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Cell-surface Biotinylation Assay

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Abstract

A cell can regulate the amount of particular proteins on its cell membrane through endocytosis, following which cell surface proteins are effectively sequestered in the cytoplasm. Once within a cell, these surface proteins can be either destroyed or “recycled” back to the membrane. The cell surface biotinylation assay provides researchers with a way to study these phenomena. The technique makes use of a derivative of the small molecule biotin, which can label surface proteins and then be chemically cleaved. However, if the surface protein is endocytosed, the biotin derivative will be protected from cleavage. Thus, by analyzing the uncleaved, endocytosed biotin label, scientists can assess the amounts of internalized surface proteins.

In this video, we review the concepts behind the biotinylation assay, delving into the chemical structure of the biotin derivative and the mechanism of its cleavage. This is followed by a generalized protocol of the technique, and finally, a description of how researchers are currently using it to study the dynamics of different cell surface proteins.

Transcript

Labeling of cell surface proteins with biotin presents a powerful tool to study cellular transport pathways involved in regulating membrane proteins. A cell maintains a tightly regulated set of proteins at the surface, so that it can receive and respond to extracellular information via several signaling pathways. Endocytosis, a process of engulfing, is suggested to be involved in regulation of these cell surface proteins by causing their internalization. Therefore, labeling these proteins before they are endocytosed with agents like biotin helps scientists quantify their internalization and study their roles in cellular pathways.

In this video, we will discuss the principles and methodology of cell-surface biotinylation assays, and explore some ways in which scientists are adapting this method today.

Let’s first review the principles behind the cell-surface biotinylation assay.

As mentioned earlier, cells use endocytic pathways to regulate the spatiotemporal density and distribution of surface proteins. Internalized proteins are transported through specific cellular pathways, following which they can be either shunted to the lysosome for degradation, or recycled back to the cell surface for continued activity. Cell-surface biotinylation is designed to measure these processes.

The tag used in this assay, biotin—also known as vitamin H—is a small, water-soluble molecule. A commonly used derivative of biotin for surface labeling experiments is the sulfo-NHS-SS-biotin, which consists of the sulfo group, the N-hydroxy succinimide ester group, the disulfide bond, and of course biotin. Let’s simplify this huge structure by replacing biotin with the letter “B.”

The sulfo group in this structure imparts a strong charge that makes this form of biotin membrane impermeant. Labeling of cell surface proteins is done by adding sulfo-NHS-SS-biotin to cells maintained at a temperature that’s restrictive to endocytosis. The NHS group reacts with the primary amines on surface proteins, forming covalent bonds.

Then, the labeled cells are moved to a temperature that is permissive to endocytosis, during which some of the labeled proteins will be internalized. Finally, cells are moved back to the restrictive temperature to stop endocytosis. In order to specifically quantify internalized proteins, a hydrophilic, membrane-impermeant reducing agent—like L-glutathione—is added. This will react with the disulfide bonds on unendocytosed sulfo-NHS-SS biotin, and cleave biotin groups off. At this point, remaining biotinylated proteins are those whose labels were protected from the reducing agent because they were previously internalized.

Cells are then lysed, and the endocytosed biotinylated proteins are isolated to be quantified. Isolation is usually performed by adding cell lysates to synthetic beads coated with streptavidin. Since streptavidin has an extremely high affinity for biotin, the biotinylated proteins attach themselves to the coated beads. A series of washing steps to remove contaminating proteins, and lastly an elution step, is performed to release bound proteins. The target proteins can then be analyzed by techniques like Western blotting.

Since now you know the concepts behind the biotinylation assay, let’s look at a generalized protocol showing how to perform this procedure to measure endocytosis of surface proteins.

Start with cultured cells cooled to 4°C. Place them on ice to maintain the temperature that is restrictive to endocytosis. Next, the membrane impermeable sulfo-NHS-SS-biotin reagent is added, and cells are incubated in the dark for approximately 30 minutes. This allows sufficient time for biotin labels to covalently attach to the surface proteins. Cells are then removed from ice and incubated at 37°C for approximately 30 minutes. At this temperature, biotinylated surface proteins are endocytosed. Following incubation, the cells are cooled to 4°C, and a hydrophilic reducing agent like L-glutathione is added. This reacts with disulfide bonds and releases the biotin groups from labeled, unendocytosed proteins.

Next, cells are lysed by centrifugation, thus breaking cell membranes and exposing biotinylated proteins. Following this, lysates are added to streptavidin-coated beads and biotinylated proteins are allowed to bind. Beads are washed with cold phosphate buffered saline and eluted with a buffer containing detergents and reducing agents. These reagents denature bound proteins off beads and enable their recovery in the eluate. Proteins in the eluate are separated on the basis of their molecular mass by gel electrophoresis. Lastly, Western blotting and probing of the blot

with protein-specific antibodies allow visualization of the target protein. Percentage endocytosed protein can be quantified from the resulting band densities.

Now that you understand the methodology of cell biotinylation, let's look at how it's used in specific experiments.

The most common application of this biotinylation protocol is to measure the endocytic rate of specific cell surface proteins. Here, scientists were interested in evaluating the internalization of dopamine transporter or DAT. By following the standard protocol, scientists were able to measure the percentage of endocytosed DATs. This is a representative immunoblot showing lane T, corresponding to "total" amount of protein before internalization, lane S is for "stripped" samples where cells were never allowed to proceed through endocytosis but treated with reducing agent, and lane I represents results of "internalized" proteins.

In addition to just measuring endocytosis of surface proteins, scientists also examine the effects of drugs on this process. In this study, researchers assessed the surface density of DATs in response to treatment with an activator of protein kinase C (PKC), whose action has been suggested to induce DAT internalization. Scientists confirmed this by adapting the *in vitro* biotinylation protocol for use in an *ex vivo* assay on mouse brain tissue. The data revealed an approximately 30% loss of DAT from the cell membrane, in response to PKC activation.

Lastly, by tweaking the biotinylation assay, scientists can also measure recycling of membrane proteins. Here, researchers were investigating the surface channel protein, CFTR, responsible for conducting chloride ions. To assess recycling, researchers performed a standard biotinylation protocol in one group of cells, and modified the protocol for the second group of cells by adding steps after cleaving biotin off of the unendocytosed surface proteins. These additional steps included raising the temperature back to 37°C to allow recycling of some of the internalized, biotin tagged receptor proteins. By calculating the difference between the internalized proteins before and after recycling takes place, these scientists were able to quantify percent CFTR recycled back to the membrane.

You just watched JoVE's introduction to cell-surface biotinylation assay. The video described the procedural steps of this assay, and explained the reaction occurring at each step. Lastly, we explored some example experiments that demonstrated the applicability of this method. As always, thanks for watching!