A high throughput screening assay to identify novel SUMOylated substrates and inhibitors of SUMOylation pathways

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Abstract

Postnatal SUMOylation is a post-translational modification often linked to disease in humans. SUMO1, -2, and -3 are highly conserved in eukaryotes. Mutations in SUMO-encoding genes or the overexpression of SUMO-related proteins have been implicated in a variety of diseases. SUMOMimics are a class of artificial peptides that have been shown to play a role in post-translational modifications in eukaryotes. SUMOMimics may be used to probe the activity of SUMOylation pathways.

Introduction

SUMOylation is a post-translational modification that plays a major role in regulating protein stability and function. Unlike ubiquitination, which is a multistep process, SUMOylation is a two-step process involving E1, E2, and E3 enzymes. SUMOylation has been shown to play a role in various processes, including DNA repair, cell division, and apoptosis. SUMOylation is also involved in the regulation of protein stability and function.

Material and Methods

SUMOylation detection assay: [Use concentration of SUMOylated protein in the reaction mixture (µM)]

1. SUMO activation reaction (SUMO-activating complex: E1, UBC9, UBC13) a. Add SUMO to E1 in a stoichiometric ratio of 1:1 b. Incubate at room temperature for 2 hours c. Add ATP and incubate at room temperature for 2 hours d. Collect the SUMOylated proteins

2. SUMOylation reaction (SUMO-conjugating complex: E2, E3) a. Add SUMO to E2 in a stoichiometric ratio of 1:1 b. Incubate at room temperature for 2 hours c. Add E3 and incubate at room temperature for 2 hours d. Collect the SUMOylated proteins

3. Methods (AlphaScreen assays)

SUMOylation detection assay: [Use concentration of SUMOylated protein in the reaction mixture (µM)]

1. SUMO activation reaction (SUMO-activating complex: E1, UBC9, UBC13) a. Add SUMO to E1 in a stoichiometric ratio of 1:1 b. Incubate at room temperature for 2 hours c. Add ATP and incubate at room temperature for 2 hours d. Collect the SUMOylated proteins

2. SUMOylation reaction (SUMO-conjugating complex: E2, E3) a. Add SUMO to E2 in a stoichiometric ratio of 1:1 b. Incubate at room temperature for 2 hours c. Add E3 and incubate at room temperature for 2 hours d. Collect the SUMOylated proteins

In Summary

- A novel application of the AlphaScreen technology was developed in order to monitor the SUMOylation of RanGAP1 with SUMO-1.
- The assay did not require any protein chemical modification and the signal was strictly dependent on the presence of E1, UBC (E2), ATP, SUMO-1, and RanGAP1, confirming the specificity of the reaction.
- The signal was generated in 90% cell viability for 24 hours using 2 µM of SUMO-1 and 2 µM of RanGAP1.
- This result was demonstrated by looking at the dependence of the reaction in the presence of various concentrations of SUMO-1 and RanGAP1. For both E1 and E2, signal intensity increased up to 2 µM, however, in order to achieve better luminescence quantitation of SUMOylation throughout screening, 0.5 µM was used for the subsequent assay optimization.