The HaloTag™ Interchangeable Labeling Technology

A New Cellular Imaging and Protein Analysis Tool

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The major challenge of modern Cell Biology is to identify the function and relation of the many different proteins in the context of the living cell. The ability to specifically label proteins is key to revealing the dynamics and functions of proteins in living cells. However, many conventional methods used to generate fluorescently tagged proteins and image them in their native environment are time consuming and difficult, requiring expertise in protein chemistry, the successful microinjection of labeled products into cells, and specialized techniques and instrumentation. On the other hand, de novo synthesis of proteins obtained through cloning and transfection of cells is more likely to result in native patterns of protein localization. The development of new methods of labeling proteins by genetic fusion is expanding our understanding of cellular function. In this seminar, we describe the new **HaloTag™ Interchangeable Labeling Technology**, a flexible system that enables efficient labeling of fusion proteins in living cells as well as *in vitro*

This novel fusion reporter technology provides new options for the rapid, site-specific and essentially irreversible labeling of proteins in living cells and in vitro. Current system comprises the **Halo Tag™ Vector** that encodes the Halo Tag Protein or a fusion protein of interest. The fusion protein is easily detected in live or fixed mammalian cells, in solution or on solid supports with **ligands** that bind specifically to the Halo Tag portion of the fusion protein. **The Halo Tag Ligands** that are cell permeable, can carry multiple functionalities specifically two ligands for labeling live cells with red (TMR) or green (diAcFAM) fluorescent tags. A third ligand linked to biotin allows capture of the Halo Tag™ fusion protein on solid supports such as beads. The strong covalent binding of the ligand to the HaloTag™ fusion protein allows labeling under gel denaturing conditions thus allowing the capability to label cells *in vivo* or in vitro as well as analysis of cell lysates on denaturing gels.

Unlike GFP constructs, the interchangeability of the Halo Tag ligands facilitates imaging at different wavelengths or incorporating functionalities without changing the underlying genetic construct. Examples of applications include: fixed and live cell imaging to observe cellular events such as trafficking and subcellular localization with multiple fluorescent ligands (which can be multiplexed with GFPs), post-translational events using cell to gel analysis of fusion proteins and capturing and immobilizing proteins on beads or solid support to look at protein-protein interactions. The versatility of the HaloTag technology can be seen in its use in performing Pulse Chase experiments, multiplexing with immunocytochemistry techniques and detection of protein-protein interaction as a pull-down system

This technology can readily be applied to different cell lines and organisms thereby providing a universal yet integrated approach for the study of proteins and protein function.