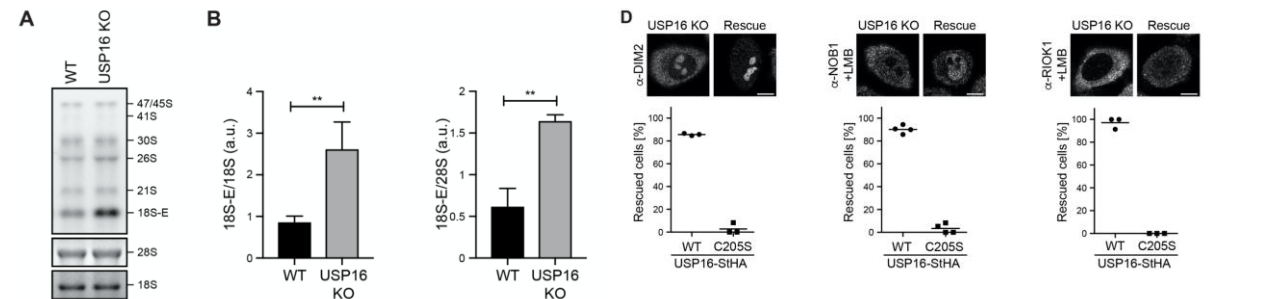
- Main focus: practical work
 - Groups of ~3 students
 - ~2 supervisors
- Lectures to teach the background of the block course
 - Given by professors, PostDocs, senior PhDs, etc.

	Tuesday	Wednesday	Thursday	Friday
9.00 am		8.45-10.30 am Lecture	8.45-10.30 am Lecture	8.45-10.30 am Lecture
10.00 am				
11.00 am		10.30 am onwards Lab work	10.30 am onwards Lab work	10.30 am onwards Lab work
12.00 pm				
1.00 pm	1.00 pm onwards Lab work			
2.00 pm				
3.00 pm				
4.00 pm				
5.00 pm				

Methods in Cellular Biochemistry Schedule

Additional elements of the block course

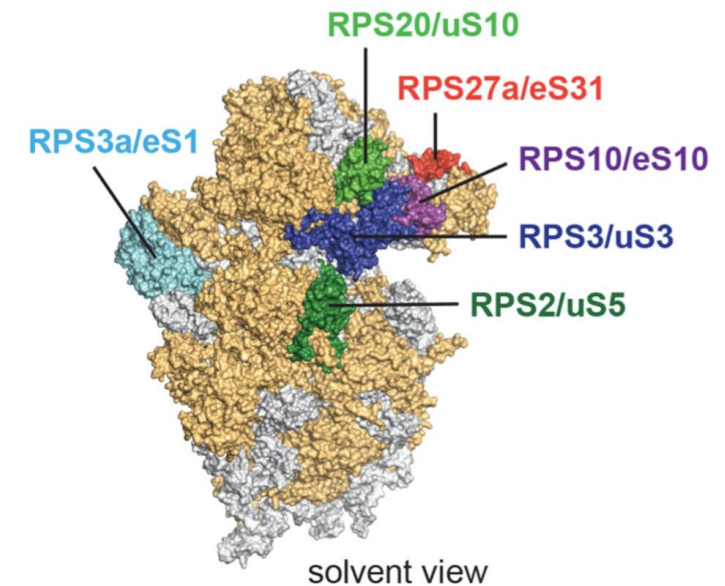
- Presenting scientific concepts and data
- ‘Chalk talks’: informal presentation allowing students to present their own projects
- Journal club:
 - Each group presents a paper
 - Or one paper for the whole course



Montellese et al, eLife (2020)

Additional elements of the block course



- Presenting scientific concepts and data
- ‘Chalk talks’: informal presentation allowing students to present their own projects
- Journal club:
 - Each group presents a paper
 - Or one paper for the whole course
- Workshop on structural biology
 - Part I: presentation of structural biology tools
 - Part II: application of tools to the respective projects



Evaluation of the students

- 50% based on practical work
 - Based on lab supervisors / PIs
- 50% based on final presentation
 - Poster session
 - Presentations
- Students get time and are supervised during presentation preparation

Role of the E3 ligase CRL4-VPRBP in tuning ribosomal protein levels


 Roberta Florea, Geo Forni, Joël Frey, Kerstin Dömer, Annamaria Gamper, Ivo Zemp and Ulrike Kutay
 Institute of Biochemistry, ETH Zürich
 

I. Introduction

Eukaryotic ribosomes consist of a small 40S and a large 60S ribosomal subunit and are required for all protein synthesis in cells [1]. Subunits comprise rRNAs and ribosomal proteins (RPs) and their biogenesis is a highly complex and energetically demanding process. Whereas rRNAs and RPs are present at 1:1 stoichiometry in mature ribosomes, RPs are produced in significant excess over rRNA in the cytosol [1]. Most RPs are imported into the nucleus and to the nucleoli, where they are incorporated into the assembling subunit precursors. Unassembled RPs are ubiquitinated and targeted for proteasomal degradation by still unknown E3 ubiquitin ligases [2]. Previous screens identified VPRBP as a factor required for 40S and 60S subunit maturation. Here we investigate the potential role of the CRL4-E3 ligase associated with the substrate recognition subunit VPRBP in degradation of the ribosomal proteins RPL6, RPL14 and RPL15.

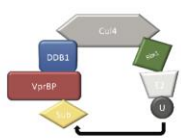


Fig. 1: The RING Type E3 ligase CRL4-VPRBP

The last step of the ubiquitination process is performed by E3 ligases which ensure the Ub transfer to the substrate. The RING type E3 ligase CRL4-VPRBP is composed of a major Cul4 subunit linking substrate recognition by VPRBP to the conjugating enzyme E2 by means of the adaptor proteins DDB1 and Rbx1 [3].

II. VPRBP does not comigrate with ribosomal subunits

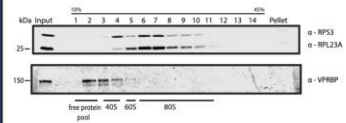


Fig. 2: The comigration of VPRBP with ribosomal subunits was tested by sucrose gradient of HeLa cell extract followed by Western Blot analysis.

The majority of VPRBP appears to be present freely or in small complexes. VPRBP does not seem to significantly interact with ribosomal subunits.

III. Characterization of inducible GFP/HAS1-tagged RPL cell lines

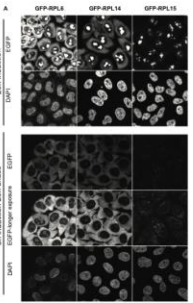
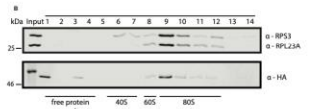



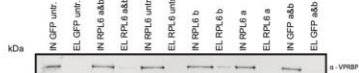
Fig. 3: (A) Expression of GFP-RPL6, GFP-RPL14 and GFP-RPL15 was induced with tetracycline in stably transfected HeLa cells, with or without tetracycline wash-out (chase) as indicated. Cells were fixed and analyzed by confocal microscopy. RPL6 and RPL14 were incorporated into mature ribosome, whereas for RPL15 no incorporation was observed.



(B) Expression of HAS1-RPL6 in HEK293 cell-lines was induced for 24h with tetracycline. Incorporation of HAS1-RPL6 into mature ribosomes was confirmed by sucrose gradient analysis followed by Western Blotting. HAS1 tagged RPL6 can be considered as functional and is incorporated into the 80S ribosome.

IV. Strep-Tactin purification of HAS1-tagged RPs, with and without bortezomib/actinomycinD treatment





Feb 15, 2022

Refresh Teaching – Ivo Zemp, Kutay Lab, Institute of Biochemistry, D-IBIOL

7

Challenges of organizing the block course

- Communication
 - Start early enough: lecturers, professors of the labs involved, lab supervisors
 - Closer to the course: students, institute staff/members
- Heterogeneity in the course:
 - Student background, motivation, etc
 - Different labs involved (style of research, supervision, etc)
- Dependence on lab supervisors and lecturers
- Did we achieve the goals of the block course? Feedback is crucial

Thank you very much for listening!