การค้นหาโปรตีนที่มีปฏิสัมพันธ์กับโปรตีน 14-3-3 จากกุ้งขาว (*Litopenaeus vannamei*)

Identification of 14-3-3 interacting proteins from white shrimp (*Litopenaeus vannamei*)

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บทคัดย่อ

โปรตีน 14-3-3 เป็นโปรตีนที่มีบริเวณอนุรักษ์สูงและมีการแสดงออกในทุกเซลล์ของยูคาริโอต การศึกษาหน้าที่ของโปรตีน 14-3-3 จากกุ้ง โดยใช้วิธียีสต์ทูไฮบริดเพื่อหาโปรตีนที่มีปฏิสัมพันธ์กับโปรตีน 14-3-3 ไอโซฟอร์ม epsilon จาก cDNA library ของกุ้ง พบว่ามีจำนวน 10 โคลน ที่มีแนวโน้มการจับกันของโปรตีน เมื่อนำทั้ง 10 โคลน มาศึกษาลำดับเบสพบว่ามี 5 ยีนที่ทราบหน้าที่แล้ว ประกอบด้วย exosome complex component, hypothetical protein, late cornified envelope protein 2B-like, ribosomal protein S3 and elongation factor 1 alpha ส่วนยีนที่เหลือ 5 ยีนยังไม่ทราบหน้าที่ ผลการทดลองนี้เป็นข้อมูลเบื้องต้นที่จะช่วยให้เข้าใจหน้าที่ของโปรตีน 14-3-3 ในกุ้งได้ดียิ่งขึ้น อย่างไรก็ตามปฏิสัมพันธ์ระหว่างโปรตีน 14-3-3 กับโปรตีนดังกล่าวจำเป็นต้องมีการศึกษาเพิ่มเติมต่อไป

ABSTRACT

The 14-3-3 proteins are a family of conserved regulatory molecules expressed in all eukaryotic cells. In order to investigate biological function of the 14-3-3 protein in shrimp, we performed yeast two hybrid method to screen proteins from shrimp cDNA library interacting with the 14-3-3 epsilon. 10 positive clones were selected and sequenced. Among 10 positive clones, 5 coding genes with known function were obtained, including exosome complex component, hypothetical protein, late cornified envelope protein 2B-like, ribosomal protein S3 and elongation factor 1 alpha. Whereas remaining genes with unknown function were found. These finding is basic knowledge that has led us to understand the function of 14-3-3 epsilon in shrimp. However the interaction between 14-3-3 epsilon and the above-mentioned interacting proteins need to further studies.

คำสำคัญ: โปรตีน 14-3-3, ยีสต์ทูไฮบริด, กุ้งขาว

Keywords: 14-3-3 protein, yeast two hybrid, *Litopenaeus vannamei*

INTRODUCTION

14-3-3 epsilon is a member of the 14-3-3 family. 14-3-3 proteins are involved in various cellular processes. 14-3-3 protein interacting with 300 target proteins and was found to be involved in widespread biological processes such as cell cycle control, apoptosis, signal transduction, proliferation and stress responses (Aitken, 2006). Several groups have useful proteomic approaches to identified 14-3-3 partner proteins to function education. For example, 170 binding proteins of 14-3-3 family member have been identified by combined affinity column-mass spectrometry (MS) approach in HEK 293 (Jin et al., 2004). Benzinger et al. identified 117 proteins associated with 14-3-3σ in mammalian cells using tandem affinity purification (TAP) (Benzinger et al., 2005).

In shrimp, 14-3-3 epsilon containing two alternatives, designated as 14-3-3EL and 14-3-3ES was reported (Wanna et al., 2012) but the function of these proteins are not known yet. In order to determine the function of 14-3-3 epsilon, the yeast two hybrid method was used to identify proteins from a shrimp hemocyte cDNA library interacting with the 14-3-3EL and 14-3-3ES.

MATERIALS AND METHODS

1. Screening of the shrimp cDNA library

Yeast two hybrid screening was carried out with the MATCHMAKER Gal4 Two-Hybrid System 3 (CLONTECH). The bait BD-14-3-3EL and BD-14-3-3ES were used to screen 10^6 independent recombinant clones of hemocyte cDNA library in *Saccharomyces cerevisiae* strain AH109. Positive clones were selected for growth on selective media that including low stringency medium (SD/-lue/-trp), medium stringency medium (SD/-ade/-lue/-trp), high stringency medium (SD/-ade/-his/-lue/-trp). After growth in high stringency medium for 5-14 days, the yeast colonies were tested β-galectosidase assay to check for expression of *lacZ* reporter gene (blue colony). Subsequently, AD-cDNA was isolated and sequenced. After that the sequences were blasted against sequences in GenBank to analyze the function of the genes.

2. Verification of the true interaction in yeast

To verify the true interaction in yeast, the existing of two plasmids (AD-cDNA and BD-14-3-3 epsilon) were isolated from positive colonies. The yeast plasmids were used as the templates for amplify each gene by using specific primers.

RESULTS AND DISCUSSION

To identify partner protein that bind to shrimp 14-3-3 epilon, we performed a yeast two hybrid screening by using 14-3-3 epsilon as bait and cDNA library form shrimp as preys. Both plasmids were co-transformed into host cell (*Saccharomyces cerevisiae* strain AH109) and screened on selective media and then checked for expression of the *lacZ* reporter gene by x-β-gal (Table1). A total of 55 clones were obtained that fulfilled the criteria of interaction between gene products. 48 out of 55 clones interacted with 14-3-3EL, 7 clones were found interacting with 14-3-3ES. Yeast plasmids were isolated from these colonies and retransformed to *E. coli*. 10 out of 55 positive clones were successfully retransformed into *E. coli* top 10F' whereas remaining clones failed. These colonies were prescreened by PCR to make sure that only colonies with different inserts were subjected to sequencing. Using the BLAST program at the National Center for Biotechnology information, 5 sequences from 10 positive colonies had high similar to known genes and remaining genes failed to match with a homologous gene in GenBank. The data are present in the Table 2.

Table 1 Yeast two hybrid screen for protein that interact with 14-3-3 protein

sample	SD/-lue/-trp	SD/-ade/-lue/-trp	SD/-ade/-his/-lue/-trp	β- Galactosidase assay
pGBKT7+ pGADT7	+	-	-	
pGBKT7-53+ pGADT7-T	+	+	+	
BD-14-3-3EL+ AD-cDNA library	+	+	+	
BD-14-3-3ES+ AD-cDNA library	+	+	+	

-; no growth, +; good growth and β-galectosidase assay was use to verify the activation of *lacZ* by interaction between two proteins. *S. cerevisiae* AH109 cells were co-transformed with (negative) pGBKT7 + pGADT7; (positive) pGBKT7-53+ pGADT7-T; pGBKT7-14-3-3EL+ pGADT7- cDNA library; pGBKT7-14-3-3ES + pGADT7- cDNA library. Blue colonies indicate positive interaction while white colonies indicate no interaction.

Table 2 Identification proteins that interact with 14-3-3 proteins by yeast two hybrid.

Sample	Identified proteins	Organism	Accession number	Positives (%)	Expect values
14-3-3EL	exosome complex component	*Gallus gallus*	XP003640625	68	3e-69
14-3-3EL	hypothetical protein	*Daphnia pulex*	FX76334	61	1e-41
14-3-3EL	late cornified envelope protein 2B-like	*Cavia porcellus*	XP003479116	100	4e-28
14-3-3EL	ribosomal protein S3	*Solea senegalensis*	AB291555	91	8e-130
14-3-3EL	Elongation factor 1alpha	*Litopenaeus vannamei*	AD051768	100	2e-139
14-3-3EL	unknown	-	-	-	-
14-3-3EL	unknown	-	-	-	-
14-3-3EL	unknown	-	-	-	-
14-3-3EL	unknown	-	-	-	-
14-3-3ES	unknown	-	-	-	-

Ribosomal protein and elongation factor 1alpha involed in biological processes as apoptosis (Lee et al., 2010 and Talpatra et al., 2002). Exosome complex component has revealed RNA degradation (LaCava et al., 2005). The function of hypothetical protein can predicted by domain homology searches. Late cornified envelope protein 2B-like (LCE2B) was precursors of the cornified envelope of the stratum corneum that involved forming epithelial linings (Marshall et al., 2001). The result of yeast two hybrid assay suggested that the shrimp 14-3-3 epsilon correlated with various cellular mechanism. However, it is highly important for this result to be validated by GST pull-down methods in future investigations.

CONCLUSION

14-3-3 protein is ability to bind a multitude of functional protein and functional of 14-3-3 protein are dependent partner protein. In this study, we found that the shrimp 14-3-3 involved in various cellular mechanism. This finding could help us to understand the function of 14-3-3 epsilon in shrimp.

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