CHAPTER I

INTRODUCTION

1.1 Two-component signal transduction

Bacterial cells have to monitor external conditions and adapt to their rapidly changing environment in order to survive (Parkinson, 1995; Parkinson; Dutta et al., 1999 and Kofoid, 1992). When environmental changes occur, regulation mechanisms involved in controlling the expression of specific genes or the activation of their products are required for the cells survival. These mechanisms frequently involve two-component signal transduction (Hoch and Varughese, 2001). This system typically consists of a histidine kinase protein and response regulator protein. The histidine kinase is responsible for detecting a change in the external environment accompanied by autophosphorylation by adenosine triphosphate (ATP) at the histidine residue. The high energy phosphoryl group is then transferred to a conserved aspartate residue in the response regulator. The response regulator is able to control the expression of the particular genes to mediate the proper cellular response to the stimulus (Stock et al., 1995; Stock, et al., 1989 and Aiba, et al., 1993).

1.1.1 Histidine kinase

Figure 1 shows the organization of the two-component signalling system (Klumpp and Krieglstein, 2002). Histidine kinase proteins contain an N-terminal signal sensing domain followed by a C-terminal transmitter domain. The signal sensing domain consists of hydrophobic amino acids that transverse the membrane. This domain functions as a membrane receptor at the external surface of the membrane

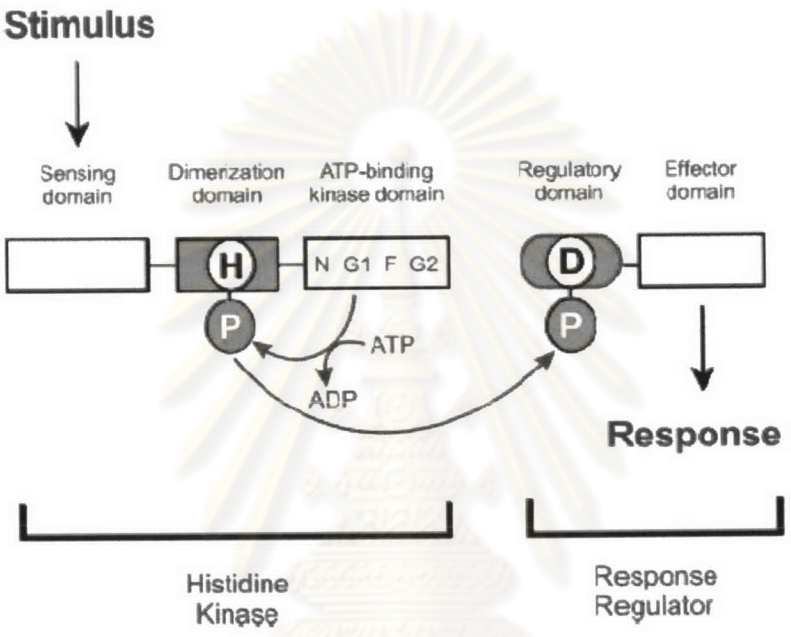


Figure 1 Typical domain organisation of the proteins involved in two-component signalling. The conserved His and Asp residues are indicated using the single letter amino acid code.

(Stock et al, 1995). The final transmembrane domain is linked to the preceding transmitter domain by linker domain. This is critical for proper signal transduction (Stock et al., 2000). However, not all input domains are membrane bound. The chemotaxis kinase, CheA and nitrogen regulatory kinase, NtrB are examples of soluble cytoplasmic domains (Stock et al., 1989).

The transmitter domain can be recognized by several short blocks of sequences. These have been designated the H, N, G1, F and G2 motifs. They are referred to by the most conserved amino acid residues. The H motif and residues around it are involved in the autophosphorylation activity. The N, G1, F and G2 motifs are involved in ATP binding. In addition, the region upstream of the N motif is proposed to be the hinge region (Stock et al., 1989; Egger et al., 1997 and Stock et al., 2000). This region contains several conserved hydrophobic residues that have been proposed to form the X motif (Hsing et al., 1998).

The transmitter domain consists of two functional subdomains. These are the dimerization domain, containing the H motif, followed by the ATP-binding domain containing conserved N, G1, F and G2 motifs (Klumpp and Krieglstein, 2002). On the basis of their domain organization, the transmitter domain has been grouped into two major classes. In Class I, the H motif containing region in the dimerization subdomain is directly linked to the ATP-binding domain. This is exemplified by EnvZ involved in osmoregulation in *E. coli*. In Class II, the H motif region is separated from dimerization subdomain and is distant from the ATP-binding domain (Dutta et al., 1999).

Histidine phosphorylation does not proceed by an intramolecular mechanism. However, this mechanism requires formation of a homodimer with the dimerization

subdomain of the histidine kinase dimerizing to undergo trans-autophosphorylation between two monomers (Cai et al., 2003). The dimerization domains of both EnvZ and CheA form antiparallel four-helix bundles with the active site histidine residue located in the middle and exposed face of helix I (Figure 2). The ATP-binding domain has an α/β structure with one layer of five stranded β-sheet and another layer of three α helices (Stock et al., 2000).

1.1.2 Response regulator protein

Response regulator proteins contain an N-terminal receiver regulatory domain and a variable C-terminal effector domain. The regulatory domain has about 120 amino acids with a conserved Asp residue, that serves as the site of phosphorylation by the histidine kinase (Egger et al., 1997). The majority of effector domains have DNA-binding activity and function to activate and/or repress transcription of specific genes. However, the specific mechanism of transcription regulation differs for each response regulator (Stock et al., 2000).

Bacterial response regulators can be subdivided into five families based on sequence homology in the conserved C-terminal domain (Egger et al., 1997). These five families are CheY, OmpR, NtrC, NarL and an unclassified family. The CheY protein is involved in chemotaxis in *E. coli*. The CheY family of 125 amino acids contains only a single conserved N-terminal domain. However, the OmpR family, involved in osmoregulation consists of 240 amino acids and the NarL family, involved in nitrate and nitrite regulation consists of 220 amino acids, both contain a C-terminal DNA binding domain. The crystal structure of the DNA-binding domain of OmpR is known as the winged-helix transcription factor. There is a recognition helix that

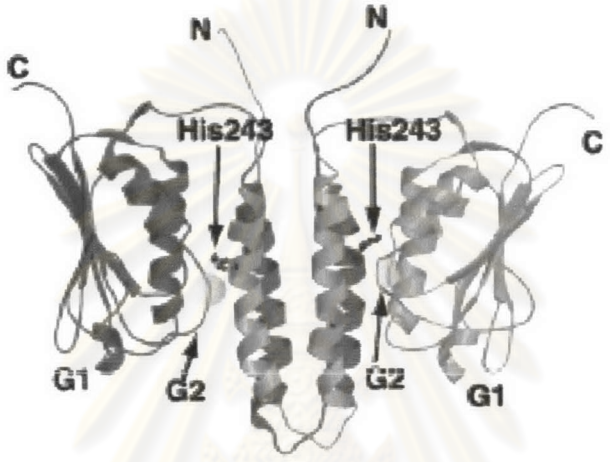


Figure 2 Proposed model of the EnvZ dimmer (Cai et al., 2003). The two subunits are shown in yellow and cyan. The active site conserved histidine side-chain (His-243) is indicated while the ATP-binding site is represented by a pink sphere. The conserved G1 and G2 boxes are shown in red.

interacts with the major groove of DNA and flanking loop or wings that are proposed to contact the minor groove (Stock et al., 2000 and Okamura et al., 2000) whereas the binding of NarL with another transcription factor regulates the NarL-regulated operon (Stock et al., 2000). The NtrC family, involved in nitrogen regulation, includes proteins of about 460 amino acids that contain an ATPase domain and a DNA-binding domain downstream of the conserved N-terminal domain. The unclassified family has a different type of effector region attached to a response regulator. The best characterized of this family is CheB. The dimethylase catalytic domain is associated with the conserved N-terminal domain (Stock et al., 1995).

The structure of the conserved N-terminal domain has been determined for CheY. The pattern of conserved residues implies that all response regulators will have similar structure. The N-terminal domain consists of five α-helices that surround a five-stranded parallel β-sheet. The conserved aspartate is located in an acidic pocket in the β-sheet. The loops connecting the β-sheet to the α-helices can have different conformations. This contributes to the specific interaction between a histidine kinase-response regulator pair (Stock et al., 2000).

The lifetime of the phospho-Asp of a response regulator varies significantly. Typical half-life ranges from seconds (Hess et al., 1988) to hours (Igo et al., 1989). Many response regulators have autophosphatase activity that decreases the lifetime of the phosphoprotein (Stock et al., 2000). The transmitter domains of histidine kinase EnvZ and PhoR also contain phosphatase activity towards their cognate response regulator OmpR and PhoB, respectively (Hsing et al., 1998 and Carmany et al., 2003). The phosphatase activity of EnvZ is in the histidine-containing substrate domain and

was enhanced by an interaction between this domain and catalytic domain (Zhu et al., 2000). In contrast, the phosphatase activity of PhoR was enhanced when the catalytic domain was deleted (Kramer and Weiss, 1999). The kinase and phosphatase activities may be regulated by the input domain. The removal of the input domain of PhoR leads to constitutive activation of the transmitter kinase activity (Yamada et al., 1990).

1.2 Phosphate uptake

Phosphate is one of the nutrients required at high levels for cell growth (Ray et al., 1991). Phosphate compounds are the major building blocks of many biomolecules, energy conservation and photosynthesis processes (Grossman and Takahashi, 2001). Phosphate is transported into the cells through both the low-affinity phosphate inorganic transport (Pit) and the high-affinity phosphate-specific transport (Pst). Whereas the Pit system is constitutive, the Pst system is repressed under the conditions of excess phosphate. Phosphate is taken up by Pit under conditions of excess phosphate while conditions of phosphate limitation leads to elevated production of the genes encoding the Pst proteins (Willsky and Malamy, 1980 and Rosenberg, 1977). Apart from transporting phosphate, the Pst system plays an important role in a repression of genes involved in phosphate sensing (Wanner, 1995 and Hoffer et al., 2001).

Phosphate can be limiting in both freshwater and terrestrial environments, since it often exists in forms not readily accessible to most organisms. Therefore phosphate sensing in *Escherichia coli* is one of the most extensively studied two-component signal transduction systems (Ray et al., 1991).

1.3 Phosphate sensing

The response of bacteria to changes in extracellular phosphate levels is mediated by the Pho system (Zundel et al., 1998). The Pho two-component signal transduction system is comprised of PhoR, the histidine kinase and PhoB, the response regulator. The latter is a transcription activator which regulates the genes belonging to the phosphate (Pho) regulon. The Pho regulon includes several genes and operons for the uptake and degradation of extracellular phosphorus sources (Su, 2003). One of the prominent proteins that accumulates during phosphate-limiting growth is an alkaline phosphatase (Ray et al., 1991). In *Escherichia coli*, the alkaline phosphatase is encoded by *phoA* which is part of Pho regulon (Wanner, 1995 and Torriani et al., 1985). This enzyme is secreted into the periplasmic space to release orthophosphate from organophosphate compounds (Wanner, 1995). PhoR senses extracellular phosphate levels through the phosphate-specific ABC transporter of the Pst system and an ancillary protein, PhoU (Wanner, 1995 and Yamada et al., 1990).

The control of the Pho regulon involves two processes: inhibition when P_i is in excess, and activation under conditions of P_i limitation. When the environmental P_i concentration falls to less than about 4 μM, the activation due to P_i limitation may lead to more than a 1,000 fold induction of an individual Pho regulon gene. The induction depends on the promoter of that gene. When an environmental Pi concentration is greater than 4 μM, the P_i repression requires an intact phosphate specific transport (Pst) system, PhoR and PhoU. The Pst system is a cell surface receptor complex for P_i uptake composed of PstS, PstA, PstB and PstC (Steed and Wanner, 1993). Moreover, the PhoU protein is proposed to be the negative regulator under phosphate sufficient

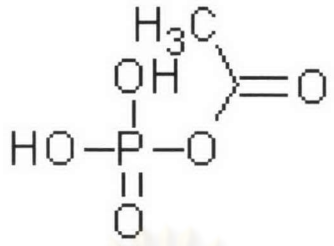
conditions. Thus the deletion of *phoU* results in an alkaline phosphatase activity in phosphate sufficient conditions (Muda et al., 1992). A role for PhoU in P_i repression may involve an association between PhoU and PhoR in a repression complex. This interaction may be necessary to maintain PhoR as $PhoR^R$ (the repressor form) (Wanner, 1995).

Under conditions of P_i limitation only PhoR and PhoB are required for activation. Because multiple components are required for P_i repression, it is likely that the process of P_i repression may involve formation of a repression complex by protein-protein interactions (Wanner, 1995).

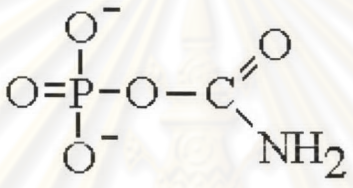
1.4 Cross regulation

Normally the regulation of two-component signal transduction usually involves interactions between pairs of environmental sensors and response regulator. They act as partner proteins (Mccleary et al., 1993). In addition some response regulators may be cross regulated by input signals from histidine kinases of different regulatory systems or by a chemical phosphorylating agent such as phosphoramidate, acetyl phosphate and carbamyl phosphate (Figure 3). Such regulatory interaction may be especially important as a way of directly linking different systems in a network to coordinate cell growth and metabolism (Wanner, 1992 and Mccleary et al., 1993).

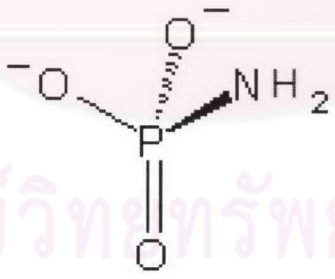
The mechanism of the phosphotransfer reaction comes from the observation that the low molecular weigh phosphorylated compounds can transfer phosphoryl groups to response regulators in the absence of a histidine kinase, *in vitro* (Hiratsu et al., 1995). Thus, it is apparent that the response regulators can catalyze their own



Acetyl phosphate



Carbamyl phosphate



Phosphoramidate

Figure 3 Small-molecule phosphodonors that can function to phosphorylate response regulators.

phosphorylation, the phosphotransfer reaction from the phosphohistidine of histidine kinase to response regulator is an autophosphorylation reaction catalyzed by the response regulator (Stock, 1995). The different response regulators show widely different reactivities toward the three small phosphorylated compounds. Acetyl phosphate is the preferred substrate for CheY, while phosphoramidate is a better substrate for NRI and CheB autophosphorylation (Mccleary, 1993).

The rates of autophosphorylation also differ dramatically between different response regulators. For example, in the presence of acetyl phosphate CheY is phosphorylated faster than NRI, which is phosphorylated faster than PhoB (Mccleary, 1993).

1.5 Acetyl phosphate metabolism

Acetyl phosphate is a high-energy phosphate compound with a ΔG of hydrolysis of –43.1kJ/mol. Acetyl phosphate was first identified as a precursor of acetic acid during fermentation and has since been shown to be involved in the activation of acetate for its metabolic utilization (Mccleary, 1993). The metabolic pathway responsible for the synthesis and degradation of acetyl phosphate is shown in Figure 4. Synthesis of acetyl phosphate from acetyl-CoA and P_i is catalyzed by phosphotransacetylase, the product of *pta* gene. Synethesis of acetyl phosphate from ATP and acetate is catalyzed by acetate kinase, the product of *ackA* gene. Both reactions are readily reversible (Mccleary, 1993). In *E. coli, Salmonella typhimurium* and related bacteria, the *pta* and *ackA* genes are contiguous on the chromosome (Kwan et al., 1988 and Wanner and Wilmes-Riesenberg, 1992). The expression of these

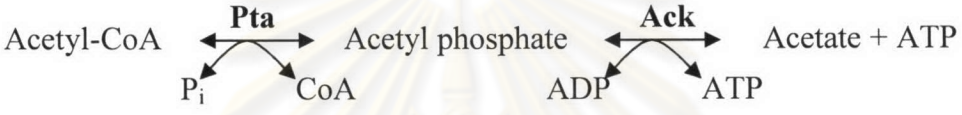


Figure 4 Acetate kinase and phosphotransacetylase pathway. Pta is phosphotransacetylase, Ack is acetate kinase.

genes is nearly constitutive, with only a two fold induction upon anaerobiosis. Nevertheless, the activities of phosphotransacetylase and acetate kinase are at least 8- to 11- fold higher in acetate-grown *Methanosarcina* cells than in cells grown on methanol, monomethylamine, dimethylamine or trimethylamine (Singh-Wissmann and Ferry, 1995). In *Sinorhizobium meliloti*, the acetate kinase and phosphotransacetylase activity is up-regulated in response to phosphate limitation (Summers et al., 1999). However, there is no report about the expression of these enzymes in cyanobacteria. It has been reported that these genes do not form an operon in *E. coli* (Wanner and Wilmes-Riesenberg, 1992). However, insertions of *ackA* have an effect on *pta* expression in *S. typhimurium* (Kwan et al., 1988). The inactivation of *pta* also eliminated the acetate kinase activity in *S. meliloti* (Summers et al., 1999), suggesting that they constitute an operon in those two organisms.

Levels of acetyl phosphate vary dramatically depending on the carbon source in the growth medium. For example, very low levels of acetyl phosphate are observed when cells are grown on glycerol under phosphate-limiting conditions. Moderate levels of acetyl phosphate are observed when cells are grown on glucose and high levels of acetyl phosphate are observed when cells are grown on pyruvate. Hence, acetyl phosphate levels may indicate an adjustment between carbon flowing through glycolysis and into the TCA cycle. Specifically, acetyl phosphate is elevated when entry of carbon into TCA cycle is slower than entry to glycolysis (Mccleary et al., 1993).

Acetyl phosphate is made via Pta and degraded via AckA during growth on glucose or pyruvate; the converse is true during growth on acetate. The AckA

mutation leads to elevated acetyl phosphate levels because of a block in its breakdown. The Pta mutation leads to lower acetyl phosphate levels because of a block in its synthesis (Wanner, 1992). In order to determine if the response regulator proteins can also be phosphorylated by endogenous acetyl phosphate in the absence of histidine kinase, the acetate kinase genes need to be interrupted and combined with a histidine kinase mutant strain.

Because of the importance of acetyl phosphate, the acetate kinases which catalyze the acetyl phosphate production have also been purified from *Bacillus stearothermophillus* (Nakajima et al., 1978), *Veillonella alcalescens* (Griffith and Nishimura, 1979), *Acholleplasma laidlawii* (Kahane and Muhlrad, 1979), *Salmonella typhimurium* (Fox and Roseman, 1986), *Clostidum acetobutylicum* (Diez-Gonzalez et al., 1997), *Thermotoga maritima* (Bock et al., 1999) and *Desulfovibrio vulgaris* (Yu et al., 2001). Acetate kinase from all these microbes is a homodimer except the enzyme from *B. stearothermophillus*, which is a homotetramer (Yu et al., 2001). There are two forms of acetate kinase from *D. vulgaris*. One acetate kinase (AK-I) is a homodimer and the other (AK-II) is a heterodimer. The molecular mass of each subunit from all organisms are in the range of 43-53 kDa. This enzyme is not found in eukaryotic cells but is found in anerobic pathogens including *Bacillus* and *Vibrio cholerae*. The specific inhibitors of this enzyme are candidates for potential new classes of antibiotics (http://www.bmb.psu.edu/faculty/ferry/lab/labmembers/sarah/). There has been no report about acetate kinase in cyanobacteria, therefore the partial purification to study some properties of this enzyme in *Synechocystis* sp. PCC 6803 was performed in this study.

1.6 Cyanobacterial phosphate sensing

Cyanobacteria grow and survive under various environmental conditions and require genetic mechanisms to adapt to environmental changes. Many genes encode two-component signal transduction systems (Ikeuchi and Tabata, 2001). Systems homologous to the *Escherichia coli* PhoR:PhoB system have been identified in *Synechococcus* sp. PCC 7942 (Aiba et al., 1993) and *Synechococcus* sp. WH 7803 (Watson et al., 1996). The histidine kinase SphS and its cognate response regulator SphR, were shown to be involved in modulating the specific response to phosphate starvation in *Synechococcus* sp. PCC 7942 (Aiba et al., 1993). Similarly, a two-component system involved in the adaptation under phosphate-limiting conditions was also identified in the marine cyanobacterium *Synechococcus* sp. WH 7803 (Watson et al., 1996). Cloning and sequencing of the DNA fragment identified a set of two-component signalling proteins homologous to the SphS-SphR proteins. The genes were shown to be induced in response to phosphate-limiting conditions.

Previously, the genes involved in the phosphate starvation response have also been identified in *Synechocystis* sp. PCC 6803. The histidine kinase (PhoR) encoded by the *sll0337* gene and its cognate response regulator (PhoB) encoded by *slr0081* gene are homologous to the proteins found in *Synechococcus* sp. PCC 7942 and *Escherichia coli* (Hirani et al., 2000). Mutations using oligonucleotide-directed mutagenesis have also been introduced into the conserved motif of the transmitter domain of Sll0337 and the DNA-binding domain of Slr0081 to examine the function of the specific amino acids, particularly where these differ from their counterparts in *E. coli* (Hirani, 2001; Simpson, 2003). The alkaline phosphatase was measured in these

mutants because it can act as a monitor for the activity of the *pho* regulon. This enzyme was normally induced under phosphate-limiting conditions whereas there was no induction of alkaline phosphatase under phosphate-sufficient conditions. The results showed that in *Synechocystis* sp. PCC 6803, His-207 was the autophosphorylation site and Lys-209, putatively located in the third turn of helix I, was required for activity through the interaction with a cluster of hydrophobic residues to form the H motif. Additionally a positively charged amino acid may be required at the 210 position. Glycine-352, Gly-354 and the Gly-387 to Leu-390 in the ATP-binding domain may interact with His-207. The Phe-364 may be involved in the binding of ATP to ATP-binding domain. The Pro-286 to Val-289 in the hinge region appeared to stabilize the interaction between the histidine-containing and the ATP-binding domain (Hirani, 2001).

It was noted previous that the PhoB response regulator protein and OmpR are members of the winged-helix-turn-helix family of DNA binding proteins. The major difference between OmpR and PhoB resides in the interdomain linker and in the transactivation loop that precedes the DNA recognition helix. The length of this loop differs between OmpR and PhoB, consisting of ten and seven residues, respectively; and is known to interact with different subunits of RNA polymerase (Walthers et al., 2003). PhoB interacts with the σ70 subunit, while OmpR interacts with the α-subunit of RNA polymerase (Makino et al., 1993). However, the residues in Slr0081 differ considerably from the corresponding residues in OmpR and PhoB suggesting that they may be involved in a specific interaction with the cyanobacterial RNA polymerase. It was confirmed that the Gly is specific for the interaction with the cyanobacterial RNA

polymerase. Moreover, the specific Trp-236 is involved in DNA binding in *Synechocystis* cells and the *pho* box is different from that of *E. coli* (Simpson, 2003).

In addition, mutation of Thr-214 in the Sll0337 resulted in up-regulation of alkaline phosphatase activity under phosphate-sufficient conditions. However, removal of negative regulator PhoU in this mutant reversed the effect under phosphate-limiting condition even though the ΔPhoU strain exhibited constitutive up-regulation of alkaline phosphatase activity. The results suggested that Thr-214 may interact with the PhoU (Simpson, 2003).

In this study, since in *E. coli* the phosphorylation site in OmpR (Asp-55) of corresponds to Asp-88 in Slr0081, mutation of Asp-88 was carried out to check whether this conserved Asp residue is the phosphorylation site. The mutation of Thr-214 to other amino acid residues was also performed in the presence and absence of PhoU protein to elucidate the interaction between PhoU and Sll0337 (PhoR) in *Synechocystis* sp. PCC 6803.

1.7 Oligonucleotide-directed mutagenesis

Oligonucleotide-directed *in vitro* mutagenesis is a widely used procedure for the study of the structure and function of DNA and its encoded protein. Many techniques are available for performing *in vitro* mutagenesis. A typical strategy is to clone the segment of DNA to be mutated into a vector whose DNA exists in both single- and double-stranded forms. An oligonucleotide conplementary to the region to be altered, except for a limited internal mismatch, is hybridized to a single-stranded copy of the DNA. A complementary strand is then synthesized by T7 DNA

polymerase using the oligonucleotide as a primer. Ligase is used to seal the new strand to the 5' end of the oligonucleotide. The double-stranded DNA, completely homologous except for the intended mutation, is then transformed into *E. coli*, resulting in two classes of progeny, the parental and those carrying the oligonucleotide-directed mutation (http://www.biochem.arizona.edu/…/Lecture6. html). The steps involved in oligonucleotide-directed mutagenesis are shown in Figure 5.

A key strategy in oligonucleotide-directed mutagenesis methods is to increase the relative number of mutated plasmids in the pooled population. The dUTP incorporation strategy which relies on the degradation and repair of template DNA has been "marked" with deoxyuridine prior to the *in vitro* DNA synthesis step. Thomas Kunkel developed the dUTP incorporation strategy by taking advantage of an *E. coli* strain that contains defects in genes responsible for preventing dUTP incorporation into DNA. Oligonucleotide-directed mutagenesis is an efficient method to generate specific nucleotide alterations in cloned DNA. dUTP incorporation into M13 DNA is done by using a phagemid vector to produce single strand DNA in an *E. coli* strain defective in the two enzymes uracil-N-glycosylase (ung) and dUTPase (dut). The mutagenic oligonucleotide is annealed to uracil-containing template DNA (U), and *in vitro* DNA synthesis is performed to generate double-stranded DNA. Following transformation into ung+, dut+ *E. coli*, the template strand is degraded and the surviving double strand plasmid is isolated and sequenced (http://www.biochem. arizona.edu/…/Lecture6.html).

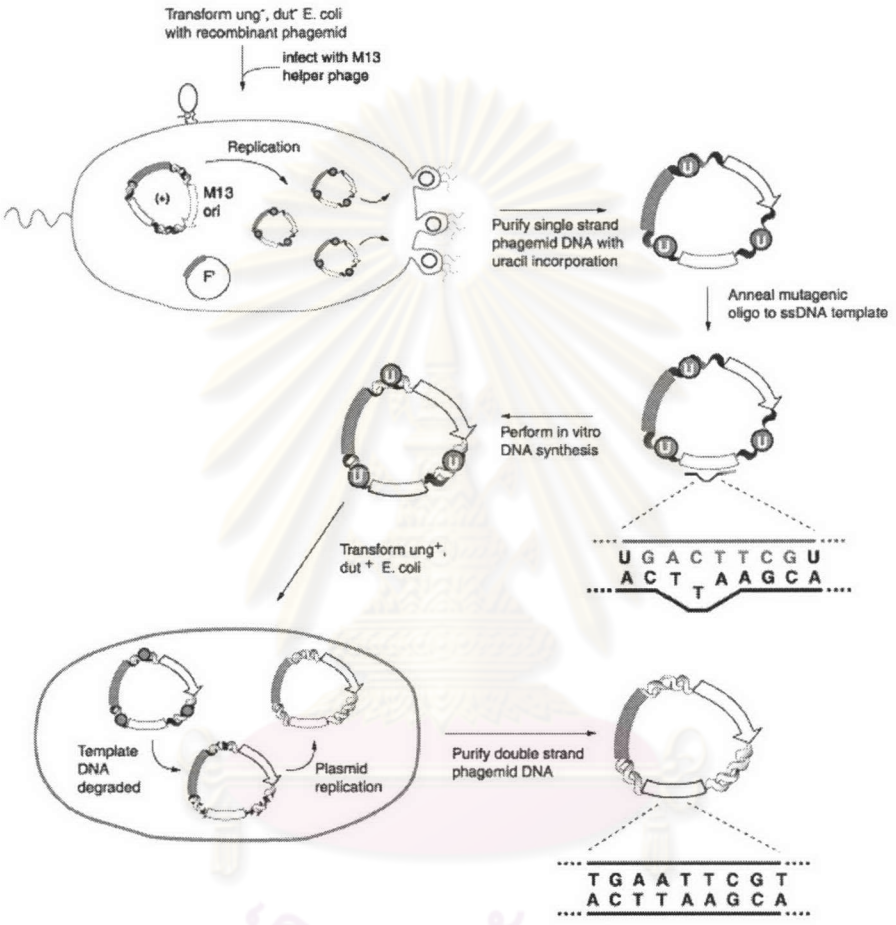


Figure 5 Steps involved in *in vitro* mutagenesis using oligonucleotide-directed mutagenesis (http://www.biochem. arizona.edu/…/Lecture6.html).

1.8 Rapid amplification of cDNA ends (RACE)

Transcription initiation points can be mapped by rapid amplification of cDNA ends (RACE). It involves production of the desired cDNA by reverse transcription, linking of a modified anchor oligonucleotide to the 5' end of cDNA, and successive amplification with a primer complementary to the anchor and primers specific for the cDNA upstream of the reverse transcriptase primer sequence (Figure 6). The addition of the anchor places a known unique sequence at the unmapped 5' end of the cDNA, which can be identified by DNA sequencing of the nested fragment (Kaebernick et al., 2002).

1.9 *Synechocystis* sp. PCC 6803

Cyanobacteria can be classified as Procaryota, Division of Cyanophyta and Cyanophyseae class (Ikeuchi et al., 2001). Single-celled blue-green algae range in size from about 0.6 μm to over 30 μm in their largest dimension. The bacteria and cyanobacteria lack mitochondria, true vacuole and endoplasmic reticulum. The cyanobacterial cell is surrounded by the cytoplasmic membrane and a multilayered cell wall similar to that of the gram-negative bacteria. The multilayer cell wall consists of an outer membrane with the unit membrane structure and peptidoglycan layer which varies in thickness between 5 and 10 nm but can be considerably thicker. The outer membrane functions more as a passive molecular sieve, whereas the cytoplasmic membrane serves as a true selective permeability barrier (Gantt, 1994).

Synechocystis cells have been defined as a unicellular coccoid, or spherical cyanobacterium. They divide by binary fission. *Synechocystis* sp. PCC 6803 is a

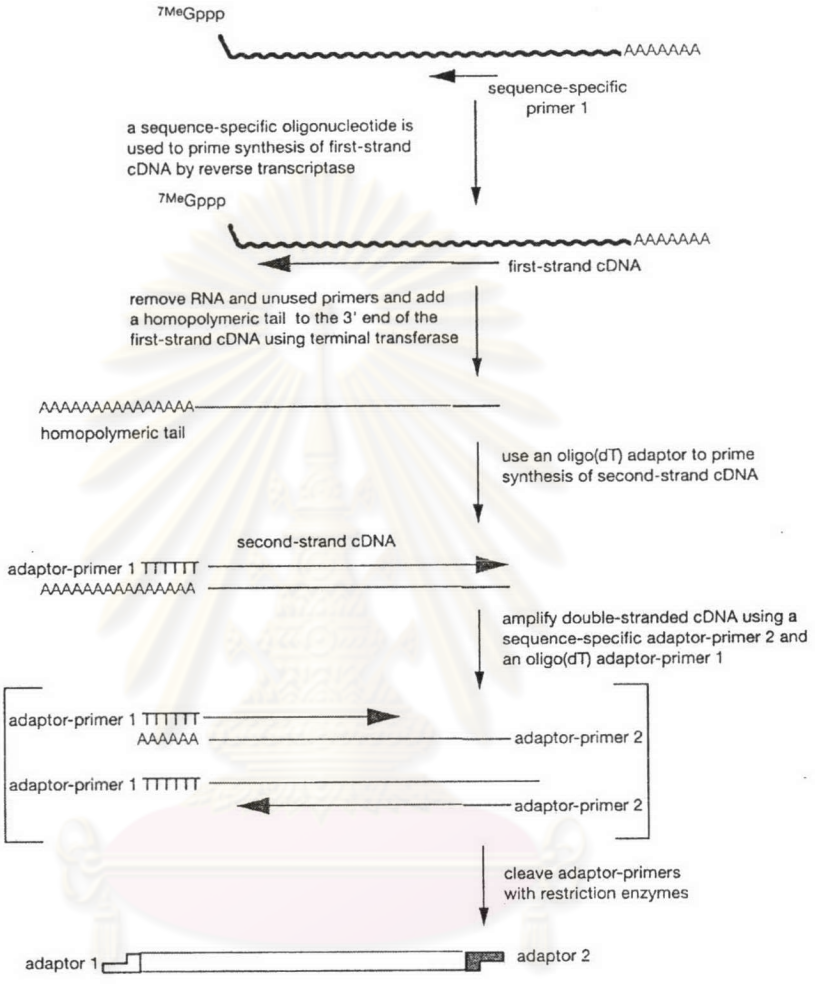


Figure 6 Determination of the 5' end of mRNA using 5' RACE.

unicellular non-nitrogen-fixing cyanobacterium and a ubiquitous inhabitant of fresh water. The entire genome of *Synechocystis* sp. PCC 6803 was sequenced in 1996. This was the first photoautotrophic organism to be fully sequenced. The circular genome was originally deduced to be 3,573,470 bp long. Since the *Synechocystis* sp. PCC 6803 is naturally transformable by exogenous DNA, it affords great simplicity for the introduction of mutations into target genes. It has been one of the most popular organisms for genetic and physiological studies of photosynthesis (Ikeuchi et al., 2001).

Objectives of this research

1. To examine the function of Thr-214 of the transmitter of Sll0337 (PhoR) in the presence and absence of the negative regulator (PhoU) and of the conserved Asp-88 of the receiver domain of Slr0081 (PhoB) in *Synechocystis* sp. PCC 6803.
2. To study the expression of acetate kinase and phosphotransacetylase genes under phosphate stress.
3. To determine if the Slr0081 response regulator proteins can be phosphorylated by endogenous acetyl phosphate.
4. To partially purify acetate kinase and characterize its properties.