

2007 TAIWAN INTERNATIONAL SCIENCE FAIR

CATEGORY : Biochemistry

**PROJECT : Elucidating the functional diversity of
BCH/SE C14 domains**

AWARDS : Biochemistry First Award

SCHOOL : Hwa Chong Institution

FINALISTS : Wee Liang En, Ian

COUNTRY : Singapore

Abstract of Exhibit
Taiwan International Science Fair

CATEGORY: **Biochemistry**

TITLE: **Functional diversity of BCH/sec14 domains: implications in cell signaling and human disease**

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Introduction

BCH (**B**nip-2 and **C**dc42GAP **h**omology) domains are a novel class of protein-interacting domains. First identified in BNIP2 and in Cdc42GAP, a GTPase-activating-protein (GAP), canonical BCH domains play important and varied roles in the control of cell morphology and apoptosis induction, amongst others. However, this domain is currently classified under lipid-interacting sec14 domains due to low sequence identity (~20%).

Given this intriguing similarity, the relationship between BCH and sec14 domains is worth further study. As BCH/sec14 domains are closely associated with cell signaling and human disease, characterization of these domains and the elucidation of their functional diversity could better aid our understanding of their cellular functions and disease pathogenesis.

Purpose Of Research

The aims of this project were threefold: to classify the BCH/sec14 domain superfamily; to elucidate the functional diversity of BCH/sec14 domains; and study their role in cell signaling and human disease.

Methodology

Classifying of BCH/sec14 domains:

501 BCH/sec14 domains were aligned using ClustalW and a phylogenetic tree was drawn. Secondary/tertiary structure of BCH/sec14 domains was also compared.

Elucidating the functional diversity of BCH/sec14 domains:

Representatives of various clades were tested for protein-interacting ability *in vitro* (using GST pulldowns) and *in vivo* (using coimmunoprecipitation). Although lipid binding was not assayed due to insufficient quantities of soluble protein, conservation of lipid-binding-motifs was studied *in silico*.

Determining the role of BCH/sec14 domains in cell signaling and human disease:

Protein interactions mediated by the BCH domains of representative proteins were studied in detail; namely the RasGAP neurofibromin and the RhoGAP p50RhoGAP.

Results and Discussion

Classification of BCH/sec14 domains: While sharing some structural similarities, BCH and sec14 domains form separate groups. The BCH/sec14 domains of RhoGEFs fall between the two groups, suggesting that they possess intermediate properties.

Functional diversity of BCH/sec14 domains: *In vitro* and *in vivo* data shows that BCH, but not sec14 domains, possess distinct protein-interacting capabilities (heterophilic complex formation and recruitment of small GTPases). Domains of RhoGEFs exhibit weak protein-interacting capabilities. However, BCH domains probably retain residual lipid-binding properties, given the high conservation of an alpha-helical motif crucial for lipid-binding in sec14.

Implications for cell signaling: We present *in vivo* evidence for intramolecular interactions between the GAP and BCH domains of p50RhoGAP. BCH-domain mediated autoinhibition of GAPs may regulate control of cell signaling; we speculate that this autoinhibition may be released through ligand binding to the BCH domain.

Implications for human disease: Our *in silico* study predicted the BCH/sec14 domain of the RasGAP tumour suppressor neurofibromin (NF1) to belong to the BCH group; contrary to previous assumptions of its lipid-binding nature. Here, we present *in vivo* evidence supporting its protein-interacting role, including the recruitment of small GTPases; a finding that may be of value in elucidating the mechanism of neurofibromatosis, one of the most common autosomal dominant disorders with an incidence of 1 in 3500. We propose that mutations in the BCH domain abolish GTPase recruitment by NF1, leading to a loss of RasGAP activity and consequently tumour-suppressor function. Neurofibromin's proapoptotic effect may also be due to its protein-interaction capabilities.

Conclusion:

BCH domains form a distinct group of protein-interacting domains from sec14, mediating inter/intramolecular interactions. In particular, disruption of the protein interactions mediated by NF1-BCH may cause neurofibromatosis. Taken together, these findings offer new insights into the complex role of the BCH/sec14 domain family in cell signaling, and human disease.

Word count: 500 (excluding headings)

評語

This project elucidates differential function of BCH/SEC14 domains in proteins interaction. The experimental data led to the hypothesis that BCH domain confers autoinhibitory function for Ras-GAP, thus regulating the tumor suppressor function of NF-1. The experimental results are conclusive in support of the hypothesis.