Simultaneous Labeling of Two Proteins in Live Cells

Introduction

Protein synthesis, transport, and degradation are highly dynamic processes. Expression, degradation and localization of a given protein can be rapidly modulated during a variety of cellular processes, including cell cycle progression, differentiation and apoptosis. Though there are a number of approaches for monitoring these processes indirectly in fixed cells and cell lysates, tools to study these events in real time are more limited.

SNAP- and CLIP-tags are novel tools for protein research, allowing the specific, covalent attachment of virtually any molecule to a protein of interest. The SNAP-tag® is based on mammalian O6-alkylguanine-DNA-alkyltransferase (AGT), a protein engineered to attach derivatives of benzylpurine or benzylpyrimidines, including benzylguanine (BG) to itself. CLIP-tag™ is a modified version of SNAP-tag, engineered to react with benzylcytosine (BC) rather than benzylguanine derivatives, enabling the simultaneous labeling of two proteins in live cells (Figure 1) (1,2).

Dual Protein Labeling in Live Cells

The ability to simultaneously label SNAP- and CLIP-tag fusion proteins provides researchers with a unique tool to explore protein dynamics in living cells. Figure 2 shows the concomitant labeling of two proteins in live cells: MEK1, a cytosolic protein kinase, and histone H2B, a nuclear protein. Briefly, MEK1 was fused to the C-terminus of CLIP-tag (CLIP-MEK1) and histone H2B was fused to the N-terminus of SNAP-tag (H2B-SNAP). CHO-K1 cells transfected with either or both fusion proteins then labeled with fluorescent substrates specific for SNAP-tag and CLIP-tag, SNAP-Cell™ 505 (NEB #S9103S) and CLIP-Cell™ TMR Star (NEB #S9219S), respectively. Excess substrate was washed away and cells were observed by fluorescence microscopy. CLIP-MEK1 or H2B-SNAP exhibited the expected localization to the cytosol and nucleus, respectively, demonstrating that SNAP- and CLIP-tag labeling work efficiently in live cells (Figure 2). Labeling can be performed simultaneously (Figure 2A) or sequentially (Figure 2B, 2C). This experiment shows clear and specific compartmental labeling of MEK1 and H2B, demonstrating that the orthogonal tagging systems can be applied to the simultaneous labeling of two proteins inside the same cell.
In a second example, MEK1 and ERK2, two protein kinases in the mitogen-activated protein kinase cascade that interact physically with one another, were used as a model system to examine the dynamic co-localization of two proteins in live cells. When expressed separately in mammalian cells, ERK2 is localized mostly in the nucleus while MEK1 is localized in the cytosol. However, when co-expressed in the same cell, ERK2 is sequestered to the cytosol (3). Figure 3 shows the expression and localization of the ERK2-CLIP and SNAP-MEK1 in live CHO-K1 cells. As expected, ERK2-CLIP is present in the nucleus when expressed alone, while co-expression of SNAP-MEK1 triggers the shuttling of ERK2-CLIP to the cytosol. These data illustrate that SNAP- and CLIP-tags are ideally suited to the simultaneous labeling of two proteins in live cells. Such analysis can be readily extended to a wide variety of other proteins. Furthermore, this image highlights the specificity of CLIP-Cell TMR-Star for ERK2-CLIP and SNAP-Cell 360 for MEK1-SNAP.

**Summary**

CLIP- and SNAP-tag are valuable tools for the selective labeling of proteins in live cells. As both tags have been engineered to react with distinct classes of substrates, the system can simultaneously visualize two proteins in the same cellular environment. The dynamics of their interaction, localization and stability can be readily examined following a variety of cellular stimuli.

**References:**