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Selective Protein Labelling to Visualize Cellular Differentiation

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ABSTRACT

Protein post-translational modifications serve to give proteins new cellular function, spatial localization, or enzymatic activity. Myristoylation is a common post-translational modification where the enzyme N-myristoyltransferase adds myristic acid onto the N-terminus of a variety of proteins. In this work we use a myristic acid analog, 12-azidododecanoic acid (12ADA) to facilitate the implementation of azide-alkyne cycloaddition reactions on myristoylated proteins. Selective protein labeling methods such as these are useful in research because they can be used to help determine the biological function of this protein lipid modification and can be extended to study disregulated protein myristoylation in disease states. To validate 12ADA incorporation onto proteins, C2C12 myoblast cell lysates were reacted with an alkyne functionalized fluorophore and analyzed via SDS-PAGE. In order to visualize 12ADA tagged proteins *in vivo*, fixed C2C12 cells were reacted with an alkyne functionalized fluorophore and were imaged with a fluorescent microscope. The results clearly indicate selective protein tagging in *in vitro* lysates and *in vivo*. There is a distinct difference in the patterning of 12ADA protein tagging between differentiated and non-differentiated cells. The purpose of this research is to develop a selective protein labeling method. In our research, this selective protein labeling method is used to studying cellular differentiation in the context of developmental biology. Currently, there is not a clear understanding of the proteins associated with cellular differentiation related to development. Understanding this can allow scientists to track development progress and understand unique proteins associated with differentiating cells.

KEYWORDS

Post-translational modifications, myristoylation, 12-azidodecanoic acid, azide-alkyne cycloaddition, differentiation, protein localization, protein tagging, protein labeling