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Doctoral College
Metabolic & Cardiovascular Disease

Medical University of Graz
UNI GRAZ TU Graz Graz University of Technology

PROTEIN SYNTHESIS FROM THE PERSPECTIVE OF ONE OF THE MAJOR TRANSLATION INITIATION FACTORS - eIF3

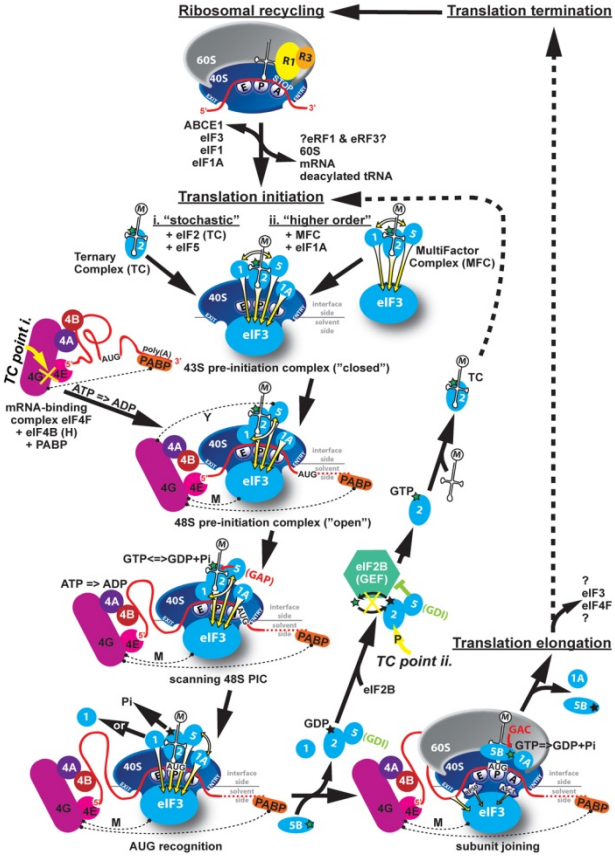
GUEST LECTURE by

Leoš Shivaya Valášek, PhD

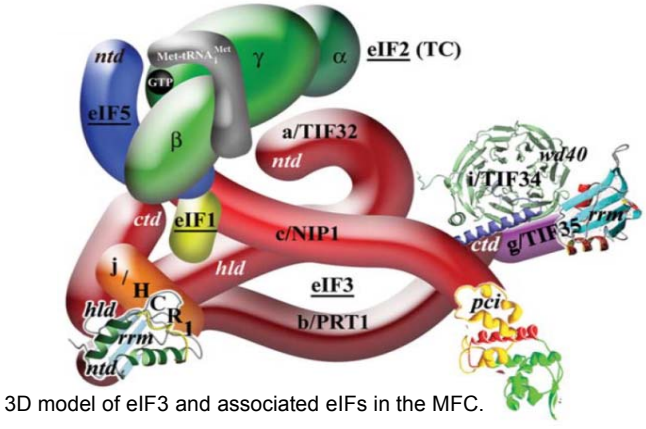
Laboratory of Regulation of Gene Expression
Institute of Microbiology ASCR
Prague / Czech Republic

Monday, 08.04.2013
12:45h

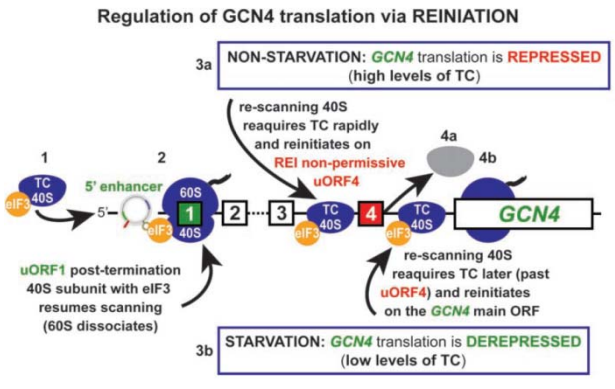
Department of Pathology, Lecture Hall
Auenbruggerplatz 25, MUG



Schematic of the canonical translation pathway in eukaryotes with the ribosomal recycling and intitiation phases shown in detail.



3D model of eIF3 and associated eIFs in the MFC.



'Ribozoomin' – Translation Initiation from the Perspective of the Ribosome-bound Eukaryotic Initiation Factors (eIFs). Valasek (2012) Curr Protein Pept Sci. 13(4):305-30

LEOŠ SHIVAYA VALÁŠEK

CURRICULUM VITAE

Leoš is head of the Laboratory of Regulation of Gene Expression (LRGE) at the Institute of Microbiology AS CR in Prague, where he also serves as the vice chairman of the Executive Board. His laboratory, established in June 2006, investigates a basic concept of translation and various aspects of its control. The studies combine the use of budding yeast *Saccharomyces cerevisiae* and mammalian cells lines, and employ tools of molecular and structural biology, biochemistry and genetics. Leos received his Master degree in Genetics and Molecular Biology in 1994 from the Charles University in Prague and subsequently his Ph.D. in Biochemistry in 1999 from the University of Vienna. His Ph.D. work focused on understanding of basic principles of translation initiation in eukaryotes resulted in three first author publications in significant scientific journals. He went on to do postdoctoral work in the Laboratory of Gene Regulation and Development, National Institute of Child Health and Human Development, NIH, under supervision of Dr. Alan Hinnebusch, where he pursued several independent projects dealing with regulation of translation in yeast. During his endeavour with Dr. Hinnebusch, he co-authored altogether 13 publications in highly prestigious journals and figured as the first author on five of them. In June 2004, Leos received the “welcome-back-home” Fellowship of J. E. Purkyne from the Academy of Sciences of the Czech Republic (ASCR), and returned to the Czech Republic to be appointed as Independent Investigator at the Institute of Microbiology ASCR in Prague. In 2005 he was awarded a Wellcome Trust International Senior Research Fellowship, became the Howard Hughes Medical Institute International Research Scholar, and received an NIH FIC Global Health Research Initiative Program Award. In June 2010, Leos became the first Czech-based researcher ever to renew the Wellcome Trust International Senior Research Fellowship. In November 2011, his group together with 6 other young groups in the Czech Republic received a highly prestigious Centre of Excellence Grant from the Czech Grant Agency. In September 2006, he co-organized the international "Translational Control and Non-Coding RNA“ meeting held in Nove Hrady, the Czech Republic; and in November 2011, he also co-organized a one-day international meeting RNA Club 2011 held in Prague. Leos’s young group currently consists of one post-doctoral fellow, 5 Ph.D., 2 Diploma and 1 Bachelor students and a lab technician. He and his lab have published over 35 publications in top-notch peer-reviewed journals such as Genes & Development, PLoS Genetics, EMBO J., Nucleic Acid Res., Mol Cell Biol etc., and won the best original research publication carried out at the Institute of Microbiology ASCR award in years 2008 and 2011.

ADDRESS

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BIOGRAPHICAL BACKGROUND

Date of birth: July 15, 1971

Place of birth: Liberec, the Czech Republic

Citizenship: Czech

WORK EXPERIENCE

5/05 - present Group Leader/Vice chairman of the Executive Board of the IM AS CR
Wellcome Trust International Senior Research Fellowship Recipient
Laboratory of Regulation of Gene Expression
Institute of Microbiology AS CR
Prague, the Czech Republic

1/99 – 5/99 Research Associate
Department of Virology
Regional Hospital Liberec,
Liberec, the Czech Republic

POSTDOCTORAL RESEARCH EXPERIENCE

6/04 – 4/05 Associate Investigator
Laboratory of Cell Reproduction
Institute of Microbiology AS CR
Prague, the Czech Republic

5/99 – 5/04 Visiting Fellow Award Recipient
NIH section on Nutrient Control of Gene Expression
Laboratory Gene Regulation and Development
National Institute of Child Health and Human Development
National Institutes of Health, Bethesda, MD
Supervisor: Dr. Alan G. Hinnebusch

9/03 – 5/04 Associate Investigator
Laboratory of Cell Reproduction
Institute of Microbiology AS CR
Prague, the Czech Republic

EDUCATION

8/94 – 11/98 **Ph.D. (2/99)**
University of Vienna, Austria
Biocenter, Institute of Biochemistry and Molecular Cell Biology
Thesis title: Characterization of RPG1, the largest subunit of yeast eIF3. (partly carried out in the laboratory of Prof. Hans Trachsel at the University of Bern, Switzerland)
Research Advisor: Prof. Helmut Ruis, Ph.D.

9/89 – 6/94 **M.S.**
Charles University, Prague, the Czech Republic
Department of Molecular Biology and Genetics
Thesis title: Yeast killer toxin K1 and its exploitation in genetic manipulations.
Research Advisor: Doc. Vladimír Vondrejs, Ph.D.

GRANT, FELLOWSHIP AND OTHER AWARDS

3/12	The best original research publication carried out at the IM ASCR in 2011 award
1/12 – 12/18	Centre of Excellence Grant, Czech Science Foundation
7/10 – 6/15	Wellcome Trust International Senior Research Fellowship - renewal
1/10 – 12/13	Czech Science Foundation Grant
1/11 – 12/14	Czech Science Foundation Grant
2/09	The best original research publication carried out at the IM ASCR in 2008 award
1/06 – 12/11	Howard Hughes Medical Institute International Research Scholar
7/05 – 6/10	Wellcome Trust International Senior Research Fellowship
5/05 – 4/10	NIH Global Health Research Initiative Program Award
5/04 – 5/09	ASCR Fellowship of Jan Evangelista Purkyne Recipient
5/99 – 5/04	NIH Visiting Fellow Award Recipient
11/98	graduated with distinction from the University of Vienna
6/94	graduated with distinction from the Charles University

PUBLICATIONS

Walker, S.E., Zhou, F., Mitchell, S.F., Larson, V.S., **Valášek, L.**, Hinnebusch, A.G., and Lorsch, J.R. (2012) Yeast eIF4B binds to the head of the 40S ribosomal subunit and promotes mRNA recruitment through its N-terminal and internal repeat domains. *RNA*, **19**(2), 191-207.

Novotný, I., Podolská, K., Blažíková, M, **Valášek, L.S.**, Svoboda, P., Staněk, D. (2012) Nuclear LSm8 affects number of cytoplasmic processing bodies via controlling cellular distribution of Like-Sm proteins. *Mol Biol Cell*, **23**(19), 3776-85.

Valášek, L.S.* (2012) Ribozoomin' – Translation Initiation from the Perspective of the ribosome-bound Eukaryotic Initiation Factors (eIFs). *Curr Protein Pept Sci.*, **13**, 305-30.

Karasková, M., Gunišová, S., Herrmannová, A., Wagner, S., Munzarová, V., and **Valášek, L.S.*** (2012) Functional Characterization of the Role of the N-terminal Domain of the c/Nip1 Subunit of eIF3 in AUG recognition. *J Biol Chem,* **287**, 28420-34.

Kouba, T., Danyi, I, Gunišová, S., Munzarová, V., Vlčková, V., Cuchalová, L., Neueder, A., Milkereit, P., and **Valášek, L.S.*** (2012) Small Ribosomal Protein RPS0 Stimulates Translation Initiation by Mediating 40S-binding of eIF3 *via* its Direct Contact with the eIF3a/TIF32 Subunit. *PLoS One*, **7**, e40464.

Kouba, T., Rutkai, E., Karasková, M., and **Valášek, L.S.*** (2012) The eIF3c/NIP1 PCI Domain Interacts with RNA and RACK1/ASC1 and Promotes Assembly of Translation Pre-initiation Complexes. *Nucleic Acid Res.*, **40**(6), 2683-99.

Herrmannová, A., Daujotytė, D., Yang, J-C., Cuchalová, L., Gorrec, F., Wagner, S., Dányi, I., Lukavsky, P.J.*, and **Valášek, L.S.*** (2012) Structural Analysis of an eIF3 Subcomplex Reveals Conserved Interactions Required for a Stable and Proper Translation Pre-Initiation Complex Assembly. *Nucleic Acid Res.*, **40**(5**)**, 2294-311.

Munzarová, V., Pánek, J., Gunišová, S., Danyi, I., Szamecz, B. and **Valášek, L.S.*** (2011) Translation Reinitiation Relies on the Interaction Between eIF3a/TIF32 and Progressively Folded cis-acting mRNA Elements Preceding Short uORFs. *PLoS Genet.*, **7**, e1002137.

Mašek, T., **Valášek, L**., and Pospíšek M. (2011) Polysome analysis and RNA purification from sucrose gradients. *Methods Mol Biol*., **703**, 293-309.

Nemoto, N., Singh, C.R., Udagawa, T., Wang, S., Thorson, E., Winter, Z., Ohira, T., Ii, M., **Valášek, L.**, Brown, S.J., and Asano K. (2010) Yeast 18 S rRNA is directly involved in the ribosomal response to stringent AUG selection during translation initiation. *J Biol Chem*., **285**, 32200-12.

Cuchalová, L., Kouba, T., Herrmannová, A., Danyi, I., Chiu, W.-l. and **Valášek, L**.* (2010) The RNA Recognition Motif of Eukaryotic Translation Initiation Factor 3g (eIF3g) Is Required for Resumption of Scanning of Posttermination Ribosomes for Reinitiation on GCN4 and Together with eIF3i Stimulates Linear Scanning. *Mol Cell Biol*, **30**, 4671-86.

Chiu, W.-L., Wagner, S., Herrmannová, A., Burela, L., Zhang, F., Saini, A.K., **Valášek, L.***, and Hinnebusch, A.G.* (2010) The C-Terminal Region of Eukaryotic Translation Initiation Factor 3a (eIF3a) Promotes mRNA Recruitment, Scanning, and, Together with eIF3j and the eIF3b RNA Recognition Motif, Selection of AUG Start Codons. *Mol Cell Biol*, **30**, 4415-34.

Groušl, T., Ivanov, P., Frýdlová, I., Vašicová, P., Janda, F., Vojtová, J., Malínská, K., Malcová,I., Nováková, L., Janošková, D., **Valášek, L**., and Hašek, J. (2009) Robust heat shock induces eIF2alpha-phosphorylation-independent assembly of stress granules containing eIF3 and 40S ribosomal subunits in budding yeast S. cerevisiae. *J. Cell Sci*., **122**, 2078-88.

ElAntak, L., Wagner, S., Herrmannová, A., Janošková, M., Rutkai, E., Lukavsky, P.J.*, and **Valášek, L.*** (2010) The indispensable N-terminal half of eIF3j co-operates with its structurally conserved binding partner eIF3b-RRM in stringent AUG selection. *J. Mol. Biol.*, **396**, 1097-116.

Szamecz, B., Rutkai, E., Cuchalová, L., Munzarová, V., Herrmannová, A., Nielsen, K.H., Burela, L., Hinnebusch, A.G., and **Valášek, L.*** (2008) eIF3a cooperates with sequences 5' of uORF1 to promote resumption of scanning by post-termination ribosomes for reinitiation on *GCN4* mRNA. *Genes & Dev*, **22**, 2414-25.

Nielsen, K.H.*, and **Valášek, L.*** (2007) In vivo deletion analysis of the architecture of a multiprotein complex of translation initiation factors. *Methods Enzymol*., Vol **431**, 15-32.

Valášek, L.*, Szamecz, B., Hinnebusch, A.G., and Nielsen, K.H.* (2007) In vivo stabilization of pre-initiation complexes by formaldehyde cross-linking. *Methods Enzymol*., Vol **429**, 163-183

Nielsen, K.H., **Valášek, L**., Sykes, C., Jivotovskaya, A.V., and Hinnebusch, A.G. (2006) Interaction of the RNP1 motif in PRT1 with HCR1 promotes 40S binding of eukaryotic initiation factor 3 in yeast. *Mol. Cell Biol.*, **26**, 2984-98.

Jivotovskaya, A.V., **Valášek, L**., Hinnebusch, A.G., and Nielsen, K.H. (2006) Eukaryotic translation initiation factor 3 (eIF3) and eIF2 can promote mRNA binding to 40S subunits independently of eIF4G in yeast. *Mol. Cell Biol.*, **26**, 1355-72.

Hašek, J., Peřinka, L., and **Valášek, L**., (2005) Specification of the monoclonal antibody PK1 reactivity in Chinese hamster ovary cells. *Folia Biol.,* **51**, 50-1.

Hinnebusch, A.G., Asano, K., Olsen, D.S., Phan, L., Nielsen, K.H., and **Valášek, L**. (2004) Study of Translational Control of Eukaryotic Gene Expression Using Yeast. *Ann N Y Acad Sci.,* **1038**, 60-74.

Valášek, L.*, Nielsen, K.H., Zhang, F., Hamilton, A.C., and Hinnebusch, A.G.* (2004) Interactions of eIF3 subunit NIP1/c with eIF1 and eIF5 promote pre-initiation complex assembly and regulate start codon selection. *Mol. Cell Biol.*, **24**, 9437-55.

Nielsen, K.H., Szamecz, B., **Valášek, L.**, Jivotovskaya, A., Shin, B-S., and Hinnebusch, G.H. (2004) Yeast eIF3 has critical functions downstream of 48S assembly that impact AUG recognition and *GCN4* translational control. *EMBO J.*, **23**, 1166-77.

Valášek, L., Mathew, A.A., Shin, B-S., Nielsen, K.H., Szamecz, B., and Hinnebusch, A.G. (2003) The Yeast eIF3 Subunits TIF32/a and NIP1/c and eIF5 Make Critical Connections with the 40S Ribosome in vivo. *Genes & Dev* **14,** 2534-46.

Valášek, L., Nielsen, K.H., and Hinnebusch, A.G. (2002) Direct eIF2-eIF3 Contact in the Multifactor Complex is Important for Translation Initiation in vivo. *EMBO J.,* **21**, 5886-98.

Valášek, L., Hašek, J., Nielsen, K.H., and Hinnebusch, A.G. (2001) Dual function of eIF3j/Hcr1p in processing 20S pre-rRNA and translation initiation. *J. Biol. Chem.,* **276**, 43351-60.

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Shalev, A., **Valášek, L**., Pise-Masison, C.A., Radonovich, M., Phan, L., Clayton, J., Brady, J.N., Hinnebusch, A.G, and Asano, K. (2001) *Saccharomyces cerevisiae* protein PCI8 and human protein eIF3e/Int-6 interact with eIF3 core complex by binding to cognate eIF3b subunits. *J Biol Chem*., 276, 34948-57.

Phan, L., Shoenfeld, L.W., **Valášek, L**., Nielsen, K., Olsen, D., and Hinnebusch, A.G. (2001) A subcomplex of three eIF3 subunits binds eIF1 and eIF5 and stimulates ribosome binding of mRNA and tRNA(i)Met. *EMBO J.,* **20**, 2954-65.

Asano, K., Shalev, A., Phan, L., Nielsen, K., Clayton, J., **Valášek, L**., Donahue, T. F., and Hinnebusch, A.G. (2001) Multiple roles for the C-terminal domain of eIF5 in translation initiation complex assembly and GTPase activation. *EMBO J.,* **20**, 2326-37.

Asano K., Phan, L., **Valášek, L**., Schoenfeld, L.W., Shalev, A., Clayton, J., Nielsen, K., Donahue, T.F., and Hinnebusch A.G. A Multifactor Complex of eIF1, eIF2, eIF3, eIF5, and $tRNA_i^{Met}$ Promotes Initiation Complex Assembly and Couples GTP Hydrolysis to AUG Recognition (2001) *Cold Spring Harbor Symposia on Quantitative Bilology*, Vol. **LXVI**, 403-415, CSHL Press

Hašek, J., Kovarik, P., **Valášek, L**., Malínská, K., Schneider J., Kohlwein S.D., and Ruis, H., (2000) Rpg1p, the subunit of the Saccharomyces cerevisiae eIF3 core complex, is a microtubule-interacting protein. *Cell Motil. Cytoskel.,* **45**, 235-46.

Valášek, L.*, Hašek, J., Trachsel, H., Imre, E.I., and Ruis, H. (1999) The Saccharomyces cerevisiae HCR1 gene encoding a homologue of the p35 subunit of human translation initiation factor 3 (eIF3) is a high copy suppressor of a temperature-sensitive mutation in the Rpg1p subunit of yeast eIF3. *J. Biol. Chem.,* **274**, 27567-72.

Valášek, L., Trachsel, H., Hašek, J., and Ruis, H., (1998) Rpg1, the *Saccharomyces cerevisiae* homologue of the largest subunit of mammalian translation initiation factor 3, is required for translational activity. *J. Biol. Chem.,* **273**, 21253-60.

Sabelli, P.A., Burgess, S.R., **Valášek, L**., and Shewry, P.R. (1998) Molecular cloning and characterisation of a maize cDNA for a homologue of the large subunit of the eukaryotic initiation factor 3 (eIF3). *Mol Gen Genet.,* **261**, 820-30.

Kovarik, P., Hašek, J., **Valášek, L**., and Ruis, H., (1998) *RPG1*: an essential gene of *Saccharomyces cerevisiae* encoding a 110 kDa protein required for passage through the G1 phase. *Curr. Genetics*, **33**, 100-9.

Vondrejs, V., Janderova, B, **Valášek, L**. (1996) Yeast killer toxin K1 and its exploitation in genetic manipulations. *Folia Microbiol.,* **41**, 379-93.

Vondrejs, V., **Valášek, L**. (1994) Comparison of different variants of the rhodamine test in terms of sensitivity of detection of the effect of zymocins in different strains of *Saccharomyces cerevisiae*. *Folia Microbiol.,* **39**, 4, 1, 0015-5632.

Vondrejs, V., **Valášek, L**. (1994) Comparison of the effect of zymocine K1 on protoplasts and cells of selected yeast species. *Folia Microbiol.,* **39**, 4, 1, 0015-5632.

ORGANIZATION OF MEETINGS

11/2006 co-organized "Translational Control and Non-Coding RNA“ international meeting held in Nove Hrady, the Czech Republic

11/2011 co-organized "RNA club 2011“ international meeting held in Prague, the Czech Republic

ORAL PRESENTATIONS

SESSION CHAIR

Translation initiation – canonical
eIF3 rocks: from initiation to termination and back to reinitiation
2011 Protein Synthesis and Translational Control Meeting
EMBL Heidelberg, Germany
September 2011

INVITED SPEAKER

Yeast translation begins and ends with translation initiation factor eIF3
Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch, France
October 2012

Yeast translation begins and ends with translation initiation factor eIF3
School of Biosciences, University of Kent, Kent, UK
October 2012

eIF3 rocks: from initiation to termination and back to reinitiation
The John Curtin School of Medical Research, The Australian National University, Canberra
February 2012

Ribozoomin': eIF3 promotes translation reinitiation
2012 Lorne Genome Conference, Lorne, Victoria, Australia
February 2012

eIF3 rocks: from initiation to termination and back to reinitiation
Georg-August University of Göttingen, Göttingen, Germany
January 2012

eIF3 rocks: from initiation to termination and back to reinitiation
Kužela Lectures
Comenius University, Bratislava, Slovakia
April 2011

eIF3 rocks: from initiation to termination and back to reinitiation
Masaryk University, Brno, the Czech Republic
April 2011

How does eIF3 affect the key translation initiation step in stringent selection of the AUG start codon?
IM, AS CR, Prague, the Czech Republic
December 2009

Hungry yeast – eIF3 duties in the gene-specific translational control mechanism termed reinitiation.
UMG, AS CR, Prague, the Czech Republic
December 2009

Hungry yeast - uncovering molecular details of the gene-specific translational control mechanism termed reinitiation.
University of South Bohemia
České Budějovice, the Czech Republic
May 2009

Uncovering molecular details of the gene-specific translational control mechanism termed reinitiation.
The Wellcome Trust Senior Research Fellows' Meeting
London, UK
October 2008

Uncovering molecular detail of the gene-specific translational control mechanism termed reinitiation.
MRC-LMB, Cambridge, UK
October 2008

In the heart of reinitiation – eIF3 as an unexpected key determinant.
University of Aarhus, Aarhus, Denmark
April 2007

KEYNOTE LECTURES

Ribozooming - mapping the contact points between yeast eukaryotic translation initiation factors, small ribosomal proteins and 18S RNA.
RNA Club 2005
Ceske Budejovice, the Czech Republic
October 2005

PLATFORM

Reinitiation revisited.
UMG & Max Planck workshop
UMG, AS CR, Prague, the Czech Republic
November 2007

In the heart of reinitiation – eIF3 as an unexpected key determinant.
2007 Translational Control Meeting
EMBL Heidelberg, Germany
September 2007

In the heart of reinitiation – eIF3 as an unexpected key determinant.
35th Annual Yeast Conference
Smolenice, Slovakia
April 2007

In depth study of physiological roles of TIF34/eIF3i and TIF35/eIF3g subunits of yeast eIF3 in eukaryotic translation initiation.
Howard Hughes Medical Institute Annual Meeting
Janelia Farm, VA
September 2006

Is yeast eIF3 involved in regulation of the AUG start codon recognition process?
XXII. International Conference on Yeast Genetics & Molecular Biology
Bratislava, Slovakia
August 2005

Regulation of the AUG start codon recognition process.
The Cytoskeleton Club 2005
Nove Mesto na Morave, the Czech Republic
April 2005

Study of the Mechanism of Eukaryotic Translation Initiation Using Yeast S. cerevisiae as a Model System.
Institute of Microbiology ASCR
Prague, the Czech Republic
October 2004

The Yeast eIF3 Subunits TIF32/a and NIP1/c and eIF5 Make Critical Connections with eIF2 and the 40S Ribosome in vivo.
BZH, University of Heidelberg
Heidelberg, Germany
September 2003

The Yeast eIF3 Subunits TIF32/a and NIP1/c and eIF5 Make Critical Connections with eIF2 and the 40S Ribosome in vivo.
EMBL, Heidelberg, Germany
February 2003

The TIF32/a and NIP1/c subunits of yeast eIF3 make critical connections with eIF2 and the 40S ribosome in vivo.
2002 Translational Control Meeting
Cold Spring Harbor, New York
September 2002

Insights into the eukaryotic translation Initiation Factor 3 (eIF3) interactions.
Washington Area Yeast Meeting
Bethesda, Maryland
November 2001