PROTEIN:PROTEIN-INTERACTION ANALYSIS

Groth et al., 2007 and Jasencakova et al., 2010

Protein:protein-interactions (PPI) govern almost all important processes in living organisms. Thus, their rapid and accurate determination and investigation is a major challenge in life sciences. The extremely efficient and fast One-STrEP (Twin-Strep) system is suitable for the isolation of functional protein complexes and subsequent mass spectrometry analysis leads to the identification of protein complex components (Junttila et al., 2005) as it is shown in the following studies.

Groth et al., 2007, analysed DNA replication in eukaryotes. In this context they isolated and characterized *in vivo* a complex in which the human histone chaperone Asf1 and MCM2-7, the putative replicase helicase, are connected through a histone H3-H4 bridge. One-STrEP (Twin-Strep)-tagged Asf1 (e-Asf1) was expressed in asynchronous HeLa S3 cells and the nuclear extract was used for the purification of the e-Asf1 complex via *Strep*-Tactin® Superflow®.

Mass spectrometry and Western blotting revealed the presence of Mcm2, 4, 6, and 7 in the nuclear e-Asf1 (a and b) complexes, together with histone H3 and H4 (Figure 1). In a second study Jasencakova et al., 2010, isolated e-Asf1 complexes from HeLa S3 cells synchronized in mid-S phase before and after treatment with the replication inhibitor hydroxyurea. In contrast to Groth et al., 2007 they used larger amounts of starting material and modified the washing conditions during the purification process. Applying this approach, they got more interactors and were not only able to identify MCM2-7, H3 and H4 as e-Asf1 complex components but also the additional interacting chaperones NASP and RbAp46/48 in the nuclear extract (Figure 2). Analysis by size-exclusion chromatography revealed that chromatin-associated Asf1 (a and b) are part of two separate nuclear complexes, a larger complex with MCM6 and a smaller one containing NASP.

Due to its high efficiency and specificity the One-STrEP (Twin-Strep) system is a reliable tool to isolate protein complexes in a simple and short one-step purification procedure. In combination with mass spectrometry it allows the identification of new protein interaction partners.

Supplemental analysis could provide the facility to identify different complexes of one and the same bait protein.

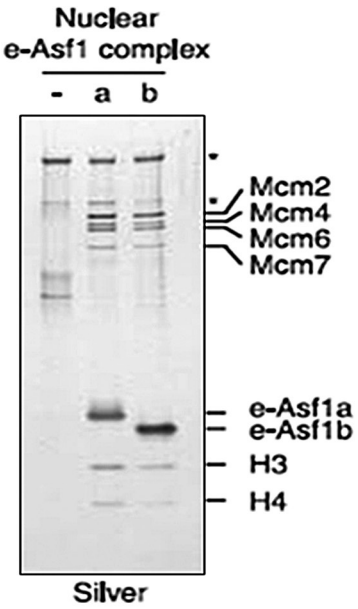

Figure 1: Purification analysis of a human Asf1-(H3-H4)-MCM2-7 complex by silver staining. Control extracts without e-Asf1 (-) was included to identify unspecific proteins (asterisks). From *`**Regulation of Replication Fork Progression Through Histone Supply and Demand**´. A. Groth, A. Corpet, A. J. L. Cook, D. Roche, J. Bartek, J. Lukas, and G. Almouzni. Science 21 Dec. 2007: 318 (5858), 1928-1931. Reprinted with permission from AAAS.*

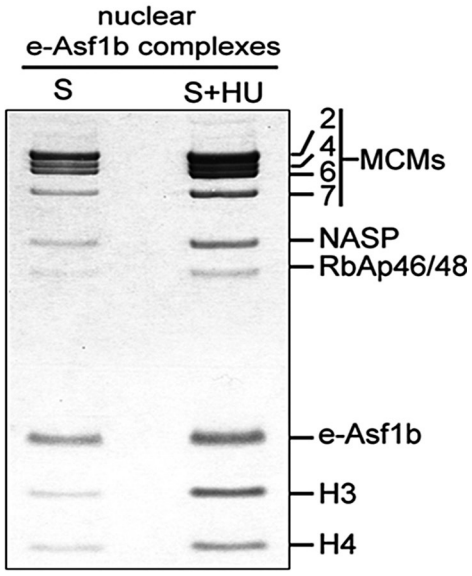

Figure 2: Coomassie staining of e-Asf1b complexes. Cells were harvested in mid-S phase (S) or after 1.5 h hydroxyurea treatment (S+HU) for complex purification. Mass spectrometry identified the indicated proteins.

(Kindly provided by Zuzana Jasencakova, Biotech Research and Innovation Centre, University of Copenhagen)

"We chose the One-STrEP (Twin-Strep)-tag for the following reasons:

*The One-STrEP (Twin-Strep) system provides a reliable, efficient and **rapid one-step purification** method useful for **isolation of protein complexes** from stable human cell lines. It is compatible with high stringency washing to obtain complexes of **high purity** and it is reasonably **low cost** compared to other systems*
."

Anja Groth, Biotech Research and Innovation Centre, University of Copenhagen

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3. Junttila MR, Saarinen S, Schmidt T, Kast J, Westermarck J, 2005: *Proteomics* **5**: 1199-1203. Single-step *Strep*-tag® purification for the isolation and identification of protein complexes from mammalian cells.

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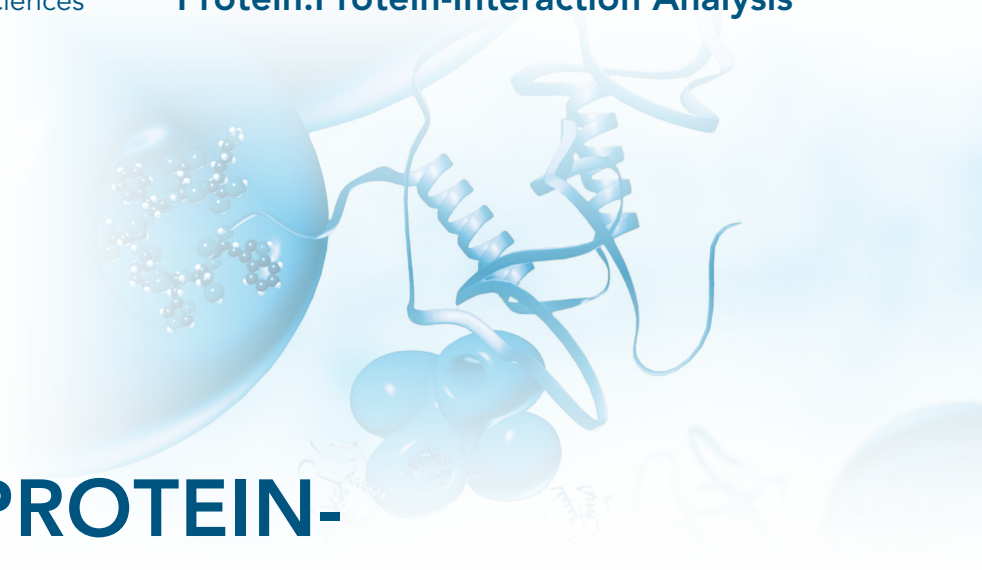

PROTEIN:PROTEIN-INTERACTION ANALYSIS

Johansen et al., 2008

Protein:protein-interactions (PPI) govern almost all important processes in living organisms. Thus, their rapid and accurate determination and investigation is a major challenge in life sciences.

The One-STrEP (Twin-Strep)-tag is a reliable tool to isolate protein complexes at high purity without losing transient binders. The advantages of the One-STrEP (Twin-Strep) system in comparison to the commonly used TAP system are that only one tag is needed and the isolation of the protein complex components is carried out in one step. An One-STrEP (Twin-Strep)-tagged bait protein is expressed in the target cell and the cell lysate containing the bait and the putatively interacting preys is subjected to tag-based affinity chromatography on *Strep*-Tactin®. The isolated protein complexes are analyzed by SDS-PAGE and potential preys are identified by mass spectrometry (Rigaut et al., 1999). In several cases the One-STrEP (Twin-Strep)-tag was already successfully applied to isolate and identify bait proteins and its interaction partners (see below for references). Johansen et al., 2008, screened for interaction partners of the IκB-kinase-associated protein, IKAP, which is involved in the neurodegenerative disease familial dysautonomia. "Previous studies of proteins that might associate with IKAP revealed no candidates that would fit with both its cytosolic localization and function in cell migration. Therefore we decided to use the One-STrEP (Twin-Strep)-tag, since it seems to be an excellent tag for the identification of even weakly-binding interactors" (Tuula Kallunki, personal comment). The One-STrEP (Twin-Strep)-tagged C-terminus of IKAP (cIKAP-strep) was used as bait and a total of 15 proteins were identified to associate specifically with cIKAP-strep (Figure 1). Two of the potential interactors (filamin A and dynein heavy chain) had been linked to neuronal migration in previous studies, what coincides with one of the functions IKAP is involved. Further analysis of IKAP and filamin A revealed their co-localization and verified their association. This example is demonstrating the effectiveness of the One-STrEP (Twin-Strep) system purifying protein complexes in combination with protein identification via MS and adjacent functional analysis.

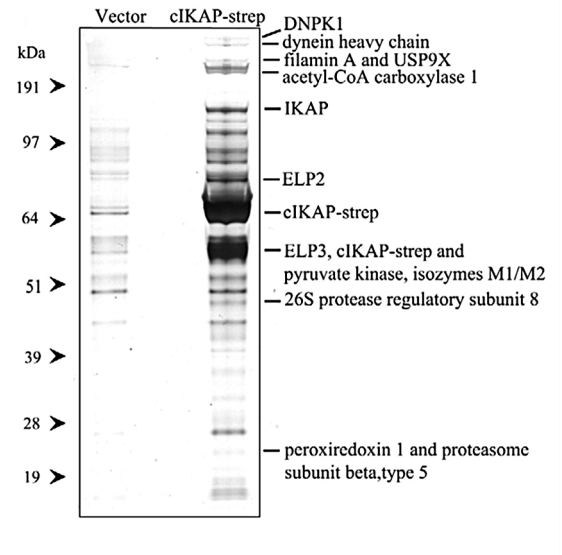

Figure 1: IKAP co-purifies with various cytosolic proteins. One-STrEP (Twin-Strep)-tag purification. HEK293 cells transiently transfected with either empty vector or cIKAP-strep were harvested 2 days after transfection and cytosolic extracts were prepared as described in the Materials and Methods. Purification was performed according to the manufacture´s instructions (IBA). Eluates were concentrated and run on 10% SDS-PAGE. Silver staining of the purified proteins. The indicated proteins were identified by MALDI-TOF-MS. DNPK1, DNA-dependent protein kinase; USP9X, ubiquitin-specific processing protease.

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