Georg Stoecklin

Posttranscriptional Control of Gene Expression

Introduction

In July 2006 the Helmholtz Young Investigator Group "Posttranscriptional Control of Gene Expression" was established at the DKFZ. Our aim is to understand how the expression of genes is regulated at the posttranscriptional level by mechanisms which control the translation efficiency and degradation rate of the mRNA. We focus on a group of unstable mRNAs containing AU-rich elements and investigate RNA-binding proteins that regulate the stability of these mRNAs. In addition we study how mRNA translation and degradation are coordinated by the sequestration of mRNAs in cytoplasmic processing bodies. We use genetic, biochemical, and cell biology approaches to examine these processes in macrophages and other mammalian cell lines.

Regulation of mRNA decay mediated by AU-rich elements

H. Sandler

The expression of several cytokines in the immune system is under control of the RNA-binding protein TTP. TTP binds to an AU-rich element (ARE) located in many cytokine mRNAs and induces rapid degradation of the mRNA (Fig. 1). Stimulation of immune cells causes activation of the p38-MAPK – MK2 cascade, which is required for stabilization of ARE-containing mRNAs. Our previous work has shown that TTP is an important target of MK2. Phosphorylation of TTP at serine 52 and 178 leads to binding of 14-3-3 adaptor proteins and reduces the activity of TTP. As a consequence, cytokine mRNAs are stabilized and cytokines are efficiently produced. The phosphatase PP2A acts as an antagonist of MK2 by dephosphorylating TTP, which causes degradation of cytokine mRNAs and inhibits cytokine production.

Our current goal is to purify proteins which interact with TTP in a phosphorylation-dependent manner. We have generated stable Hela cell lines expressing tetracycline-inducible TAP-tagged TTP. One line expresses wild-type TTP, and a second line expresses the non-phosphorylatable mutant TTP-AA. This mutant is constitutively active as it cannot be inhibited by MK2. We are now in the process of purifying proteins associated with both forms of TTP.

Georg Stoecklin

g.stoecklin@dkfz.de

1997	Dr. med., University of Basel, Switzerland
2000	Dr. phil. II, Biology, University of Basel, Switzerland
2000-2001	Postdoctoral Fellow at the University of Basel, Switzerland
2002-2004	Postdoctoral Fellow at Brigham and Women's Hospital, Boston, USA
2004-2006	Instructor at Harvard Medical School, Boston, USA
2006-present	Helmholtz Young Investigator, DKFZ

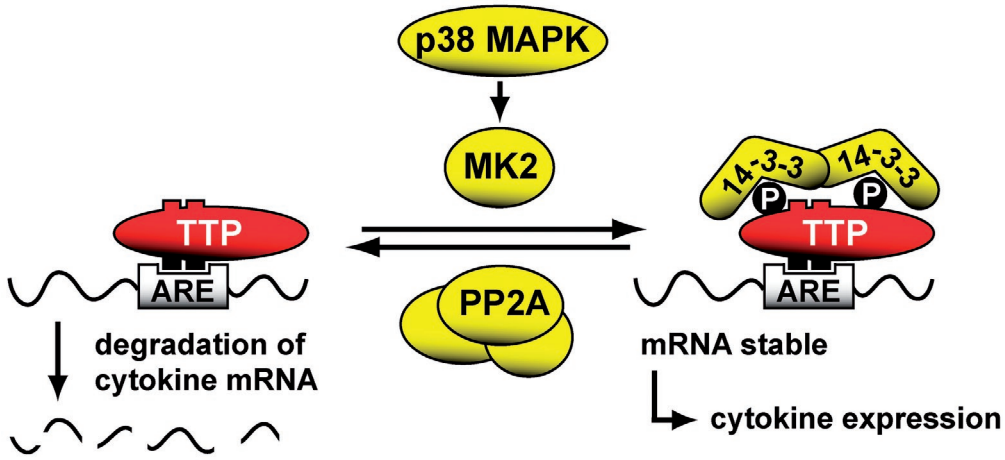

***Fig. 1: Regulation of cytokine mRNA decay by TTP**. In the unphosphorylated state, TTP binds to the ARE and promotes rapid degradation of cytokine mRNAs. Immune cell activation causes phosphorylation of TTP by MK2. As a consequence, 14-3-3 adaptor proteins bind to TTP, which leads to stabilization of the ARE-containing cytokine mRNA and efficient cytokine production. The phosphatase PP2A acts as an antagonist of MK2 by dephosphorylating TTP and activating mRNA decay.*

Genomic screen for mRNA decay factors in Drosophila cells

M. Spasic

In order to identify novel factors that are required for ARE-mediated mRNA decay, we are setting up a genome-wide screen using cultured Drosophila cells. To this end we have generated firefly luciferase reporter constructs. Introduction of the ARE from mouse interleukin-3 leads to a 6-10 fold reduction in luciferase expression. By knocking down Tis11, the Drosophila ortholog of TTP, expression of the luciferase-ARE reporter is restored to 80% of the level observed in the absence of the ARE. This indicates that the mechanism of ARE-mediated mRNA decay is well conserved in Drosophila cells. Our current work focuses on optimizing the reporter system. The genome-wide screen will be conducted in collaboration with M. Boutros (DKFZ) using a library of 14'000 dsRNAs.

Control of mRNA translation and decay in processing bodies

S. Özgür

The yeast protein Pat1 acts both as an inhibitor of translation and an activator of mRNA decapping. By homology searches we have identified a possible ortholog of Pat1 in humans. The putative human (h)Pat1 ortholog localizes in processing bodies (Fig. 2). Processing bodies are cytoplasmic foci which contain mRNAs that are translationally silenced and subject to degradation. In agreement with its localization, we found that hPat1 interacts with various proteins known to reside in processing bodies including the helicase Rck, the activator of decapping Helds, the exoribonuclease Xrn1, and the scaffold protein Lsm1. We are currently investigating whether hPat1 serves as a regulator of translation and/or mRNA decay in human cells.

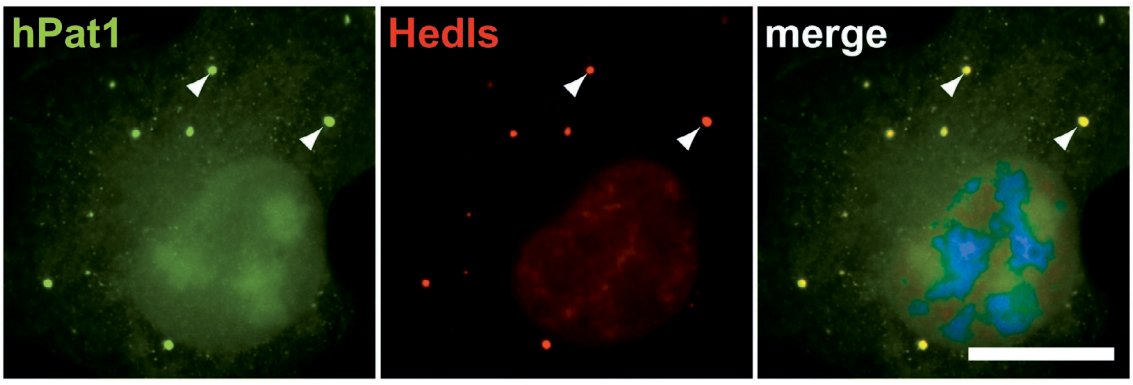

***Fig. 2: Human Pat1 localizes to processing bodies.** COS7 cells were transiently transfected with HA-tagged human Pat1, fixed and processed for immunofluorescence microscopy. Pat1 was stained in green with anti-HA antibody, and processing-bodies were counterstained in red with an antibody against the decapping enhancer Hedls. DNA was stained in blue with Hoechst dye. Arrowheads indicate processing-bodies, the scale bar is 10 µm.*

Collaborations

We would like to thank our collaborators S. Tenenbaum (Albany, NY, USA), T. Shanley (Ann Arbor, MI, USA), J. Lykke-Andersen (Boulder, CO, USA), W. Filipowicz (FMI, Basel), R. Bartenschlager (Univ. Heidelberg), M. Boutros (DKFZ, Heidelberg), P. Lichter (DKFZ, Heidelberg), C. Clayton (ZMBH, Heidelberg), T. Ruppert (ZMBH, Heidelberg).

External funding

Our current research is supported by a Helmholtz Young Investigator grant.

PUBLICATIONS 2005 - 2008

Schwede, A., L. Ellis, J. Luther, M. Carrington, G. Stoecklin and C. Clayton. A role for Caf1 in mRNA deadenylation and decay in trypanosomes and human cells. Nucl Acids Res: in press.

Stoecklin, G., S. A. Tenenbaum, T. Mayo, S. V. Chittur, A. D. George, T. E. Baroni, P. J. Blackshear and P. Anderson. Genome-wide analysis identifies interleukin-10 mRNA as target of tristetraprolin. J Biol Chem: in press.

Sun, L., G. Stoecklin, S. Van Way, V. Hinkovska-Galcheva, R. F. Guo, P. Anderson and T. P. Shanley (2007). Tristetraprolin (TTP)-14-3-3 complex formation protects TTP from dephosphorylation by protein phosphatase 2a and stabilizes tumor necrosis factor-alpha mRNA. J Biol Chem 282(6): 3766-3777.

Yamasaki, S., G. Stoecklin, N. Kedersha, M. Simarro and P. Anderson (2007). T-cell intracellular antigen-1 (TIA-1)-induced translational silencing promotes the decay of selected mRNAs. J Biol Chem 282(41): 30070-30077.

Stoecklin, G. and P. Anderson (2007). In a tight spot: ARE-mRNAs at processing bodies. Genes Dev 21(6): 627-631.

Stoecklin, G., T. Mayo and P. Anderson (2006). ARE-mRNA degradation requires the 5'-3' decay pathway. EMBO Rep 7(1): 72-77.

Benjamin, D., M. Colombi, G. Stoecklin and C. Moroni (2006). A GFP-based assay for monitoring post-transcriptional regulation of ARE-mRNA turnover. Mol Biosyst 2(11): 561-567.

Newbury, S. F., O. Muhlemann and G. Stoecklin (2006). Turnover in the Alps: an mRNA perspective. Workshops on mechanisms and regulation of mRNA turnover. EMBO Rep 7(2): 143-148.

Stoecklin, G. and P. Anderson (2006). Posttranscriptional mechanisms regulating the inflammatory response. Adv Immunol 89: 1-37.

Kedersha, N., G. Stoecklin, M. Ayodele, P. Yacono, J. Lykke-Andersen, M. J. Fitzler, D. Scheuner, R. J. Kaufman, D. E. Golan and P. Anderson (2005). Stress granules and processing bodies are dynamically linked sites of mRNP remodeling. J. Cell Biol. 169(6): 871-884.

THESES

Diploma

Simon Schäfer (2008): Analysis of the 3' untranslated region of interleukin-2 and leukemia inhibitory factor.

Contact:

DKFZ-ZMBH Alliance
Im Neuenheimer Feld 280
69120 Heidelberg, Germany
Tel: +49 6221 546887
Fax: +49 6221 545891
g.stoecklin@dkfz.de
http://www.dkfz.de/en/posttrans-genreg/
http://www.zmbh.uni-heidelberg.de/Stoecklin/

STOECKLIN GROUP (approx. 6 members in steady-state)

Group Leader
Stöcklin, Georg, Dr.

Postdoctoral Fellow
Spasic, Milan, Dr.

PhD Student
Sandler, Heike

Masters and Diploma Students
Schäfer, Simon
Özgür, Sevim

Techn. Assistant
Kreth, Jochen

*only part of the time reported