4. Discussion

In an attempt to identify Pm-syntenin binding proteins that could identify a role for Pm-syntenin in the cell, we performed a yeast two-hybrid screen using full length Pm-syntenin as bait to screen the cDNA library from WSSV infected shrimps. We identified a clone that is a homologue of human α_2M as a novel binding protein. Although homologues of human α_2M have been identified in numerous species, including primitive invertebrates (Sottrup-Jensen, 1989a; Starkey and Barrott, 1982; and Quigley et al., 1983, Hoffmann et al., 1999; Gudderra et al., 2002), the function of this protein remains incompletely defined. The vertebrate α_2M has been defined as a ubiquitous high molecular weight proteinase inhibitor. The mechanism of proteinase inhibition is initiated by the protease cleaving at a unique N-terminal part of α_2M and this induces a conformational change in the α_2M which physically traps the proteinase

within an interior cavity (Sottrup-Jesen et al., 1989b). Consequent cleavage of the α_2M-protease molecule at the internal thiol ester results in the exposure of the carboxy terminal receptor region and the clearance of the proteinase-α_2M complex (Tapon-Bretaudierc et al., 1985; Sottrup-Jesen et al., 1986, 2002). The binding of the receptor region to a cell-surface receptor is followed by endocytosis (Maxfield et al., 1981; Hanover et al., 1983; Yamashiro et al., 1989; Huang, 1998). The role of α_2M in immunity and the importance of regulation of proteolysis in invertebrates has been explored previously (Hergennahn, et al., 1988; Aspan et al, 1990; Bender et al., 1992; Melchior et al., 1995; Kanost, 1999; Armstrong, 2001). A purified α_2M from white shrimp (*P. vannamei*) was shown to have proteinase inhibitory properties (Gollas-Galván et al., 2003). In 2004, Rattanachai et al cloned α_2M from *M. japonicus* and demonstrated its high expression in shrimp, 7 days after feeding with peptidoglycan, an immune stimulant, and concluded that shrimp α_2M is an important molecule in the immune system.

Meanwhile, syntenin has been identified as an adaptor protein that couples various kinds of molecules in a signal transduction pathway. The binding can occur through the N-terminal or the PDZ domains of the molecule. Of particular interest to us is the recent report that syntenin regulates p53-dependent apoptosis by interacting with eIF5A and balances the regulation of eIF5A signaling to p53 for cell death or cell survival (Li et al., 2004).

Pm-syntenin is a 322 residue long protein, it was predicted to contain a tandem of PDZ domains (PDZ1 and PDZ2) preceded by an N-terminal fragment of 135 amino acids. Two putative PDZ domains were identified by an 83 residue stretch of PDZ1 amino acids followed by a stretch of another 81 PDZ2 amino acids. The yeast two-hybrid assay between Pm-syntenin that was separately cloned in a BD-vector and an AD-vector demonstrated the self interaction of the proteins through the PDZ domain.

To investigate the binding domain of Pm-syntenin to α_2M, several plasmids with different domains of PM-syntenin were constructed and used in the yeast two-hybrid assay. The N-terminal sequence spanning amino acids 1-131of Pm-syntenin binds to the 181 amino acid residue fragment of SA1 and the 286 amino acid residues of SA2. Therefore the binding site was located at the N-terminal of Pm-syntenin and the C-terminal of the α_2M-like protein. More mutants are required to map this binding site precisely.

We also used semi-quantitative RT-PCR to demonstrate that α_2M is expressed constitutively in uninfected shrimp and inducible in WSSV infected shrimp. This result indicates a correlation between the expression level of α_2M and its novel partner, Pm-syntenin, to the viral infection process.

Théry et al (2001) isolated proteins from exosomes that were purified from a growth factor-dependent dendritic cell line (DC cell line). The proteins were loaded on 10 or 15% SDS gels. All bands obtained were subjected to trypsin digestion and peptide mass mapping by MALDI-TOF mass spectrometry. This systematic proteomic approach allowed them to identify 21 new exosomal proteins that included detection of α_2M and syntenin together in dendritic cell-derived exosomes. In humans, dendritic cells (DC) are potent antigen-presenting cells (APCs) that act as initiators and modulators of the immune response against virus, microbes, tumors and self antigens. In addition to cytokines, dendritic cells produce a specific population of small membrane vesicles: exosomes with a unique molecular composition that are involved in the initiation of T-cell immunity (Théry et al., 2001; Banchereau and Steiman, 1998; Mellman et al., 1998; Lanzavecchia, 1992; Stockinger, 1992; Nelson et al., 1994).

Results from Théry's work can indicate that although α_2M has been described as an extracellular protein and syntenin as an intracellular protein, under a certain condition both can be present in the same intracellular space. Taken together with our results that provides here the first evidence of a Pm-syntenin and α_2M interaction *in vitro* and that also shows that both are

up-regulated during viral infection, we suggest that the binding could be involved in downstream activation of the signaling pathway. The physiological significance of these interaction for the invertebrate immune response remains to be determined.