$SNAP_f$ based pulse labeling for analysis of protein turnover in living cells

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Introduction

SNAP-tag® based pulse labeling allows for analysis of protein turnover in living cells (1-3). Since the timing of labeling of a given fusion protein is under experimental control, questions about protein trafficking, protein turnover, organelle dynamics and macromolecular assembly become open to investigation. Sensitivity and temporal resolution of such measurements are limited by reaction kinetics. An improved version of SNAP-tag has been developed, termed $SNAP_f$, that displays better reaction kinetics *in vitro* and *in vivo* (4,5).

Pulse Labeling of SNAP-CENP-A Fusion Protein

To assess $SNAP_f$-tag performance in living cells, both SNAP26m (earlier mutant version) and $SNAP_f$, along with 3 copies of a Haemagglutinin (HA) tag each, were fused to the N-terminus of Centromeric Protein A (CENP-A) (Fig. 1). This histone variant localizes to centromeres producing a unique pattern of nuclear foci (1). HeLa cells were transfected with either fusion protein and labeled with SNAP-Cell® TMR-Star (NEB#S9105) at concentrations ranging from 1-2 µM for 5-15 minutes. Excess substrate was washed away. Cells were fixed and processed for immunofluorescence to detect HA as a measure of SNAP-tag fusion protein expression level. Finally, TMR/HA ratios were obtained as a measurement of SNAP-tag activity per protein amount. $SNAP_f$ outperformed SNAP26m *in vivo* by three-fold across dye concentration and incubation times tested (Fig. 2).

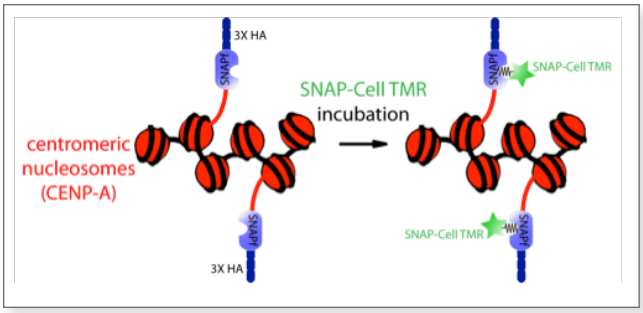

Figure 1. *A proportion of centromeric histones are N-terminally tagged with $SNAP_f$ and an HA epitope tag and incorporated into centromeric chromatin in vivo. Pulse labeling with SNAP-Cell TMR-Star results in specific $SNAP_f$ dependent fluorescence.*

General Protocol

Labeling of SNAP26m and $SNAP_f$-tag fusion proteins

1. Seed cells on a glass coverslip.
2. After 24-48 hours transfect cells with a construct expressing SNAP26m or $SNAP_f$-tag fusion proteins.
3. Incubate for 48 hours at 37°C, 5% CO_2, in a cell culture incubator.

Materials

- Cells expressing SNAP-CENP-A fusion proteins
- SNAP-Cell TMR-Star (NEB# S9105)
- HeLa cells
- DMEM medium
- Anti-HA antibody (Covance)
- 4´,6-diamidino-2-phenylindole (DAPI)
- Glass coverslips
- Phosphate buffered saline (PBS)

4. Label cells with 1-2 µM SNAP-Cell TMR-Star (NEB#S9105) diluted in pre-warmed complete medium.
5. Incubate for 5-15 minutes at 37°C, 5% CO_2, in a cell culture incubator.
6. Wash cells 2X with pre-warmed PBS, followed by incubation in fresh complete medium for 30 minutes.
7. Wash cells 2X with pre-warmed PBS.
8. Fix cells.

Immunofluorescence

9. Perform immunofluorescence using standard procedures to detect the HA-tag (Covance) or protein of interest.
10. Stain with 4´,6-diamidino-2-phenylindole (DAPI) before mounting.

Microscopy

11. Image cells using a standard wide field fluorescence microscope using appropriate filters.

Results

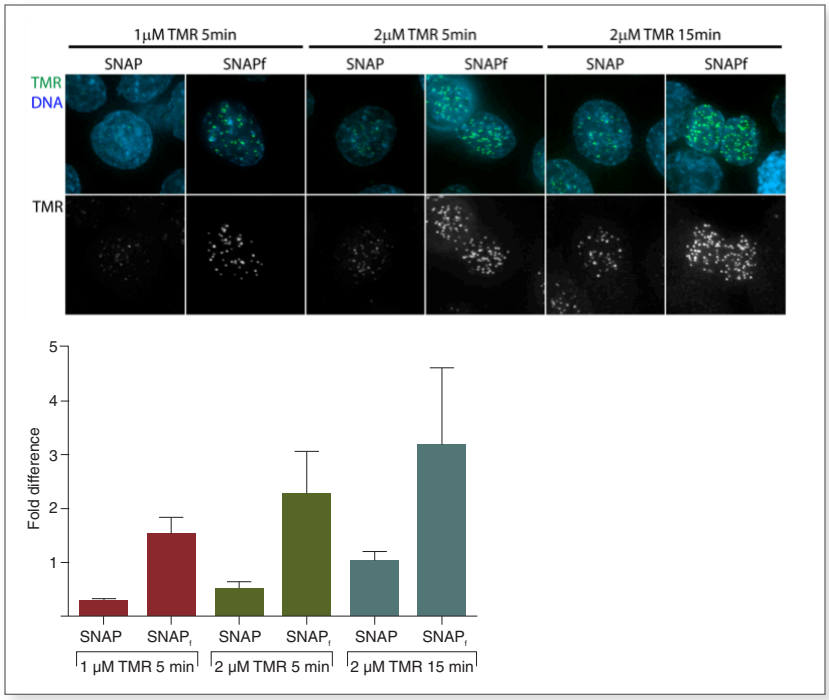

Figure 2. *HeLa cells transiently transfected with centromeric SNAP26m or $SNAP_f$ fusion proteins were labeled with SNAP-Cell TMR-Star at indicated concentrations and incubation times and processed for immunofluorescence with anti-HA. Representative images of cells are shown with SNAP-Cell TMR-Star signals in green and DAPI (DNA) in blue. TMR/HA ratios are used as a measure of $SNAP_f$ activity. Results are plotted as fold difference normalized against signals obtained after incubation with 2 µM SNAP-Cell TMR-Star for 15 minutes.*

Summary

Pulse labeling with SNAP-Cell TMR-Star results in specific $SNAP_f$ dependent fluorescence. The $SNAP_f$ displays a 3-fold better performance in living cells compared to SNAP26m. Faster $SNAP_f$ kinetics allows for reduction of substrate concentration and incubation times. This is a significant improvement that will facilitate sensitivity, better temporal resolution of turnover studies and live cell imaging of $SNAP_f$ pulse labeled cells.

References:
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