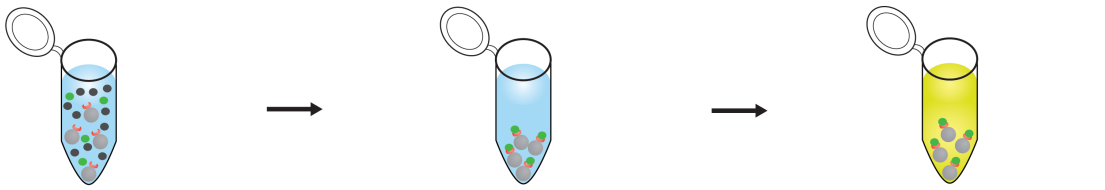
One step purification of GFP-tagged enzymes for enzymatic activity test



1. incubate cell lysate with GFP-Trap® beads
2. remove unbound material and isolate enzyme-GFP-Trap® complex
3. test enzyme activity

Background

Expression of fluorescent fusion proteins in cells is a common method. However, a critical question is whether the fluorescent fusion proteins expressed in cells are active and have properties similar to their endogenous counterparts. Using the GFP-Trap®, fluorescent fusion proteins can be efficiently pulled out from cellular extracts for further evaluation in biochemical studies. DNA methyltransferase 1 (DNMT1) is the most abundant DNA methyltransferase in mammalian cells. In our experiment we analyzed the enzymatic activity of GFP-DNA methyltransferase 1 (DNMT1) bound to GFP-Trap® beads and compared the activity of the fluorescent fusion protein to an enzymatically inactive version of DNMT1 and recombinant DNMT1 (1).

Materials and Methods

GFP-DNMT1 or GFP-DNMT1 (P1229W), a catalytically inactive version of DNMT1, was expressed in HEK293T cells. GFP fusion proteins were immunoprecipitated from cell extracts using the GFP-Trap® as described (1). After pull down, GFP-Trap® A beads binding GFP-DNMT1 were washed extensively in dilution buffer containing 300 mM NaCl and afterwards twice in 500 ml assay buffer (100 mM KCl, 10 mM Tris pH 7,6, 1 mM EDTA, 1 mM DTT). Amount of immunoprecipitated GFP-DNMT1 bound to GFP-Trap® beads were analysed by SDS-PAGE followed by Coomassie staining. 30 µl of methylation mixture (0,1 mCi of S-(^{3}H)adensoylmethionine (Amersham Biosciences, 1.67 pmol/ml hemimethylated double-stranded 35-bp DNA (50 pmol/ml), 160 ng/ml BSA) were added to 4 µg GFP-DNMT1 bound to beads. As a positive control 2 µg of recombinant purified DNMT1 was used. Incubation was carried out for 2,5 h at 37 °C, and the reactions were spotted onto DE81 cellulose filters. Subsequently, filters were washed three times with 0,2 M $(NH_4)HCO_3$, once with 100% EtOH. After drying at 80°C the filter was transferred into a Mini-Poly-Q vial with 5 ml of Ultima Gold LSC mixture (PerkinElmer Life Sciences) and each sample was measured for 1 min in a scintillation counter (Beckman LS1801). A sample without enzyme was used as negative control.

Results

Both GFP-DNMT1 variants were efficiently pulled out from cellular extracts. In an enzymatic activity test GFP-DNMT1 showed a specific DNA methyltransferase activity similar to purified recombinant DNMT1, proving that GFP fusion proteins retain their enzymatic activity after purification with the GFP-Trap® while bound to beads. In comparison no enzymatic activity was measured with the catalytically inactive DNMT1 variant, revealing that endogenous DNMT1 activities of mammalian cells were efficiently removed by this quick one step purification (Fig1).

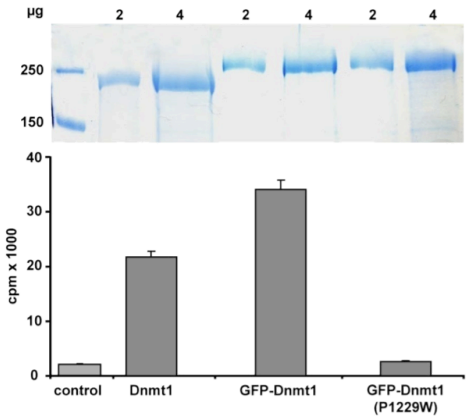


Fig. 1 Soluble protein extracts containing GFP-DNMT1 or GFP-DNMT1 (P1229W) were one step purified with GFP-Trap® beads and analyzed by SDS- Page followed by Coomassie Blue staining (Top). DNMT1 activity of purified GFP fusion proteins (~ 4µg each) and recombinant DNMT1 (~ 2 µg each) were determined (bottom). n=4; error bars show standard deviation

Conclusion

In summary, we showed that the GFP-Trap® is a powerful tool for quick and reliable biochemical characterization of fluorescent proteins. Enzymatically active fluorescent fusion proteins can be efficiently isolated with the GFP-Trap® and enzymatic assays are feasible without further purification or elution of the fluorescent fusion protein from beads.

References/relevant literature

(1) Rothbauer, U. et al. A versatile nanotrap for biochemical and functional studies with fluorescent fusion proteins. Mol Cell Proteomics 7, 282-289 (2008)

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Wang, Y., et al. Dictyostelium huntingtin controls chemotaxis and cytokinesis through the regulation of myosin II phosphorylation. Mol. Biol. Cell: 2270-2281 (2011)

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Ordering Information

Product	Quantity	Code
GFP-Trap® A	20 reactions	gta-20
GFP-Trap® A kit	20 reactions	gtak-20

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