SITE-SPECIFIC PROTEIN UBIQUITYLATION AND SUMOYLATION USING GENETIC-CODE EXPANSION AND SORTASE-MEDIATED TRANSPEPTIDATION

Ref-Nr: TA-B78098

HINTERGRUND

The covalent attachment of ubiquitin (Ub) to target proteins represents one of the most versatile and common posttranslational modifications (PTMs) in eukaryotic cells and many fundamental cellular processes are regulated by this modification. Ubiquitylation, in which the C-terminal carboxylate of Ub is attached to a lysine in a substrate protein to form an isopeptide-bond, is naturally mediated by E1/E2/E3-enzymes. Once attached, further ubiquitins can be added either to additional lysine residues within the substrate protein or to an already attached ubiquitin via one of the seven lysines of ubiquitin itself. In a similar fashion, target proteins can also be covalently modified by ubiquitin-like-proteins (Ubls) like SUMO. Ubiquitylation and modification of target proteins with Ubls play crucial roles in a variety of cellular processes, such as protein degradation, DNA repair, nuclear transport, endocytosis, and chromosomal organization. Hence, many different human diseases, including different types of cancer and neurodegenerative diseases, are being linked to dysfunction of ubiquitylation pathways. A major obstacle consists in the generation of defined protein-Ub and protein-Ubls conjugates for subsequent biochemical analysis and consequently only a small fraction of regulatory events triggered by ubiquitylation has been studied in detail.

LÖSUNG

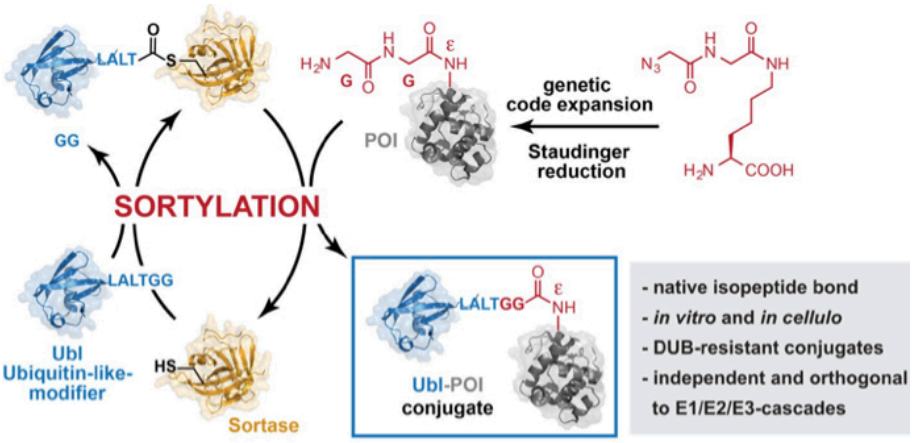
This technology provides a new approach to site-specifically modify target proteins - both in vitro and in cellulo. It combines genetic-code expansion, bioorthogonal Staudinger reduction and sortase-mediated transpeptidation to develop a general tool to ubiquitylate proteins in an inducible fashion. It can be employed to ubiquitylate and SUMOylate a target protein or to conjugate site-specifically any other ubiquitin-like protein or polypeptide to a protein of interest. It can for example also employed to attach a dye-bearing polypeptide via a peptide bond to a target protein. This new approach, which is termed sortylation, overcomes current limitations and allows the site-specific attachment of Ubls to non-refoldable, multidomain proteins. It furthermore enables inducible and E3-ligase-independent ubiquitylation of proteins in mammalian cells, providing a powerful tool to dissect the biological functions of ubiquitylation with temporal control.

Bayerische Patentallianz GmbH

089 5480177-0
kontakt@baypat.de
www.baypat.de

CATEGORIES

//Diagnostik



VORTEILE

- Incorporation of a novel unnatural amino acid that serves as a platform for sortase-mediated transpeptidation.
- Preparative in vitro ubiquitylation and SUMOylation of various target proteins.
- Transferring “Sortylation” into living cells which enables the inducible in cellulo ubiquitylation and SUMOylation of proteins.
- Building an orthogonal ubiquitylation pathway in mammalian cells.