CHAPTER I

INTRODUCTION

1.1 General information

The black tiger shrimp, *Penaeus monodon* Fabricius (1798), is one of the main shrimp cultured world wide in term of production and value. The global production of *P. monodon* has increased from 21,000 MT in 1981 to 200,000 MT in 1988. Its production was sharply increased to nearly 500,000 MT in 1993 and peaked at 730,404 MT in 2003 (FAO 2013). The major producers of *P. monodon* include Thailand, Philippines, Malaysia, Indonesia, Myanmar and Viet Nam.

Thailand has been the world's leader in shrimp exports. The largest export markets for the cultured shrimp were the United States of America and Japan (Table 1.1). Shrimp farms and hatcheries are located along the coastal areas of Thailand where Nakorn Sri Thammarat and Surat Thani are the major parts of shrimp cultivation. In addition, Chanthaburi (eastern Thailand), Samut Sakhon and Samut Songkhram (central region) also significantly contribute on the country production.

The production of *P. monodon* has been dramatically decreased since the last several years. Thai shrimp farmers have faced the outbreak of diseasess. Much of decline in production of *P. monodon* can be attributed by yellow-head virus (YHV) and white spot syndrome virus (WSSV) diseases. Moreover, breeding of pond-reared *P. monodon* is difficult and rarely produced the sufficient amount and quality of larvae required by the industry (Menasveta et al., 1993; Jackson and Wang, 1998). Therefore, farming of *P.* monodon relies almost entirely on wild-caught broodstock for the seed supply.

Besides problems from diseases, the lack of high quality wild and/or domesticated broodstock of *P. monodon* has possibly caused an occurrence of a large portion of stunted shrimp at the harvest time and the culture period needs to be extended from 4 to 5 month cultivation period. Unlike *P. monodon*, the Pacific white shrimp *(Litopenaeus vannamei)* have been successfully domesticated on a commercial

Table 1.1 The exportation of the giant tiger shrimp from Thailand during 2005-2010

Country	2005		2006		2007		2008		2009		2010	
	Quantity (MT)	Value (MB)	Quantity (MT)	Value (MB)	Quantity (MT)	Value (MB)	Quantity (MT)	Value (MB)	Quantity (MT)	Value (MB)	Quantity (MT)	Value (MB)
USA	97,681	36,011	89,115	29,032	58,365	17,026	29,116	17,026	34,537	8,847	7,979	1,909
Japan	16,644	13,813	33,235	11,916	27,977	9,586	20,182	9,586	15,709	3,832	3,711	1,067
Canada	6,455	3,890	11,216	3,412	6,490	2,072	3,249	2,072	2,798	744	1,762	462
Singapore	5,251	3,138	3,317	1,258	3,383	538	1,934	538	1,580	236	401	63
Australia	4,481	1,326	4,817	1,252	2,418	1,042	2,097	1,042	1,418	446	658	225
China	1,649	352	993	215	833	163	1,003	163	711	86	1,629	236
U.Kingdom	661	211	184	64	506	182	162	182	242	71	242	73
Taiwan	4,917	1,276	3,051	799	2,965	564	1,673	564	607	170	692	194
Hong kong	1,365	533	1,438	340	1,396	410	1,027	410	921	257	1,569	366
Total	**139,104**	**60,550**	**147,406**	**86,274**	**104,331**	**31,579**	**58,521**	**31,761**	**58522**	**14,686**	**18,643**	**4,593**

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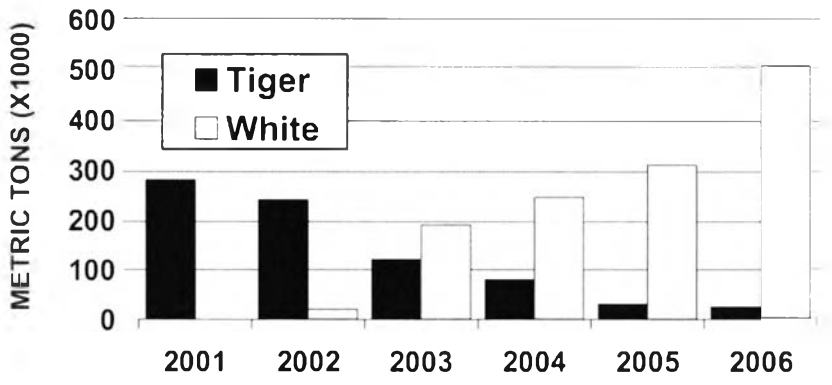

Figure 1.1 A diagram of production of *P. monodon* and *L. vannamei* during 2001-2006 in Thailand (Thai DOF, 2007).

scale (Pascual et al., 2004; Clifford and Scura, 2004; Argue et al., 2002). As a result, domesticated *L. vannamei* has been introduced to Thailand as a new cultured species and initially contributed approximately 20,000 MT of the cultured production in 2002 and dramatically increased to nearly 500,000 MT in 2006 (Figure 1.1). At present, *L. vannamei* is the main cultured species in Thailand. The production of *P. monodon* and *L. vannamei* since January to October 2012 is shown by Table 1.2.

Table 1.2 The production of *P. monodon* and *L. vannamei* since January to October in 2012 in Thailand (Thaishrimpnews, 2012)

Month	Quantity (MT)		
	P. monodon	***L. vannamei***	**Total**
January	324.80	30,524.36	30,849.16
February	370.55	30,774.27	31,144.82
March	558.90	37,174.23	37,723.13
April	1,383.52	35,869.22	37,252.74
May	1,323.54	52,337.30	53,660.84
June	716.56	50,612.34	51,328.90
July	1,016.82	45,818.82	46,835.64
August	1,182.03	33,776.19	34,958.22
September	1,729.65	29,932.64	31,662.29
October	2,411.58	39,449.51	41,861.09
Total	11,017.95	386,258.88	397,276.83

1.2 Objectives of this thesis

The objectives of this thesis are identification of single nucleotide polymorphisms (SNPs) in growth-related genes (*calponin 1, cyclin C* and *cdc25*) of *P. monodon* and determination of their relationships with growth parameters (e.g. body weight, total length, hepatopancreatic weight and hepatosomatic index, HSI). In addition, association between SNPs in these genes and their expression levels in hepatopancreas of *P. monodon* was also examined.

1.3 Biology of the black tiger shrimp (*P. monodon*)

1.3.1 Taxonomy

Penaeid shrimp are taxonomically recognized as members the largest phylum in the animal kingdom, the Arthropoda. This group of animals is characterized by the presence of paired appendages and a protective cuticle or exoskeleton that covers the whole animal. The taxonomic definition of the giant tiger shrimp, *P. monodon* is as follows (Bailey-Brook and Moss, 1992).

Phylum Arthropoda;

Subphylum Crustacea;

Class Malacostraca;

Subclass Eumalacostraca;

Order Decapoda;

Suborder Natantia;

Infraorder Penaeidea;

Superfamily Penaeoidea;

Family Penaeidae Rafinesque, 1985;

Genus Penaeus Fabricius, 1798;

Subgenus *Penaeus*.

The scientific name of this species is *Penaeus monodon* (Fabricius, 1798) and the common name is giant tiger prawn or black tiger shrimp.

1.3.2 Morphology

The external morphology of penaied shrimp is distinguished by a cephalothorax with a characteristic hard rosthum, and by a segmented abdomen (Fig. 1.2). Most organs are located in cephalothorax, while the body muscles are mainly in the abdomen. The internal morphology of penaeid shrimp is outlined by Fig. 1.3. Penaeids and other arthropods have an open circulatory system and, therefore, the blood and the blood cells are called hemolymph and hemocytes, respectively.

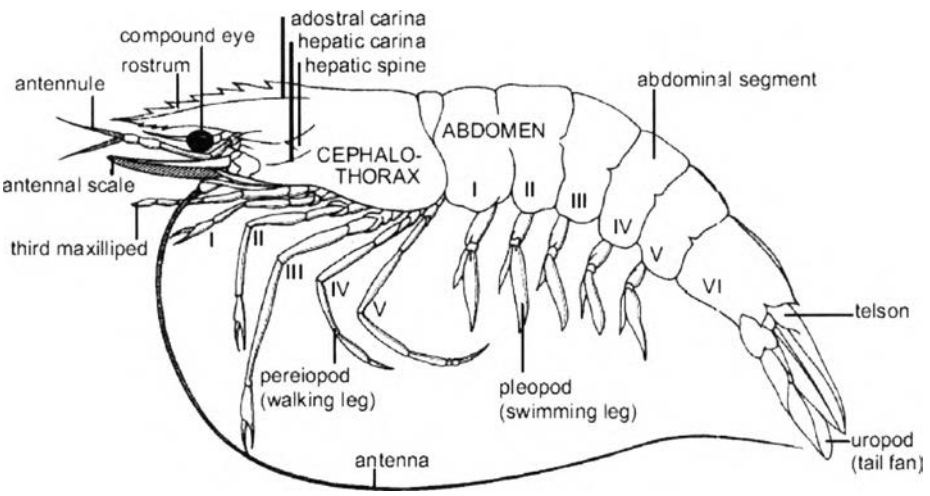

Figure 1.2 Lateral view of the external morphology of *P. monodon*. (Primavera, 1990)

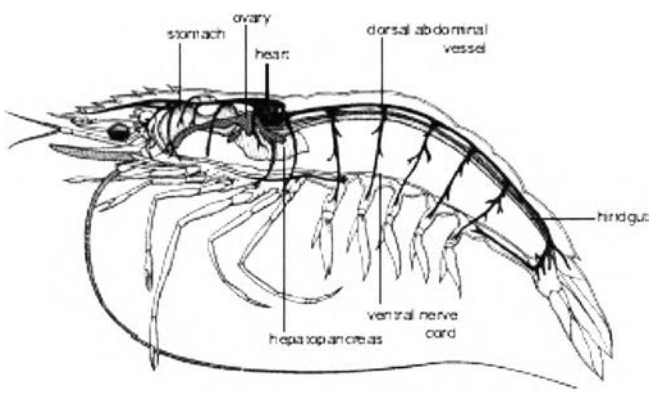

Figure 1.3 Lateral view of the internal anatomy of a female *P. monodon*. (Primavera, 1990)

1.4 Domestication and genetic improvement of aquatic animals

In aquaculture, genetic improvement through selective breeding programs is not commonly used by the industry. For many species, the aquaculture production still relies almost entirely on wild-caught broodstock owing to the lack of efficient breeding programs and difficulties to breed the cultured species in captivity. The latter is mainly due to poor reproductive performance of captive-reared broodstock, for example, in *P. monodon*.

The aquaculture production can be significantly increased through genetic improvement. However, before genetic improvement programs in the target species is successful, the cultured species need to be domesticated, the traits affecting productivity identified, breeding objectives defined and genetic parameters estimated. The advantages of domestication are increasing the accuracy of selection for desired traits, elevating production, improving disease resistance and improving the product quality.

Although there are several examples of successful breeding programs applied to aquatic species, the information about phenotypic and genetic parameters of economically important traits is rare. Therefore, the information about correlations between phenotypes and genotypes are critical for genetic improvement of economically important species. Advances in molecular genetics have introduced a new generation of molecular markers for the genetic improvement of livestock for a period of time. Molecular genetics have been used to identify several genes and markers associated with quantitative traits including genetic variation explaining phenotypic differences in growth (Salem et al., 2012). Therefore, the appropriate molecular markers for specific species need to be developed.

Molecular markers can be used to identify the genetic variation at the DNA sequences underlying phenotypes. This marker revealing the polymorphism at the DNA sequences are now key player in animal genetics, including *P. monodon*. Well-designed studies using genetic markers will undoubtedly accelerate identification of genes involved in quantitative trait loci (QTL) for marker-assisted selection (Liu and Cordes, 2004). Accordingly, appropriate mapping populations of desired traits of *P. monodon* should be generated. Fine QTL mapping should be

examined allowing traits-linked markers to be identified and used to ensure accuracy and effective selection of genetically improved shrimp. Associated studies between genotypes (e.g. SNP) and production traits for *P. monodon* must be accomplished and applied in the shrimp industry.

Selective breeding in aquaculture provide remarkable economic benefits to the industry, as it reduces production costs due to faster turnover rates. This is because of faster growth rates, decreased maintenance rates, increased energy and protein retention, and better feed efficiency (Gjedrem and Baranski, 2009). Applying such genetic improvement program to aquaculture species will increase productivity to meet the increasing demands of growing populations.

1.4.1 Domestication and selective breeding program of *P. monodon*

Domestication and selective breeding programs aim to increase commercially important traits in selected populations. Basically, short generation time and high fecundity of shrimps suggested that genetic improvement of *P. monodon* is promising. This will in turn lead to the sustainable aquaculture of *P. monodon*. (Goyard et al., 2003; Dixon et al., 2009).

The benefits of heterosis have been demonstrated in shrimp. Domestication and selective breeding programs of Thai *P. monodon* should take the advantage of population genetic differentiation (genetic differences among geographically different samples previously reported by molecular genetic markers between major stocks of *P. monodon*: the Andaman, the upper Gulf of Thailand (Trat) and the lower Gulf of Thailand (Chumphon and Surat) (Supungul et al., 2000; Klinbunga et al., 2001).

In terms of aquaculture, the establishment of appropriate domesticated stocks of *P. monodon* will require samples from different geographic locations as the founder stocks for genetic improvement through selective breeding programs. Loss of genetic variation in small populations as a consequence of genetic drift and inbreeding is commonly observed within captive stocks (Sbordoni et al., 1986). The low levels of genetic variability may reduce the mean fitness of a population, affecting its viability. Inbreeding increases homozygosity, which in some species can reduce growth, viability and reproductive performance (Hansson and Westerberg, 2002). Inbreeding has also been linked to biochemical disorders and deformities from lethal and

sub-lethal recessive alleles (Dunham, 2004). Inbreeding can be avoided if a wide genetic variation is secured in the base or founder population, and even better if parentage of animals is known without mating among relatives.

Determining the relative effects between male and female broodstock quality on reproductive performance parameters (i.e. hatching rate, number of spawning, etc.) will enhance reproductive performance of domesticated stocks. Previous studies on the reproductive performance of reciprocally crossed wild and pond-reared broodstock found that the wild females outperformed domesticated females in terms of maturation, spawning and total egg production in *P. monodon* (Menasveta et al., 1993).

In general, the effective number of founders (N_e) = 50, which is not a census number of male and female in the hatcheries, is minimally required for producing an inbreeding coefficiency of 1% per generation. This seems to be practical for fish but rather difficult for *P. monodon*. The most important limitation of selective breeding programs in *P. monodon* is the low degrees of maturation and spawning of captive *P. monodon*. Practically, as high N_e as possible should be maintained through generations. Microsatellites can be applied to identify different families reared together in the same earth-pond. Additional researches on maturation of *P. monodon* through feed technology and hormonal systems are also important to appropriately solve the problems.

1.5 Molecular markers and their used for genetic improvement of aquatic species

Molecular markers are classified into two categories: type I which are markers associated with coding sequences of genes, while type II markers are those associated with anonymous genomic segments (Liu and Cordes, 2004; Table 1.3). Various genetic markers used to evaluate DNA polymorphism in the aquaculture have been identified by a rang of molecular techniques include random amplified length polymorphic DNAs (RAPDs), amplified fragment length polymorphism (AFLPs), restriction fragment length polymorphism (RFLPs) and microsatellites etc. Nevertheless these markers are cumbersome to use in large-scale and may not be widely and evenly distributed in the genome. Alternatively, single nucleotide polymorphisms (SNPs) have several advantages over other markers. SNPs are

especially important if they cause differences in economic traits, or the mutations are linked to the phenotypes of interest. Recent developments of sequencing technologies have allowed whole genome sequencing and SNPs discovery at fast, accurate and affordable scale. This make SNP as a marker of choice for association analysis of commercially important traits in various species.

1.5.1 Single Nucleotide Polymorphisms (SNPs)

Single nucleotide polymorphism (SNPs) are one base changes including substitutions, insertion or deletion occurring in the same genomic position of the DNA segments of different individual distributed with the frequency of more than 1% in the examined populations. SNPs are becoming especially important in molecular marker development since they are the most abundant polymorphism in any organism, adaptable to automation, and reveal hidden polymorphism not detected with other markers and methods. There are four major reasons for the increasing interest in the use of SNPs as molecular markers for genetic analysis (Bruzen et al., 2000). Firstly, SNPs are prevalent and occur more often in the genome or near in any locus of interest than other types of markers. In the human genome, for instance, they are present at one SNP in every 1,000 bp. Secondly, some SNPs found within coding region, called cSNPs, are directly affected the functions of proteins. This class of SNPs may be directly responsible for some of the variations among individuals in economic traits. Thirdly, SNPs are co-dominantly inherited, make them suites as long-term selection markers. Finally, SNPs are most suitable for high throughput genotypic analysis.

SNPs are biallelic markers, indicating a specific polymorphism mostly two alleles in a position. SNPs may affect gene and/or protein functions. For instance, allelic variation due to a single nucleotide transition (cytosine [C] to thymine [T] transition that results in a non-synonymous mutation from Arg to Cys) has been demonstrated to be associated with higher *leptin* mRNA levels in adipose tissues and increased fat deposition in mature beef (Kononoff et al., 2005).

Table 1.3 Types of DNA markers, their characteristics, and potential applications. (Liu and Cordes, 2004).

Marker type	Acronym	Requires prior molecular information?	Mode of inheritance	Type	Locus under investigation	Likely allele number	Polymorphism or power	Major applications
Allozyme	-	Yes	Mendelian, Codominant	Type I	Single	2-6	Low	Linkage mapping, population studies
Mitochondrial DNA	mtDNA	No*	Maternal Inheritance	-	-	Multiple haplotypes	-	Maternal lineage
Restriction fragment length polymorphism	RFLP	Yes	Mendelian, Codominant	Type I or II	Single	2	Low	Linkage mapping
Random amplified polymorphic DNA	RAPD, AP-PCR	No	Mendelian, Dominant	Type II	Multiple	2	Intermediate	Fingerprinting for population studies, hybrid identification
Amplified fragment length polymorphism	AFLP	No	Mendelian, Dominant	Type II	Multiple	2	High	Linkage mapping, population studies
Microsatellites	SSR	Yes	Mendelian, Codominant	Mostly type II	Single	Multiple	High	Linkage mapping, population studies, paternity analysis
Expressed sequence tags	EST	Yes	Mendelian, Codominant	Type I	Single	2	Low	Linkage mapping, physical mapping, comparative mapping
Single nucleotide polymorphism	SNP	Yes	Mendelian, Codominant	Type I or II	Single	2, but up to 4	High	Linkage mapping, population studies?
Insertions/deletions	Indels	Yes	Mendelian, Codominant	Type I or II	Single	2	Low	Linkage mapping

* Conserved PCR primers can be adopted from sequence information from a related species

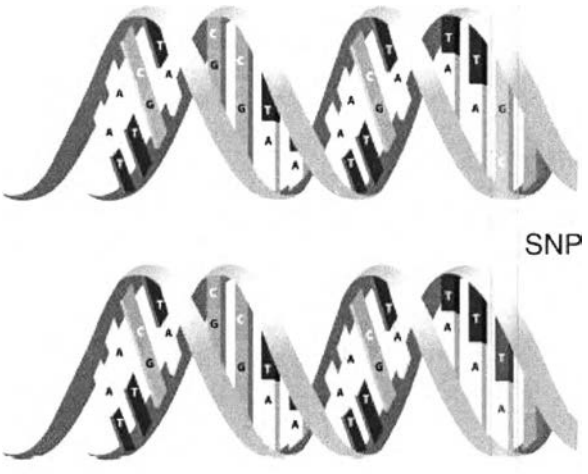

Figure 1.4 General illustration of single nucleotide polymorphism (SNP) (http://www.mdsupport.org/library/genetics.html)

1.5.1.1 Classification of SNP

SNPs are classified into two types. There are non-coding SNP and coding SNP. Coding SNP or types I SNP, located in coding sequences, that does not change amino acid sequence of the protein is called synonymous SNP. The coding SNP resulting in changes amino acid sequence of the protein is called non-synonymous SNP. Non-coding SNP or types II SNP is found outside the coding sequences composed of 5' or 3' untranslated regions, intronic region and intergenic spacers.

1.5.1.2 SNP discovery

SNP can be detected in the DNA sequences by several methods. DNA sequencing is the direct and accurate approach for SNP discovery. Alternatively, SNP can be detected by indirect methods include denaturing gradient gel electrophoresis (DGGE), single strand conformational polymorphism (SSCP), allele-specific amplification (ASA), denaturing HPLC (DHPLC), matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry and pyrosequencing. Each approach has its advantages and limitation, selection of a particular assay depends on

budget, labor constraints, high/low throughput, cutting-edge equipment and difficulty of assays (Table 1.4).

Table 1.4 A comparison of selected mutation screening methods (Shastry, 2002)

Method	Fragment length (bp)	Advantage	Disadvantage	Efficiency (%)
Single strand conformationl polymorphism	~300	No expensive equipment	Small fragments. Temperature variation	80
Heteroduplex analysis	300-600	No expensive equipment,	Conditions to be determined	80
Denaturing gradient gel electrophoresis	100-1000	Simple, long and short fragments	Gradient gel required, mutation in GC region may not be detected	100 with GC clamp
Enzymatic mismatch detection	300-1000	Long and short fragments	Identifies all kinds of mutations	100
Base excision sequence scanning	50-1000	Accurate	Expensive instruments	100
RNAase cleavage	1.6 kb	Longer fragment and rapid analysis	Requires special kit	100
Chemical cleavage	1-2 kb	Large fragment	Multi-steps, labor intensive and hazardous chemicals.	100
DNA sequencing	500	Rapid and easy, no additional sequencing	Labor intensives	100

1.6 Molecular technique used for studies for SNP in this thesis

1.6.1 Polymerase chain reaction (PCR)

The introduction of the *in vitro* amplification of DNA by polymerase chain reaction (PCR) by Kary Mullis in 1987 (Mullis et al., 1987) has become a standard technique in molecular biology. PCR is based on enzymatic replication of DNA, without using a living organism. This technique involves creating oligonucleotide primers that are complementary to sequences of the gene of interest (DNA template). The primers are designed to flank the gene sequence of interest, usually 18 to 30 nucleotides in length. Over a million copies from a low amount of starting DNA template can be obtained using specific oligonucleotide primers within a few hours.

The PCR reaction utilizes a DNA template, a pair of primers for the target sequence, dNTPs (dATP, dCTP, dGTP and dTTP), buffer and heat-stable DNA polymerase (usually *Taq* polymerase). The principle of the PCR is requiring a three-step: (1) denaturation of double-stranded DNA at high temperature, (2) annealing of primers, for which primers are attached to the dissociated DNA strands at the optimal temperature, (3) extension of the annealed primers by polymerization with a heat-stable DNA polymerase. The product synthesized in one cycle serves as a template in the next, resulting in an exponential increase of the target DNA (Figure 1.5). The cycle is repeated until the desired amount of DNA is obtained, usually about 30-40 cycles, resulting in an exponential increase of the target DNA. The amplification product is determined by agarose gel electrophoresis.

1.6.2 Single-Stranded Conformational Polymorphism Analysis

Single-stranded conformational polymorphism (SSCP) analysis was originally described by Orita et al. (1989). SSCP is a genetic screening technique that allows rapid detection of single nucleotide substitutions, deletions, insertions or single nucleotide polymorphism (SNP) in fragment of PCR-amplified genomic DNA (Lin et al., 1993; Fujioka et al., 1995). SSCP relies on the principle that the electrophoretic mobility of a single-stranded DNA molecule in a non-denaturing polyacrylamide gel (Figure 1.6). Single-stranded DNA molecules can alter the conformation are secondary and tertiary structure due to differences in sequence and consequently its electrophoretic profile. The PCR-SSCP technique includes four steps: PCR amplification using primers that flank the DNA region of interest, denaturation of the resulting double-stranded PCR product, followed by rapid chilling to prevent re-annealing of the single strands, electrophoretic separation of the single-stranded DNA on a nondenaturing gel, and bands are detected by silver staining, and the pattern is interpreted.

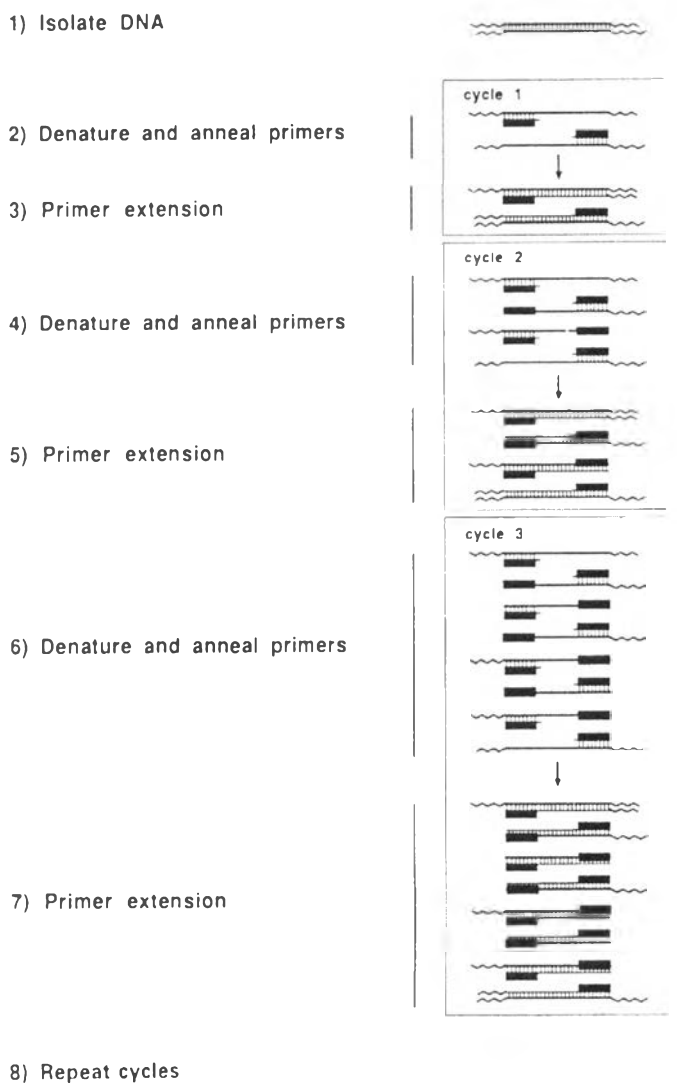

Figure 1.5 A schematic illustration of the polymerase chain reaction (PCR) for amplifying DNA.

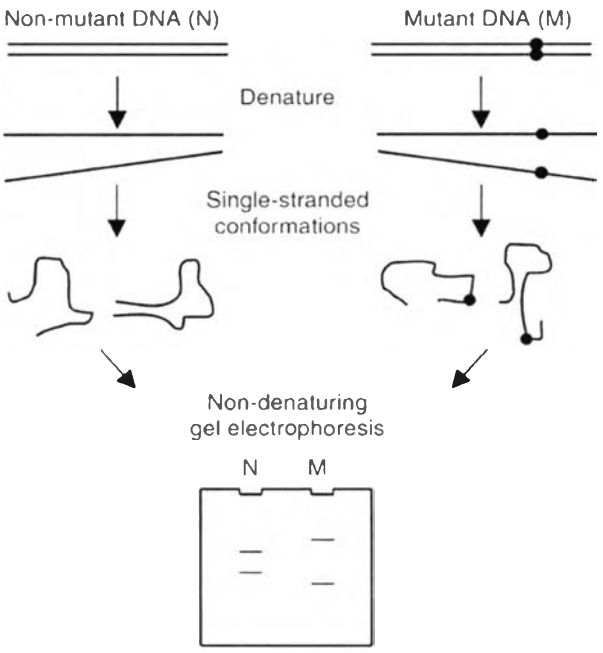

Figure 1.6 A schematic diagram of SSCP analysis for determination of polymorphism of DNA (Gasser et al., 2006).

PCR-SSCP have significant advantages over many other nucleic acid techniques for the accurate analysis of allelic and mutational sequence variation (Gasser et al., 2006). SSCP is one of the easiest and sensitive, nonradioactive methods for detecting mutations based on PCR. Monoduplex, heteroduplex and single-stranded DNA can be occasionally resolved by a different location; bottom, middle and top of the gel, respectively.

The disadvantage of the technique is that reproducibility for the separation pattern may not be highly consistent due to SSCP patterns are affected by temperature and degree of crosslinking. A temperature rise during electrophoresis is especially hazardous for obtaining reproducible results. Additionally, multi-allelic patterns of some nuclear DNA markers may cause the SSCP patterns too complicate for estimation of allele frequencies precisely.

1.6.3 DNA sequencing

DNA sequencing is the process of determine the order of the nucleotides bases; adenine, guanine, cytosine and thymine, in a strand or fragment of DNA. There are two main methods of DNA sequencing: the "chemical cleavage" procedure described by Maxam and Gilbert (1977) and the "enzymatic chain termination" procedure was described by Sanger, (1977). The disadvantage of the former method is that the procedure requires the use of several hazardous chemicals. Therefore, the latter is commonly used at present.

The dideoxy or enzymatic method of DNA sequencing utilizes the principle that a dideoxyribonucleotide triphosphate can be incorporated into a growing DNA chain, but cannot continue synthesis. DNA synthesis is terminated and the type of dideoxyNTP (ddNTP) added reflect the last nucleotide incorporated DNA (Figure 1.7). The Sanger method require the production of fragments of DNA from the piece of DNA which is being sequenced. This requires resolving DNA fragments which differ in length by one nucleotide and can be carried out by denaturing polyacrylamide gel electrophoresis.

DNA sequencing is the molecular biology technique for determined sequence of a piece of DNA. This technique provides high resolution and facilitating interpretation. However, sequencing of a large number of individuals using conventional method is tedious and prohibitively possible. At present, automated DNA sequencing has been introduced and commonly used. This greatly allows wider application of DNA sequencing analysis for various applications of genetic studies.

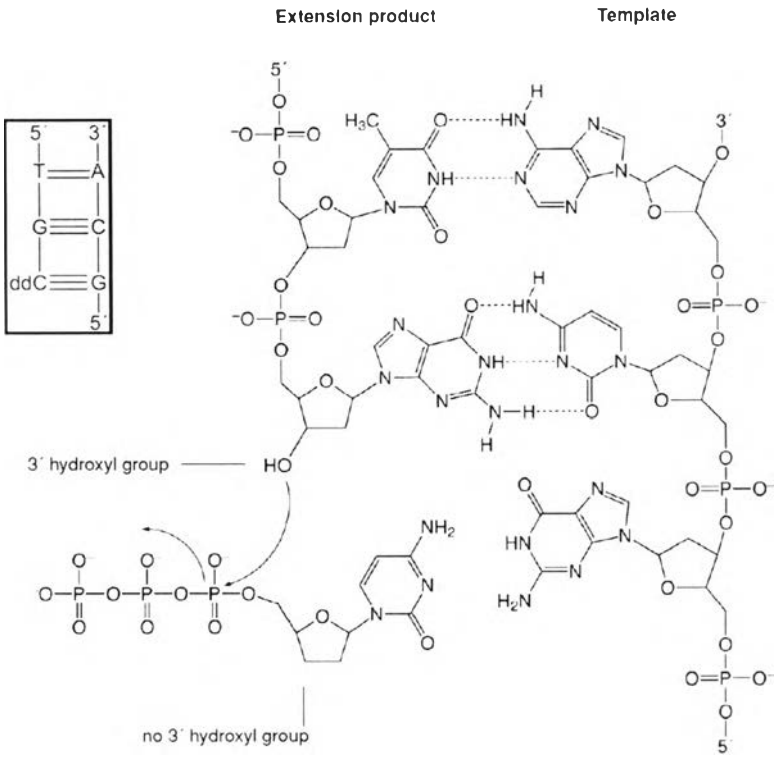

Figure 1.7 A schematic representation of sequencing an oligonucleotide by Sanger method. DNA strand synthesis by formation of phosphodiester bonds. In this example, the chain is terminated by the use of dideoxycytidine triphosphate (ddC) in place of deoxycytidine triphosphate (dCTP).

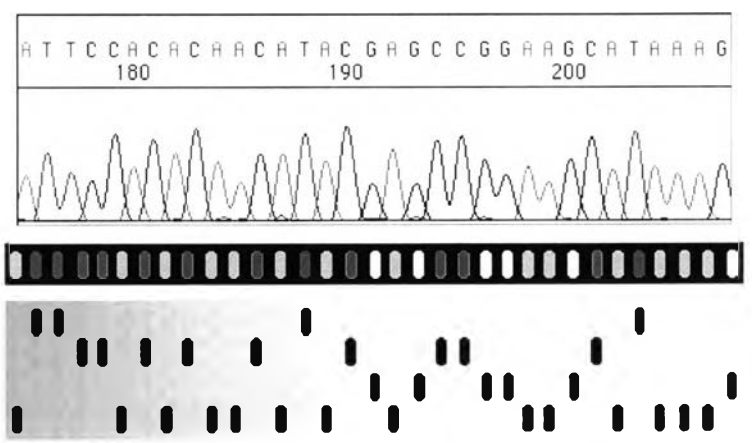

Figure 1.8 An illustration of the results of automated DNA sequencing.

1.6.4 Restriction Fragment Length Polymorphism (RFLP)

RFLP was first developed in 1980 (Botstein et al., 1980). It was among the first technique used for detection of DNA polymorphism. Restriction endonuclease are bacterial enzymes that specific nucleotide sequences 4, 6 or 8 base pair and cut the DNA at the recognition site. RFLPs are based on the analysis of patterns derived from a DNA sequence digested with known restriction enzymes. Nucleotide changes in the DNA sequence due to indels or base substitutions involving the recognition site result in the position of a restriction endonuclease recognition site, then the DNA sequence acquires or loses the ability to be cleaved by a particular restriction endonuclease. Loss of a restriction site within the locus, then digestion with the relevant restriction enzyme will generate a long fragment. On the other hand, if a recognition site is present will obtained two shorter fragments after digest with the restriction enzyme. Moreover, digestion will produce two different fragments include a long fragment and two shorter fragments. The procedure and principles of RFLP markers are illustrated by Fig. 1.9.

The traditional methods were separated fragments using Southern blot analysis, which is slow and tedious. Presently, this technique is carried out based on the polymerase chain reaction (PCR). The main advantages of RFLP are that they are co-dominant markers, which mean that it allows discrimination of homozygotic and heterozygotic states in diploid organisms, stable and reproducible. The major disadvantage of RFLP is the relatively low level of polymorphism. Additionally, sequence information for PCR-RFLP is required, making it difficult and time-consuming to develop markers in species lacking known molecular information.

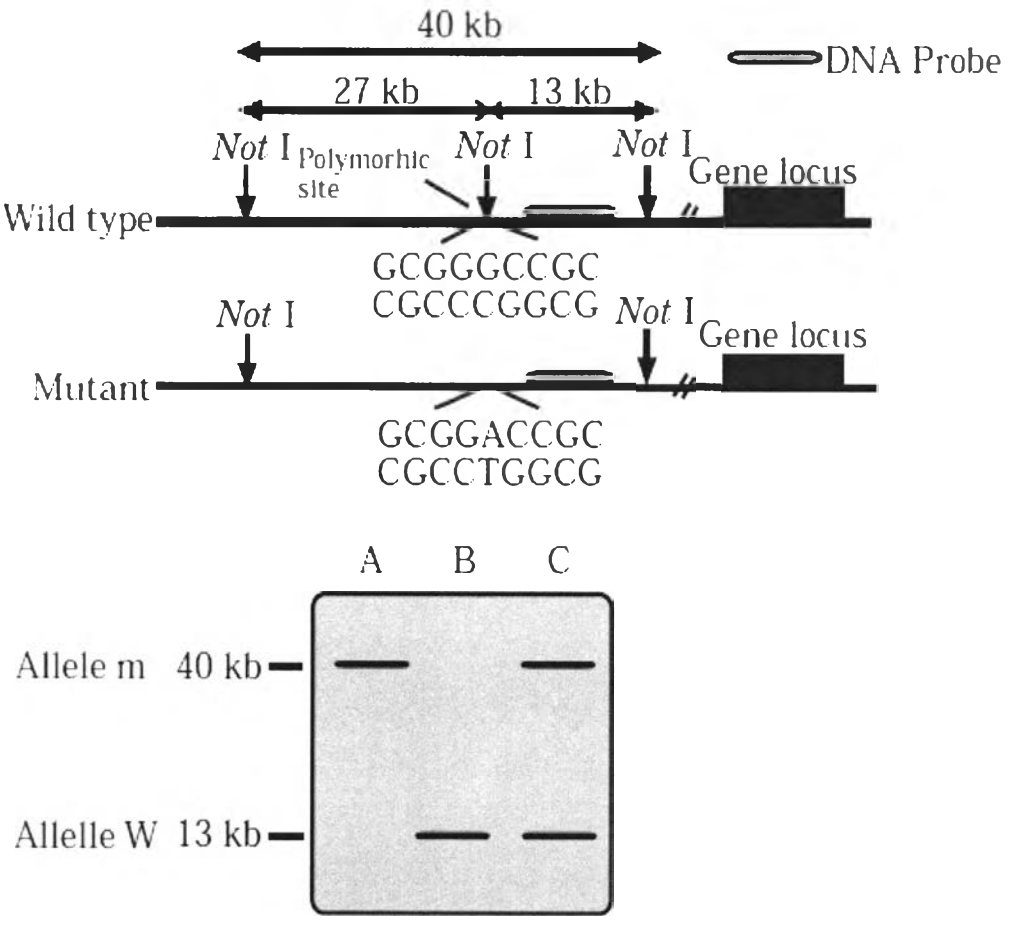

Figure 1.9 A schematic diagram of RFLP analysis (Bruzen et al., 2000).

1.7 SNP studies in functionally important genes of various organisms

SNPs are commonly used in several applications including gene discovery, population genetic studies and construction of genetic linkage maps and linkage disequilibrium mapping for identification of quantitative trait loci (QTL). SNPs are especially important if they cause differences in economic traits, or the mutations are linked to the phenotypes of interest. Identification of functionally important growth genes in agricultural and aquaculture species that can be used to increased the accuracy of selection for desired traits, thereby increasing the rate of genetic gain and production efficiency.

Growth hormone (*GH*) directly or indirectly plays an essential role in the regulator of postnatal somatic growth and stimulates anabolic processes such as cell division, skeletal growth and protein synthesis. In cattle, McCormack et al. (2009) cloned and sequenced the GH cDNA from Brooksville miniature Brahman cattle and this gene was aligned against the normal Brahman cattle. Sequence alignment revealed that at base number 641 of miniature cattle differed from normal cattle because there was a cytosine (C) instead of a thymine (T). This substitutions lead to a non-synonymous Thr to Met at position 200 of the GH peptide. This SNP was detected by PCR-RFLP with *Bsm* BI restriction enzyme in 12 individuals of miniature cattle and 9 individuals of normal Brahman cattle. All miniature cattle were homozygous for the mutation (-/-). While 7 individuals and 2 individuals of normal Brahman were homozygous for the wild-type (+/+) and heterozygous (+/-) alleles, respectively.

Gross and Nilsson, (1999) studied association between variation of the Atlantic salmon *growth hormone 1* (*GH1*) gene with the weight of one-year-old progeny fish of the hatchery strain (graded into three size groups). Two novel polymorphisms were detected by PCR-RFLP using *Taq* I. Significant heterogeneity of the *GH1* haplotype and genotype frequencies among the size groups was detected ($P<0.05$).

Prolactin (PRL) plays an important role growth and development, osmoregulation, reproduction, immunomodulation, endocrine and metabolic

regulation. In beef cattle, several studies have explored the association between *prolactin* gene polymorphism and milk production. For example, polymorphism of the *PRL* gene in Russian Red Pied cattle (Alipanah et al., 2007) and montebeliard cows (Ghasemi et al., 2009). In chicken, Bhattacharya et al. (2011) reported SNPs in the 5′UTR region of this gene and its association with egg production traits in White Leghorn chickens, which chickens with genotypes AA had significantly higher egg yields (up to 52 and 64 weeks of age) than those genotypes AC (144.5 ±5.06 and 143.2 ±4.67 eggs, respectively).

He et al. (2011) identified polymorphism within the *PRL* gene of the Asian seabass (*Lates calcarifer*). The result revealed five polymorphism include c.264+980_983delTTGT, c.264+127C>G, c.264+138T>G, c.264+269T>C and c.330C>G in 521 individuals. Only a T/C SNP substitution at position c.264+269 was significantly associated with body weight (BW), total length (TL), standard length (SL) and Fulton's condition factor (KTL and KSL).

Myostatin, a member of the transforming growth factor-β (TGF-β) superfamily, is the regulation of skeletal muscle as a negative regulator of muscle development in animals. The disrupted of *myostatin* in mice showed that null mutations exhibited a 2-3 times increase in the muscle mass due to a combination of muscle cell hypertrophy and hyperplasia, compared with wild-type mice (McPherron et al., 1997). In addition, it was demonstrated that the loss of the mature region of the *myostatin* protein due to 11-nucleotide deletion in the third exon or a missense mutation occurring in the *myostatin* coding sequence have been associated with the double muscle phenotype found in Belgian Blue and Piedmontese cattle.

In the 5′ and 3′ regulatory region of chicken *myostatin* gene, SNP has been demonstrated to be associated with adipose tissue in chicken (Zhiliang et al., 2004). In mollusk, Wang et al. (2004) identified SNP in *myostatin* of the 103 cultivated *Chlamys farreiri* individuals using PCR-SSCP and DNA sequencing method. SNP in exon 2 of primer M5 was significantly associated with body mass, soft-tissue mass, adductor muscle mass, shell length, shell height, absolute growth rate of shell height (ASH) and body mass (ABM). Results showed that the *C. farreri* genotype GG had significantly examined traits than those of genotype AG and AA ($P < 0.05$).

In crustacean, *actin* and *crustacean hyperglycemic hormone (CHH)* has been investigated in several decapods species, including shrimp, crab, crayfish and lobster (Zhue et al., 1997; Fanjul-Moles, 2006). *Actin* plays important roles muscle contraction, cell mobility and cell division (Pollard and Cooper, 1986). *CHH* plays major roles in carbohydrate and lipid metabolism, and also influences molting, reproduction, and osmoregulatory functions (Santos et al., 1997; Fanjul-Moles, 2006). Than et al. (2010) screen SNPs in *actin* and *CHH* genes and evaluate correlation between SNPs with individual growth performance in the giant freshwater prawn *Macrobrachium rosenbergii*. Four SNPs in the *actin* gene (single SNP in the AC2 fragment and three SNPs in the AC3 fragment) and ten SNPs in the *CHH* gene (single SNP in the 5′UTR and nine SNPs in the intronic region) of 243 individuals of offspring. No association with growth traits in the *actin* gene in the groups while four intronic SNPs of *CHH* exhibited highly significant associations with individuals growth performance (body weight, carapace length and standard length) ($P < 0.05$). Of these, homozyhous individuals (GG) at CH3 g.2402 had a significantly slower growth rate than did heterozygous individuals (GT). In contrast, individuals with the AA genotype at CH3 g.2561 had a significantly faster growth rate than those with the GA genotype. Homozygotes at CH3 g.2407 and g.2409 showed faster growth rate than did heterozygotes, however, a significantly effect was only on the body weight. A further haplotype-trait association analysis confirmed that these four SNP markers were in linkage disequilibrium, and the specific haplotype TGAA had significant associations with high growth ($P<0.01$). The implications of these findings with relevance to increase the efficiency of the selection process in giant freshwater prawn.

Marker assisted selection (MAS) is a major potential application used for expanding research on the genomics of farmed species. DNA marker technologies have already been used routinely for stock identification and family assignment of various shrimp species (Klinbunga et al., 2001; Ball and Chapman, 2003; McMillen-Jackson and Bert, 2004; Jerry et al., 2006; Khamnamtong et al., 2009). However, no validated marker linked to performance and production traits has been identified in penaeid shrimp up to date.

In *P. monodon*, Prasertlux, et al. (2010) was the first report on the correlation between SNP in *RuvBL2* and growth rate of *P. monodon*. *ATP-dependent DNA*

helicase (*RuvBL2*) is essential for growth in eukaryotes (Qiu et al., 1998; Makino et al., 1999). The full-length cDNA of *P. monodon RuvBL2* was 3791 bp and contained a 1392 bp open reading frame (ORF) corresponding to a polypeptide of 463 amino acids. Polymorphism of the amplified *PmRuvBL2* gene segment (484 bp containing an intron of 259 bp) was examined in commercially cultivated shrimp from the same pond (approximately 3 months old, average BW = 17.39 ± 4.36 g, N = 359) by single strand conformational polymorphism (SSCP) analysis. Results indicated that the *P. monodon* with genotypes A (average BW = 19.277 ± 3.640 g, N = 37) and B (average BW = 19.293 ± 4.548 g, N = 79) was significantly greater body weight than those genotype C (average BW = 16.528 ± 3.847 g, N = 93) and D (average BW = 16.365 ± 4.378 g, N = 124). One exonic (G-A81) and two intronic (A-T196 and G-T248) SNPs corresponding to ATG, A[T/A]G, GAG and GAT for respective SSCP genotypes were found.

In this thesis, relationships between SNP of *calponin1* (*PmCnn1*), *cyclin C* (*PmCyC*) and *cell division cycle 25* (*PmCdc25*) and growth traits of *P. monodon* juveniles are examined.

Calponins are actin-binding proteins that are implicated in the regulation of actomyosin (Jiang et al., 1997). *Calponin* is implicated in actin-linked regulation because it inhibits smooth muscle actin-activated myosin MgATPase and this inhibition can be reversed by phosphorylation or binding of Ca^{2+}calmodulin *in vitro* (Winder and Walsh, 1993; Takahashi et al., 1988; Gimona et al., 1996). Actin-myosin interaction also powers cell proliferation by driving cytoplasmic streaming, which may contribute to the division of the cytosolic components of the cell during cytokinesis. Accordingly, through the inhibition of actin-myosin interaction, *calponin* may play a role in regulating the functions of the actin cytoskeleton, such as coordinating changes in cell shape and intracellular molecular trafficking, both of which are critical events in cytokinesis (Han et al., 1993). Interestingly, forced expression of chicken gizzard calponin in cultured smooth muscle cells and fibroblasts showed an inhibition of cell proliferation (Jiang et al., 1997). Therefore, calponin, through its regulation of actin-myosin interaction and possibly actin filament stability, may function as a negative controlling factor for cytokinesis and the rate of cell proliferation.

Recently, the full-length genomic sequence of *P. monodon calponin 1* (*PmCnn1*) was isolated by genome walking. The *PmCnn1* gene contained 3 exons (185, 206 and 169 bp) and 2 introns (214 and 306 bp) with the open reading frame (ORF) of 561 bp deducing to a polypeptide of 186 amino acids (Buaklin, 2005). In this thesis, primers were designed and further tested to identify whether SNPs of this gene are related with various growth parameters in domesticated *P. monodon*.

Cyclin C is a highly conserved protein that functionally involved in both the positive and negative regulation of transcription in all eukaryotes from yeast to humans. This function is accomplished in cooperation with cyclin-dependent kinases (CDKs) by binding to CDK8 and CDK3 during cell cycle progression (Katona et al., 2006). *Cyclin C* plays a novel role in the regulation of G0/G1/S and G2M phases of cell cycle (Liu et al., 1998). Prior to this study, *cyclin C* has not been identified and characterized in penaeid shrimp. The full length cDNA of this gene in *P. monodon* is characterized in this thesis.

In addition, SNPs in *PmCdc25* were also carried out. Cdc25 phosphatases are essential regulators of the cell cycle. They dephosphorylate and activate cyclin-dependent kinases (CDK) that, in association with their cyclin regulatory subunits, control progression at various stages of the cell cycle (Contour-Galcera et al., 2007). The partial cDNA sequence of *PmCdc25* was previously identified by EST analysis (OV-N-S01-1905-W, *E*-value = 1.0E-16) but the full-length cDNA of *PmCdc25* in penaeid shrimp has not been characterized.

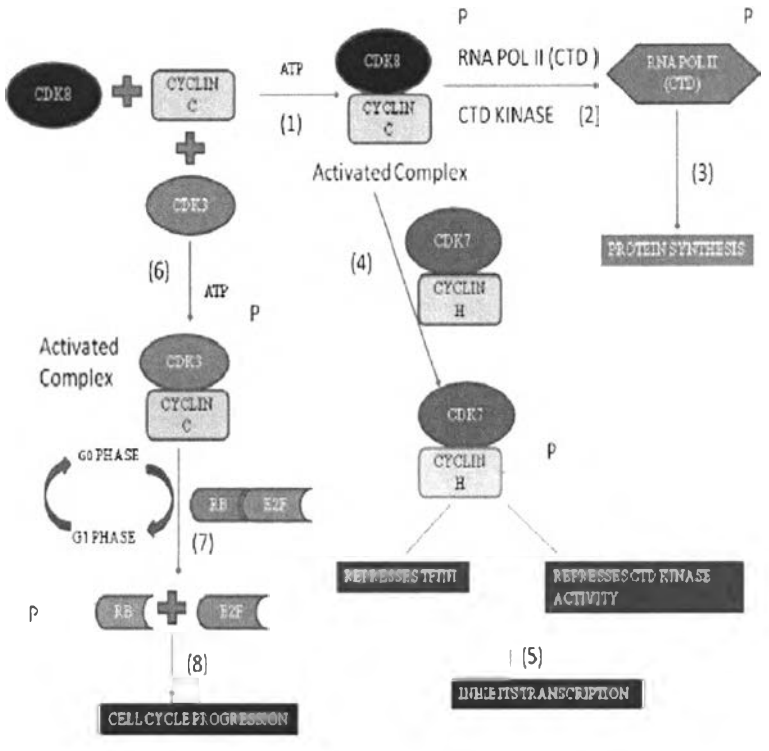

Figure 1.10 Prominent reactions of *cyclin C*. *Cyclin C* combines with CDK8 and CDK3 to regulate cell cycle progression. In response to growth factors CDK3/cyclin C complex becomes activated, phosphorylates and inactivates Rb promoting GO exit of cells by releasing E2F (transcription factor). Cell cycle re-entry from G0 may be normally suppressed by Rb family proteins in association with E2F transcription factors. CDK8 combines with its partner *cyclin C* and plays pivotal role in cell cycle progression in all eukaryotes. The negative regulatory functions of CDK8/cyclin C that operate in higher eukaryotes exert their effects at two crucial steps in the transcription–initiation pathway. CDK8 mediated phosphorylation of mammalian cyclin H represses transcription factors (TFIIH) to activate transcription and kinase activity (Ragender et al, 2010).

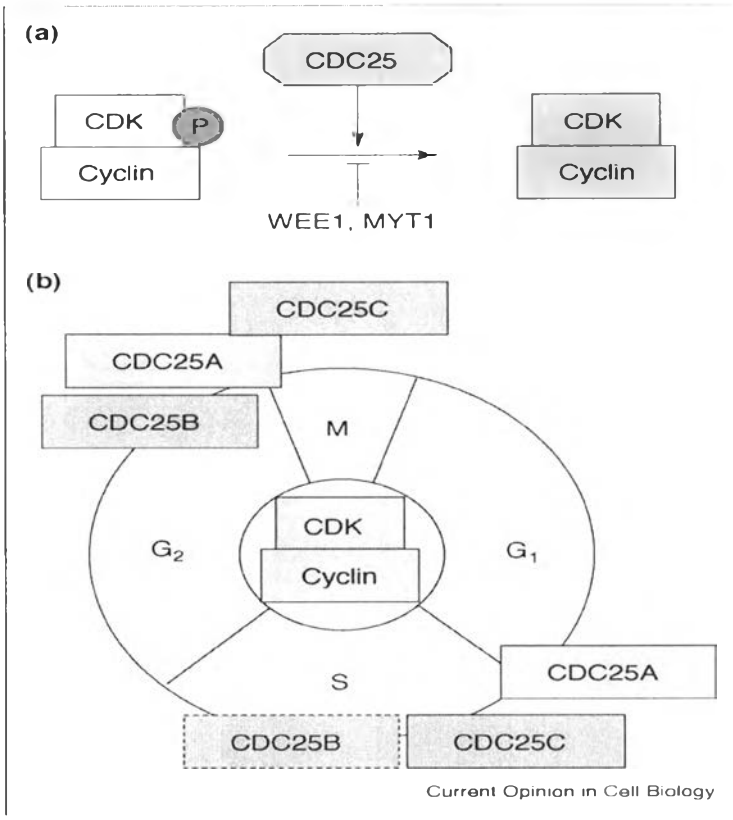

Figure 1.11 Cdc25 phosphatases control cell cycle progression. (a) Cdk/cyclin complexes are dephosphorylated and activated by Cdc25-dependent dephosphorylation. On the contrary, they are kept inactive by phosphorylation by the Wee1 and Myt1 kinases. (b) Activation of Cdk/cyclin complexes during the mammalian cell cycle by three Cdc25 phosphatases. Cdc25A is active both at G1/S and at mitosis. Cdc25B is active at G2/M and has also been proposed to be involved in S-phase (dotted box). Cdc25C has long been known to be active at mitosis, and has also recently been reported to be active in S-phase (Contour-Galcera et al., 2007).