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CONCLUSIONS

1. Identification of Pm-syntenin binding proteins using yeast two hybrid screening

Six putative Pm-syntenin binding proteins were identified from yeast two-hybrid screening of the cDNA library of haemocytes of WSSV-infected shrimp and subtractive cDNA library of WSSV-infected shrimp.

1.1 Alpha-2-macroglobulin (α_2M) is composed of 181 amino acids and has 78% identity with the C-terminal receptor-binding domain of α_2M from the kuruma prawn (*Masupenaeus japonicus*).

1.2 Proteasome subunit alpha type 6 is composed of 64 amino acids and has 66% identity with the C-terminal of proteasome alpha subunit 6 from red flour beetle (*Tribolium castaneum*).

1.3 Elongation factor-1-α (EF1α) is composed of 207 amino acids and has 94% identity with the C-terminal of EF1α from the pill bug (*Armadillium vulgare*).

1.4 Elongation factor-2 (EF2) is composed of 82 amino acids and has 88% identity with the N-terminal part of EF2 from the spider crab (*Libinia emarginata*).

1.5 Lysozyme is composed of 132 amino acids and has 91% identity with the C-terminal of EF2 from the green tiger shrimp (*Penaeus simisulcatus*).

1.6 β-actin is composed of 193 amino acids and has 100% identity with the C-terminal of β-actin from the pacific white shrimp (*Litopenaeus vannamei*).

2. Pm-syntenin interaction with C-terminal of α_2M using yeast two hybrid system

2.1 Pm-syntenin interacts with 181 and 286 amino acids in the C-terminal portion of α_2M in yeast two-hybrid assay.

2.2 Pm-syntenin binds α_2M through its N-terminal 131 amino acids *in vivo* based on results from the yeast two-hybrid assay.

3. Pm-syntenin interacts with C-terminal of α_2M *in vitro* binding assay

3.1 GST-α_2M, not GST alone, was capable of co-precipitating 6xHis-syntenin using GST-pull down assay.

3.2 GST-syntenin, not GST alone, was interacted with in vitro translated ^{35}S-α_2M using GST pull down assay.

Pm-syntenin and Pm-α_2M interact both *in vivo* and *in vitro*. These results indicated that the binding could be involved in downstream activation of the signaling pathway. The physiological significance of these interactions for the invertebrate immune response remains to be determined.

4. Expression of α_2M transcripts in WSSV infected shrimp

Pm-α_2M is expressed constitutively in uninfected shrimp and inducible in WSSV infected shrimp. Pm-α_2M expression is correlated with Pm-syntenin expression in the WSSV infected shrimp.

Future works

1. Preparation of the high quality antibody of Pm-syntenin and Pm-α_2M to use for determination of the protein-protein interaction by co-immunoprecipitation technique from shrimp protein lysate.
2. The function analysis of Pm-syntenin using siRNA experiment in shrimp cell.
3. The determination of the interaction of Pm-syntenin and other Pm-syntenin binding proteins.