SPINE: A method for the rapid detection and analysis of protein-protein interactions *in vivo*

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INTRODUCTION

Protein-protein interactions are among the few essentials that make up life. Therefore, their analysis is of prime importance to understand what's going on in living cells. The available methods allow their study *in vitro* (immunoprecipitation, affinity chromatography) or *in vivo* (two-hybrid systems, cross-links). However, most of these methods do not easily distinguish the relevant interactions from those that are non-specific. Moreover, many of the potential interactions did so far escape detection. With the transition of *B. subtilis* research from genomics to systems biology the elucidation of the full picture of protein-protein interctions is even more urgent.

THE METHOD

Scientific background

In *B. subtilis* the expression of the *gltAB* operon encoding the glutamate synthase is activated by the transcription factor GltC (1). GltC can activate the transcription of the *gltAB* operon in the presence of the preferred carbon source glucose (2). In the absence of glucose and in the presence of arginine the activator protein GltC is inhibited (3). All the genetic data indicate, that the catabolic glutamate dehydrogenase RocG inhibits the transcription factor GltC by a direct protein-protein interaction (4). We developed the Strep-protein interaction experiment (SPINE) to test this protein-protein interaction *in vivo*. This technique combines two powerful techniques (as outlined below).

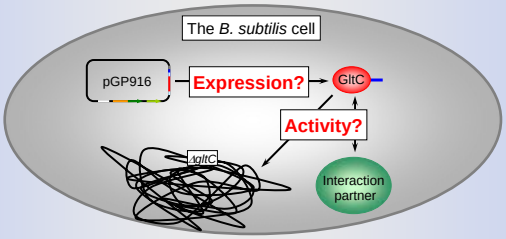
Hallmarks of the method

(i) **Expression of a protein carrying a Strep-tag (high purity in one-step purification)**

(ii) **Use of formaldehyde (FA) as a cross-linker that can be used *in vivo* and that links proteins in close proximity (~ 2 Å)**

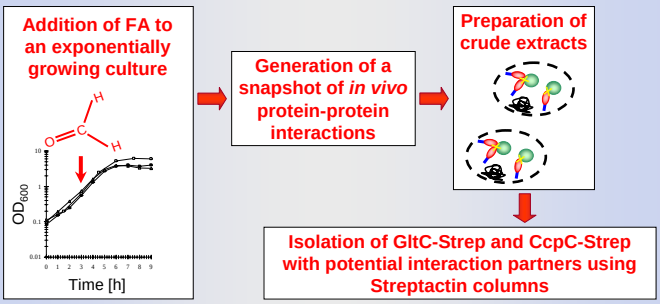
The cross-link is reversible and allows simple analysis of interaction partners (5)

1 Overexpression and functional analysis of the Strep-tagged regulator protein GltC in *B. subtilis*

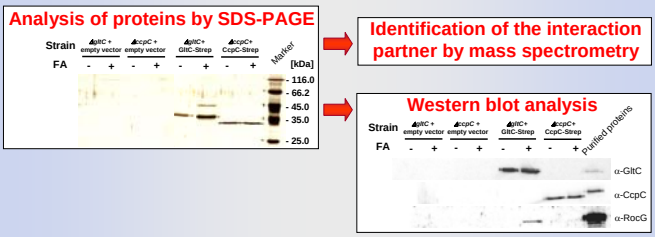


The *gltC* gene was cloned in an overexpression vector for *B. subtilis* giving plasmid pGP916. The reverse primer attached the sequence coding for the Strep-tag to the *gltC* reading frame. As a control we cloned the *ccpC* gene in a similar manner. Expression and functional activities of the C-terminal tagged proteins were demonstrated by Western blot analyses and β-galactosidase assays, respectively, using the corresponding mutant strains (4, 6).

2 Work flow to obtain cross-linked protein complexes



3 Analysis of the isolated protein complexes



The elution fractions of each purification (obtained from strains either carrying the empty vectors or expressing the regulatory proteins) were analyzed by SDS-PAGE, mass spectroscopy and Western blotting. The SDS-PAGE indicates that only one protein was co-purified with the activator protein GltC in the presence of FA. Analyses of this protein by mass spectrometry and Western blotting revealed, that this protein is RocG, as expected from genetic studies(4).

Summary and Outlook

(i) **The combined use of FA and the one step Strep-tag/Streptactin purification system allows the rapid identification of protein-protein interactions in *B. subtilis***

(ii) **For high throughput analysis of protein-protein interactions we constructed two plasmids to express proteins, carrying N-terminal and C-terminal Strep-tags, in *B. subtilis*, respectively (6)**

(iii) **Our preliminary results obtained from other interaction studies show that SPINE is well suited to study the *B. subtilis* interactome**

Acknowledgements

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References

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