1. INTRODUCTION

Fortilin or TCTP (Translationally Controlled Tumor Protein) was initially identified as a growth-related protein in mouse *Ehrlish ascites* tumor cells and erythroleukemia cells (Yenofski et al., 1983; Chitpatima et al., 1988; Boehm et al., 1989; Gross et al., 1989). The protein has also been known as P21 (Yenofsky et al., 1982; 1983), Q23 (Thomas and Luther, 1981; Thomas, 1986), P23 (Benndorf et al., 1988; Boehm et al., 1989), and IgE-dependent histamine releasing facter (HRF) (MacDonald et al., 1995). It is widely expressed among various organs, among animals and among plants (Thiele et al., 2000). A comparison of cDNA sequences has revealed a high degree of conservation among all eukaryotes investigated, suggesting that the protein is important in cellular process. Fortilin/TCTP expression is highly regulated both at the transcriptional and translation level and by a wide range of extracellular signals (Bommer et al., 2002). Fortilin/TCTP has been implicated in important cellular processes such as cell growth, cell cycle progression (Gachet et al., 1999), malignant transformation, in the protection of cells against various stress conditions (Bommer et al., 2002; Bonnet et al., 2000; Xu et al., 1999), and apoptosis (Li et al., 2001). Li et al. (2001) found that an overexpression of human-fortilin/TCTP prevents HeLa and U2OS cells from undergoing etoposide-induced apoptosis. Anti-sense depletion of human-fortilin/TCTP caused human breast cancer cell line, MCF-7, to undergo spontaneous cell death. Taken together, these findings have established a unique antiapoptotic protein. Because the amino acid sequence of human-fortilin/TCTP does not resemble that of either Bcl-2 family proteins or Inhibitor of

Apoptosis Proteins (IAPs); and because human-fortilin/TCTP specifically interacted with MCL1, an anti-apoptotic Bcl-2 family protein, the antiapoptotic function of human-fortilin/TCTP could be mediated through MCL1. Intriguingly, human-fortilin/TCTP interacted only with MCL1, but not with other Bcl-2 family member molecules suggesting that human-fortilin/TCTP might be a MCL1-specific cofactor in the regulation of apoptosis. Moreover, a human-fortilin/TCTP point-mutant failed to interact with MCL1 (human-fortilin/$TCTP_{R21A}$) and degraded far more quickly than was wild type human-fortilin/TCTP *in vivo*. These data suggested that human-fortilin/TCTP binds MCL1 (Zhang et al., 2002a).

White spot syndrome virus (WSSV) was the most serious viral disease of farmed penaeid shrimp in the past decade (Lightner, 1996; Zhang et al., 2002b). Due to the extremely strong virulence, once introduced the viruses spread rapidly, and also infected other species of aquatic organisms including crabs and crayfish (Chen et al., 1997; Flegel, 1997; Lo et al., 1996a). It has been suggested that apoptosis induced by WSSV may be a part of the pathophysiology leading to shrimp death (Flegel and Pasharawipas, 1998; Flegel, 2001), as has been shown in *Penaeus monodon* infected with yellow-head virus (Khanobdee et al., 2002). Because shrimp lack a humoral immune response, apoptosis may be a primitive viral defence response and may provide a mechanism for preventing viral replication in host cells. Sahtoul et al. (2001) reported TdT-mediated dUTP nick-end labeling (TUNEL)-positive cells in various tissues of naturally WSSV-infected *P. monodon*. In their report, shrimp with gross signs of WSSV contained up to 40% apoptotic cells and it was suggested that apoptosis might be implicated in shrimp death. As cell death is also caused by necrosis, it is yet not clear whether the main event leading to cell death in WSSV-

infected shrimp is apoptosis or necrosis. Moreover, in naturally infected shrimp, it is not possible to determine how long they have been infected with WSSV and how soon after infection apoptosis occurs. To obtain this information, Wongprasert et al. (2003) experimentally infected *P. monodon* with WSSV and monitored the progression of necrosis and apoptosis by morphological and biochemical methods which included (1) light microscopy (LM) and transmission electron microscopy (TEM) of various tissues; (2) fluorescent LM of nuclear DNA and TdT mediated dUTP nick-end labeling (TUNEL) techniques; and (3) determination of caspase-3 activity. The data from these experiments strongly suggests that apoptosis occurs following WSSV infection in *P. monodon*.

In our previous work, we performed subtraction hybridization of mRNAs from healthy and WSSV-infected haemocyte (Bangrak et al., 2002). Several hundred positive clones were obtained. One of the clones showed a statistically significant similarity with translationally controlled tumor protein (TCTP) also known as fortilin. A similar result has been reported by Rojtinnakorn et al. (2002) in WSSV-infected shrimp by EST approach. In addition, He and colleague (2005) reported by suppression subtractive hybridization (SSH) and differential hybridization (DH) that TCTP had high expression in virus-resistant shrimp and implies TCTP plays a critical role in the defense process. Intriguingly, as WSSV-infected shrimp start to exhibit signs of serious illness and death, the messages of fortilin/TCTP showed an abrupt decrease (Bangrak et al., 2004). In addition, we found that Pm-fortilin/TCTP binds to Ca^{2+}, the same as the human counterpart does. On the basis of these observations, the gene isolated from shrimp is now designated as Pm-fortilin/TCTP.

In order to investigate the function of Pm-fortilin/TCTP gene and due to an unavailability of a good shrimp cell culture system, we choose the human cell line. The first part of this thesis involved the investigation of antiapoptotic function of Pm-fortilin/TCTP in mammalian cell culture. And the second part concentrated on the involvement of fortilin/TCTP in the apoptotic pathway by using siRNA technique to knock down the human-fortilin/TCTP gene. Results obtained from this study will allow us to have a better understanding of the function of this highly conserved molecule.