Exhaustive identification of interaction domains using a high-throughput method based on two-hybrid screening and PCR-convergence: Molecular dissection of a kinetochore subunit Spc34p (*)

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The Dam1 complex, also known as DASH complex, is the outer kinetochore protein complex of yeast that plays a crucial role in attachment of kinetochore to microtubule. The Dam1 complex is formed by at least 9 proteins including Dam1p, Duo1p, Dad1p, Spc19p, and Spc34p. In this study, domains of Spc34p that physically interact with other subunits of the complex were mapped using a high-throughput methodology. The method is a combination of two-hybrid screening of a random truncation library of the Spc34 gene and a unique PCR-based amplification that converge the selected DNA fragments to a few short fragments. Duo1p, Dam1p, Dad1p and Spc19p binding domains of Spc34p were mapped on M1-E59, M1-D47, M1-D47 or T207-E295, and S154-Q294, respectively. Most of the boundaries were located at less conserved regions among fungal Spc34p homologs, which is consistent with the boundaries of the putative secondary structures. The accuracy of the mapped domain boundaries was verified using truncated Spc34p polypeptides.

The results and methodology we demonstrated herein not only shed light on the molecular architecture of the protein complex but also pave the road to the high-throughput identification of specific interaction domains of proteins whose possible interaction partners have been identified in genome-scale analyses.

Together with these results, I will show some other successful examples of domain-boundary identification. An applicability of the method for proteome analysis is also discussed in my presentation.