CHAPTER 1
INTRODUCTION

Protein Phosphorylation

Living cells must be able to respond rapidly to changing environmental conditions in order to survive. To respond quickly to changing conditions, the cell must be able to rapidly and specifically "turn on" and "turn off" enzyme activities. Perhaps the most common molecular "switch" employed by cells for this purpose is *regulatory protein phosphorylation*.

At its core, a regulatory system involving protein phosphorylation consists of a protein kinase, a phosphoacceptor protein whose structure (and thus, function) is altered by phosphorylation, and a protein phosphatase. Protein kinases catalyze the transfer of the gamma phosphoryl group from a nucleoside triphosphate (usually ATP) to an amino acid on the acceptor protein. Protein phosphatases catalyze the hydrolysis of phosphoamino acids on proteins, thus restoring the modified protein to its initial state (Fig. 1.1).

The phosphorylation of a single amino acid on even a very large protein can have dramatic effects on the structure and function of that protein (1). One common effect is to stimulate or attenuate an enzymatic activity. The ability to dramatically alter a protein's function with a chemical modification that is readily attained and hydrolyzed enzymatically (2), has made protein phosphorylation a "method of choice" for rapidly and reversibly regulating enzyme activities and other protein functions in the cell. It has recently been estimated that there may be as many as 3000 genes encoding protein kinases and phosphatases in the mammalian genome (3) and that as many as 30% of intracellular proteins are modified post-translationally by phosphorylation (4).

[1]Johnson, L. N. and D. Barford. (1993). The effects of phosphorylation on the structure and function of proteins. *Annu. Rev. Biophys. Biomol .Struct*. **22**: 199-233.

[2]Westheimer, F. H. (1987). Why nature chose phosphates. *Science* **235**: 1173-78.

[3]Hunter, T. (1995). Protein kinases and phosphatases: the yin and yang of protein phosphorylation and signalling. *Cell* **80**: 225-36.

[4]Barford, D., Z. Jia, and N. Tonks. (1995). Protein tyrosine phosphatases take off. *Nature Struct. Biol.* **2** (12): 1043-53.
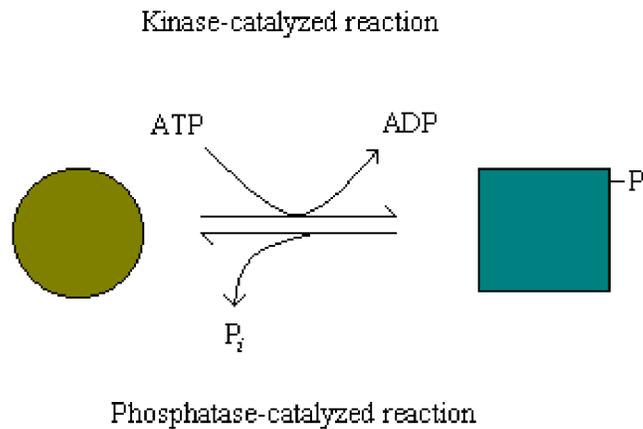
Kinase-catalyzed reaction

ATP   ADP

P$_i$

Phosphatase-catalyzed reaction

Figure 1.1
Modification of a protein by phosphorylation. Protein kinases catalyze
the transfer of a phosphoryl group from ATP to the acceptor protein.
Protein phosphatases catalyze the hydrolysis of the phosphoamino acid,
releasing inorganic phosphorus and the de-modified protein.

Protein Kinases

Protein kinases catalyze the phosphorylation of specific amino acid residues on
acceptor proteins and are classified as serine / threonine kinases, tyrosine kinases, or
histidine kinases depending upon the nature of the amino acid side chain they phosphorylate.
The serine/threonine and protein tyrosine kinases share a high degree of structural and
sequence similarity despite their differences in substrate specificity (5)(6). The histidine
kinases form a completely separate structural class that autophosphorylate on a histidine
residue prior to transferring the phosphate moiety onto an aspartyl residue of a response
regulator (see below).

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[5]Hunter, T. (1987). A thousand and one protein kinases. *Cell* **50**: 823-39.

[6]Hanks, S. K., and A. M. Quinn. (1991). Protein kinase catalytic domain sequence
database: identification of conserved features of primary structure and
classification of family members. *Meth. Enzymol*. **200**: 38-62.

Protein Phosphatases

Protein phosphatases "reverse" the action of protein kinases by restoring phosphoproteins to their unmodified state. Protein phosphatases were once thought to act constitutively , essentially performing a "house-keeping" role. The actual task of regulation was delegated to the protein kinases, many of which were dramatically responsive to second messengers such as cyclic nucleotides and Ca^{++}/calmodulin. Today we know that the activities of most protein phosphatases are regulated as well (7) and that protein phosphatases function in partnership with protein kinases to modulate signal transduction events (8)(9)(10).

Like protein kinases, protein phosphatases are classified according to the type of (phospho)amino acid residue upon which they act. Two major classes of protein phosphatases were recognized under this classification scheme: the protein serine / threonine phosphatases, which show restricted specificity for phosphoserine and phosphothreonine residues on proteins, and the protein tyrosine phosphatases, which show restricted specificity for phosphotyrosine residues. In addition, there are a number of non-specific alkaline and acid phosphatases that do not share any sequence similarities with either of the other two groups. These enzymes are thought to target the phosphoryl moieties on small organic molecules *in vivo*, although both exhibit protein phosphatase activity *in vitro*.

The protein serine / threonine phosphatases are further catagorized as Type 1 or Type 2, based upon their preference for "standard" substrates and response to heat-stable protein inhibitors (11). Type 1 phosphatases preferentially dephosphorylate the β subunit of

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[7]Tonks, N. K. (1990). Protein phosphatases: key players in the regulation of cell function. *Curr. Opinion Cell Biol*. **2**: 1114-24.

[8]Missiakas, D. and S. Raina. (1997). Signal transduction pathways in response to protein misfolding in the extracytoplasmic compartments of *E. coli*: role of two phosphoprotein phosphatases PrpA and PrpB. *EMBO J*. **16**: 1670-85.

[9]Koretzky, G. A., J. Picus, M. L. Thomas, and A. Weiss. (1990). Tyrosine phosphatase CD45 is essential for coupling T-cell antigen receptor to the phosphatidylinositol pathway. *Nature* **346**: 66-68.

[10]Tonks, N. K., M. F. Cicirelli, C. D. Diltz, J. E. Weiel, E. H. Fischer, and E. G. Krebs. (1990). Microinjection of a protein tyrosine phosphatase inhibits insulin action in *Xenopus* oocytes. *Proc. Natl. Acad. Sci. USA* **87**: 5514-18.

[11]Cohen, P. (1989). Structure and regulation of protein phosphatases. *Annu. Rev. Biochem*. **58**: 453-508.

phosphorylase kinase and are potently inhibited by two proteins called inhibitor 1 and inhibitor 2. Type 2 protein phosphatases preferentially dephosphorylate the α subunit of phosphorylase kinase and are insensitive to inhibitor 1 or inhibitor 2. The catalytic mechanism of the protein serine/threonine phosphatases appears to involve a direct attack of water on the target phosphoprotein (12).

Tyrosine-specific protein phosphatases (PTPs) are classified as either intracellular or receptor-like. All of the protein-tyrosine phosphatases share a conserved catalytic domain of ~250 amino acids (13) containing the active site sequence $HCXaa_5R$ (or HAT, for His-Arg-Thiolate) (14). Many receptor-like PTPs possess two catalytic domains within this cytoplasmic region, though usually only one of these is active. The extracellular portion of receptor PTPs often possess ligand receptors or adhesion factors (15)(16). An important subclass of the intracellular PTPs is a group of so-called dual-specificity protein phosphates (DSPs) which possess the HAT sequence characteristic of other PTPs, yet exhibit the ability to dephosphorylate phosphoserine and phosphothreonine residues with high efficiency (17). Crystallographic data suggest that a deep active site pocket on PTPs is responsible for their specificity for phosphotyrosine, while a much shallower pocket on the DSPs allow them to

[12]Martin, B. L. and D. J. Graves. (1986). Mechanistic aspects of the low-molecular-weight phosphatase activity of the calmodulin-activated phosphatase, calcineurin. *J. Biol. Chem*. **261**: 14545-50.

[13]Barford, D., Z. Jia, and N. Tonks. (1995). Protein tyrosine phosphatases take off. *Nature Struct. Biol.* **2** (12): 1043-53.

[14]Stone, R. L., and J. E. Dixon. (1994). Protein tyrosine phosphatases. *J. Biol. Chem*. **269**: 31323-26.

[15]Tonks, N. K. (1990). Protein phosphatases: key players in the regulation of cell function. *Curr. Opin. Cell Biol*. **2**: 1114-24.

[16]Stone, R. L., and J. E. Dixon. (1994). Protein-tyrosine phosphatases. *J. Biol. Chem*. **269**: 31323-26.

[17]Keyse, S. M. (1995). An emerging family of dual specificity MAP kinase phosphatases. *Biochim. Biophys. Acta*. **1265**: 152-60.

act upon all phosphoamino acid phosphoester linkages (18)(19). Unlike the protein serine/threonine phosphatases, the PTPs form a phosphoenzyme intermediate during the catalytic cycle. (20).

Protein Phosphorylation in the Bacteria

Protein phosphorylation as a regulatory mechanism was first described in the 1950's as a means of controlling glycogen metabolism in skeletal muscle (21). For many years this particular mode of regulation was assumed to be restricted to the mammalian cell, particularly since initial studies on bacterial systems proved negative (22). Not until the late 1970's was it conclusively demonstrated that protein phosphorylation occurs in both unicellular eukaryotes (i.e., yeast) as well as prokaryotes (23)(24)(25). Still, it was generally believed throughout the 1980's that protein phosphorylation developed independently in prokaryotes and eukaryotes, because their phosphorylation reactions were so fundamentally different. In particular, it appeared that bacteria predominantly phosphorylated proteins on histidine and aspartic acid residues (employing several low molecular weight phospho-donor molecules) while eukaryotes preferentially phosphorylated serine, threonine, and tyrosine

[18]Jia, Z., D. Barford, A. J. Flint, and N. K. Tonks. (1995). Structural basis for phosphotyrosine peptide recognition by protein tyrosine phosphatase 1B. *Science* **268**: 1754-58.

[19]Ref to DSP crystal structure.

[20]Guan, K., and J. E. Dixon. (1991). Evidence for protein-tyrosine-phosphatase catalysis proceeding via a cysteine-phosphate intermediate. *J. Biol. Chem*. **266**: 17026-30.

[21]Krebs, E. G., and E. H. Fisher. (1956). The phosphorylase b to a converting enzyme of rabbit skeletal muscle. *Biochem. Biophysica. Acta*. **20**: 150-57.

[22]Cozonne, A. J. (1993). ATP-dependent protein kinases in bacteria. *J. Cellular. Biochem.* **51**: 7-13.

[23]Wang, J. Y. J. and D. E. Koshland, Jr. (1978). Evidence for protein kinase activities in the prokaryote *Salmonella typhimurium. J. Biol. Chem.* **253**: 7605-08.

[24]Garnak, M. and H. C. Reeves. (1979). Phosphorylation of isocitrate dehydrogenase of *Escherichia coli. Science* **203**: 1111-12.

[25]Manai, M. and A. J. Cozzone. (1979). Analysis of the protein kinase activity of *Escherichia coli* cells. *Biochem. Biophys. Res. Commun.* **91**: 819-26.

residues (using ATP-dependent kinases).

In the 1990's it has become clear that the prokaryote / eukaryote dichotomy that dominated the thinking of the '80's does not accurately reflect nature. Protein phosphorylation among all organisms (including the archaea) is, in fact, far more similar than dissimilar. "Bacterial" signal transduction enzymes, such as protein histidine kinases, have recently been found in eukaryotic organisms (see below) and numerous serine / threonine- and tyrosine phosphorylated proteins have been discovered in bacteria (see below). The ubiquity and commonality of protein phosphorylation is now becoming well established (26). As a result, we now realize that the microbial world offers unique experimental opportunities for the study of protein phosphorylation as a mechanism of regulation. Such studies will be facilitated by the easy maneuverability and relatively small genome size of bacteria.

The Two Component Regulatory System

The name "two-component regulatory system" refers to a signal transduction unit consisting of a *sensor kinase* and a *response regulator* (27)(28). The sensor kinases share a common catalytic domain that autophosphorylates on a conserved histidine (using ATP as the phospho-donor) in response to an environmental stimulus. The phosphorylated sensor kinase then tranfers the phosphoryl-group to a conserved aspartate on the response regulator protein / domain, altering its enzymatic activity or affinity for other proteins or DNA. More than a dozen two-component systems have been described in various bacterial species (29) and, more recently, in eukaryotes, including *Saccharomyces cerevisiae* (30), *Neurospora*

[26]Kennelly, P. J. and M. Potts. (1996). Fancy meeting you here! a fresh look at "prokaryotic" protein phosphorylation. *J. Bacteriol*. **178**: 4759-64.

[27]Swanson, R. V., L. A. Alex, and M. I. Simon. (1994). Histidine and aspartate phosphorylation: two-component systems and the limits of homology. *TIBS* **19**: 187-92.

[28]Saier, M. H. Jr. (1993). Introduction: protein phosphorylation and signal transduction in bacteria. *J. Cell. Biochem*. **51**: 1-6.

[29]Stock, J. B., A. J. Ninfa, and A. M. Stock. (1989). Protein phosphorylation and adaptive responses in bacteria. *Microbiol. Rev*. **53**: 450-90.

[30]Maeda, T., S. M. Wurgler-Murphy, and H. Saito. (1994). A two-component system that regulates an osmosensing MAP kinase cascade in yeast. *Nature* **369**: 242-45.

crassa (31), and *Arabidopsis thaliana* (32).

Protein Serine / Threonine Phosphorylation

Genes encoding known or potential protein serine/threonine kinases have recently been cloned from several bacterial species. In 1991, Munoz-Dorado and coworkers cloned a gene, *pkn1*, from the spore-forming bacterium *Myxococcus xanthus* whose predicted gene product, Pkn1, contained a region that shares ~30% sequence homology with the catalytic domains of the well-characterized kinases PKA and PKC (33). The catalytic domain of the gene product, Pkn1, shares ~30% sequence homology with the well-characterized kinases PKA and PKC. When Pkn1 is expressed in *E. coli*, it is able to autophosphorylate on serine and threonine residues, a common trait of eukaryotic protein kinases. Since this seminal report, genes encoding potential protein serine/threonine kinases have also been found in the cyanobacterium *Anabaena* PCC 7120 (34), *Streptomyces ceolicolor* A3[2] (35), and several archaebacteria (36). Although the physiological roles for these protein kinases are largely unknown, two of them, Pkn1 and PknA (from *Anabaena* PCC 7120), appear to participate in cell differentiation.

Recently, models have been proposed in several eukaryotic species involving cross-

[31]Oda, K., and K. Hasunuma. (1994). Light signals are transduced to the phosphorylation of 15kDa proteins in *Neurospora crassa. FEBS Lett*. **345**: 162-66.

[32]Kakimoto, T. (1996). CKI1, a histidine kinase homolog implicated in cytokinin signal transduction. *Science* **274**: 982-85.

[33]Munoz-Dorado, J., S. Inouye, and M. Inouye. (1991). A hene encoding a protein serine / threonine kinase ir required for normal development of *M. xanthus*, a gram-negative bacterium. *Cell* **67**: 995-1006.

[34]Zhang, C-C. (1993). A gene encoding a protein related to eukaryotic protein kinases from the filamentous heterocystous cyanobacterium *Anabaena* PCC 7120. *Proc. Natl. Acad. Sci USA* **90**: 11840-44.

[35]Urabe, H., and H. Ogawara. (1995). Cloning, sequencing, and expression of serine / threonine kinase-encoding genes from *Streptomyces coelicolor* A3(2). *Gene* **153**: 99-104.

[36]Smith, R. F., and K. Y. King. (1995). Identification of a eukaryotic-like protein kinase gene in Archaebacteria. *Protein Sci*. **4**: 126-29.

talk between two-component systems and serine/threonine phosphorylation cascades (37). The models suggest that an autophosphorylated protein histidine kinase transfers its phosphate group to a response regulator which subsequently regulates a protein serine/threonine kinase activity. In light of these proposed mechanisms, it is interesting to the note the recent discovery of a gene (*hstK*) encoding a 200kDa protein in *Anabaena* PCC 7120 possessing a protein serine/threonine kinase domain at its N-terminus and a histidine kinase domain at its C-terminus (Zhang, personal communication). The gene is downstream of an ORF, *msnA*, coding for a protein possessing two hydrophobic domains and a domain homologous to a bacterial membrane sensor domain usually part of a His-kinase. The proteins encoded by these genes may therefore by instrumental in the elucidation of a here-to-fore undiscovered mechanism of signal transduction in bacteria, as well as detailing how cross-talk between two-component systems and Ser/Thr phosphorylation cascades might occur in both prokaryotes and eukaryotes.

Protein serine/threonine phosphatase genes have also been cloned from several prokaryotes in recent years, including *Escherichia coli*, the archaeons *Sulfolobus sulfataricus* and *Methanosarcina thermophila*, and the cyanobacteria *Microcystis aeruginosa* PCC 7120 and *M. aeruginosa* UTEX 2063. All are homologs of the PP1/2A/2B superfamily of protein serine/threonine phosphatases from the eukaryotes. The gene for the first archaeal protein serine/threonine phosphatase, PP1-Arch1 from *Sulfolobus sulfataricus*, was cloned and sequenced in 1995 (38). The DNA-derived amino acid sequence exhibits ~30% sequence identity with eukaryotic PP1/2A/2B protein phosphatases. Another archaeal protein serine/threonine phosphatase gene has been cloned more recently from *Methanosarcina thermophila* (39). The deduced amino acid sequence of the gene product displays high homology with PP1-Arch1. Additionally, the detection of a protein serine/threonine phosphatase activity in *Haloferax volcanii* (40) with similar characteristics to PP1-Arch1 *in*

[37]Appleby, J. L., J. S. Parkinson, and R. B. Bourret. (1996). Signal transduction via the multi-step phosphorely: not necessarily the road less traveled. *Cell* **86**: 845-8.

[38]Leng, J., A. J. Cameron, S. Buckel, and P. J. Kennelly. (1995). Isolation and cloning of a protein-serine / threonine phosphatase from an archaeon. *J. Bacteriol*. **177**: 6510-17.

[39]Solow, B., J. C. Young, and P. J. Kennelly (1997). Gene cloning and expression and characterization of a toxin-sensative protein phosphatase from the methangenic archaeon *Methanosarcinia thermophilia* TM-1. *J. Bacteriol*. **179**: 5072-75.

[40]Oxenrider, K. A., and P. J. Kennelly. (1993). A protein-serine phosphatase from the halophilic archaeon *Haloferax volcanii*. *Biochem. Biophys. Res. Commun*. **194**:

vitro suggests the presence of a family of PP1/2A/2B protein phosphatases in the *Archaea*.

The cyanobacterial serine/threonine protein phosphatases, PP1-Cyano1 (from *M. aeruginosa* PCC 7120) and PP1-Cyano2 (from *M. aeruginosa* UTEX 2063), are 96% identical in amino acid sequence to one another and possess sequence features highly conserved among all Type 1 serine/threonine protein phosphatases (41). Notably, both protein phosphatases lack a binding motif for the PP1/PP2A specific inhibitor microcystin-LR and are resistant to the effects of this cyanobacterially-produced toxin to 0.1mM. The discovery of PCR products homologous with eukaryotic protein serine/threonine phosphatases from five cyanobacterial strains (42) suggests that, as with the Archaea, a family of serine/threonine protein phosphatases exists within the cyanobacteria.

Protein Tyrosine Phosphorylation

With the discovery of protein-tyrosine phosphorylation as a covalent modification event in eukaryotic cells, a search was quickly mounted for protein-tyrosine phosphorylation in bacteria. Several researchers reported the presence of tyrosine-phosphorylated proteins in various bacterial strains in the late 1980's (43)(44). However, these early reports were rendered suspect by the finding that the chemical methods employed to detect phosphoamino acids could generate phosphotyrosine as a degradation product of nucleotidylated proteins (45). Subsequent research using differential ^{32}P-labelling (i.e., [γ-^{32}P]-ATP vs. [α-^{32}P]-ATP)

1330-35.

[41]Shi, L. (1996). Cloning and sequencing of PP1-Cyano1 and PP1-Cyano2, two protein serine / threonine family I phosphatase genes, from microcystin-producing strain *Microcystis aeruginosa* PCC 7820 and non-microcystin-producing strain *M. aeruginosa* UTEX 2063. Thesis Diss.

[42]Shi, L. (1996). Cloning and sequencing of PP1-Cyano1 and PP1-Cyano2, two protein serine / threonine family I phosphatase genes, from microcystin-producing strain *Microcystis aeruginosa* PCC 7820 and non-microcystin-producing strain *M. aeruginosa* UTEX 2063. Thesis Diss.

[43]Cozzone, A. J. (1984). Protein phosphorylation in the bacteria. *Trends Biochem. Sci*. **9**:400-403.

[44]Londesborough, J. (1986). Phosphorylation of proteins in *Clostridium thermohydrosulfuricum*. *J. Bacteriol*. **165**: 595-601.

[45]Foster, R., J. Thorner, and G. S. Martin. (1989). Nucleotidylation, not phosphorylation, is the major source of the phosphotyrosine detected in enteric bacteria. *J*.

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and anti-phosphotyrosine antibodies, however, has confirmed that bacterial proteins can be phosphorylated on tyrosine both *in vivo* and *in vitro*.

In the past few years, dozens of bacterial proteins ranging in size from 6kDa to 206kDa, from more than a dozen bacterial species, have been reported to contain phosphotyrosine (Table 1.1). Despite the accumulation of reports describing tyrosine-phosphorylated proteins of bacterial origin, few reports have appeared concerning the protein kinases and protein phosphatases presumably responsible for regulating their state of phosphorylation. Nevertheless, some progress has been made in recent years. For example, Duclos and coworkers recently reported the discovery of a PTK in the bacterium *Acinetobacter johnsonii* (46). They originally detected the enzyme on SDS gels as an 81kDa phosphoprotein. Partial purification of the phosphoprotein revealed that it is localized specifically to the inner-membrane fraction of *A. johnsonii*. Upon renaturation following SDS-PAGE, the protein was shown to incorporate ^{32}P in the presence of [γ-^{32}P]-ATP by autophosphorylation at multiple tyrosine residues.

Table 1.1: Bacterial Phosphotyrosyl-phosphoproteins

Table 1.1: Bacterial Phosphotyrosyl-phosphoproteins		
Strain	**Apparent Molecular mass (kDa)**	**Reference**
Sulfolbus sulfataricus ATCC 35091	15-65*	(47)
Haloferax volcanii	30-65*	
Methanosarcina thermophilia TM-1	45-70*	
Anabaena PCC 7120	27, 36, 52	(48)

Bacteriol. **171**: 272-79.

[46]Duclos, B., C. Grangeasse, E. Baganay, M. Riberty, and A. J. Cozzone. (1996). Autophosphorylation of a bacterial protein at tyrosine. *J. Mol. Biol*. **259**: 891-95.

[47]Smith, S. C., P. J. Kennelly, and M. Potts. (1997). Protein-tyrosine phosphorylation in the *Archaea. J. Bacteriol*. **179**: 2418-20.

[48]McCartney, B., L. D. Howell, P. J. Kennelly, and M. Potts. (1997). Protein tyrosine phosphorylation in the cyanobacterium *Anabaena* sp. strain PCC 7120. *J. Bacteriol*. **179**: 2314-18.

Table 1.1: Bacterial Phosphotyrosyl-phosphoproteins		
Strain	**Apparent Molecular mass (kDa)**	**Reference**
Nostoc commune UTEX 584	85	(49)
Pseudomonas syringae	66	(50)
Aeromonas hydrophilia	45	(51)
Myxococcus xanthus	40, 83	(52)
Acinetobacter calcoaceticus	19, 52, 61, 81	(53)
Pseudomonas solanacearum	85	(54)
Acinetobacter johnonsii	81	(55)

[49]Potts, M. H. Sun, K. Mockaitis, P. J. Kennelly, D. Reed, and N. K. Tonks. (1993). A protein-tyrosine / serine phosphatase encoded by the genome of the cyanobacterium *Nostoc commune* UTEX 584. *J. Biol. Chem*. **268**: 7632-35.

[50]Ray, M. K., G. S. Kumar, and S. Shivaji. (1994). Tyrosine phosphorylation of a cytoplasmic protein from the antarctic psychrotrophic bacterium *Pseudomonas syringae. FEMS Microbiol. Lett*. **122**: 49-54.

[51]Thomas, S. R., and T. J. Trust. (1995). Tyrosine phosphorylation of the tetragonal paracrystalline array of *Aeromonas hydrophila*: molecular cloning and high-level expression of the S-layer protein gene. *J. Mol. Biol*. **245**: 568-81.

[52]Frasch, S. C., and M. Dworkin. (1996). Tyrosine phosphorylation in *Myxococcus xanthus*, a multicellular prokaryote. *J. Bacteriol*. **178**: 4084-88.

[53]Dadssi, M. and A. J. Cozzone. (1990). Evidence of protein-tyrosine kinase activity in the bacterium *Acinetobacter calcoaceticus. J. Biol. Chem*. **265**: 20996-99.

[54]Atkinson, M., C. Allen, and L. Sequeira. (1992). Tyrosine phosphorylation of a membrane protein from *Pseudomonas solanacearum. J. Bacteriol*. **174**: 4356-60.

[55]Duclos, B., C. Grangeasse, E. Vaganay, M. Riberty, and A. J. Cozzone. (1996). Autophosphorylation of a bacterial protein at tyrosine. *J. Mol. Biol*. **259**: 891-95.

Table 1.1: Bacterial Phosphotyrosyl-phosphoproteins		
Strain	**Apparent Molecular mass (kDa)**	**Reference**
Salmonella typhimurium	144	(56)
Streptomyces spp.	25-206*	(57)
*numerous proteins in the range given		

Another protein kinase, PutA, has been identified in *Salmonella typhimurium* (58). This protein kinase was found to be phosphorylated on serine, threonine, and tyrosine residues both *in vitro* and *in vivo*. The enzyme shares sequence similarities with eukaryotic protein kinases, including tyrosine-specific protein kinases, and can efficiently phosphorylate serine, threonine, or tyrosine residues of acceptor proteins. Consequently, the enzyme has been classified as a dual-specificity protein kinase (DSK). In addition, homogeneous PutA autophosphorylates *in vitro* in the presence of [γ-^{32}P]-ATP (59).

PutA is a multifunctional protein. In the absence of proline, it represses its own gene expression, as well as the expression of *putP*, a gene encoding a proline permease. In the

[56]Ostrovsky, P. C., and S. Maloy. (1995). Protein phosphorylation on serine, threonine, and tyrosine residues modulates membrane-protein interactions and transcriptional regulation in *Salmonella typhimurium. Genes & Development* **9**: 2034-41.

[57]Waters, B., D. Vujaklija, M. R. Gold, and J. Davies. (1994). Protein tyrosine phosphorlylation in streptomycetes. *FEMS Microbiol. Lett*. **120**: 187-90.

[58]Ostrovsky, P. C., and S. Maloy. (1995). Protein phosphorylation on serine, threonine, and tyrosine residues modulates membrane-protein interactions and transcriptional regulation in *Salmonella typhimurium. Genes & Dev*. **9**: 2034-41.

[59]Ostrovsky, P. C., and S. Maloy. (1995). Protein phosphorylation on serine, threonine, and tyrosine residues modulates membrane-protein interactions and transcriptional regulation in *Salmonella typhimurium. Genes & Dev*. **9**: 2034-41.

presence of proline, PutA catalyzes the oxidation of proline to glutamate (60)(61). The function of PutA (transcriptional repressor vs. proline oxidase) is determined by both the intracellular concentration of proline and the phosphorylation state of the protein (62).

With regard to PTPs, the gene for a dual-specificity protein phosphatase, IphP, has been cloned from the heterocystous cyanobacterium *Nostoc commune* UTEX 584 (63). While the physiological role of IphP is not known, the discovery by Potts and coworkers of a protein in *N. commune* UTEX 584 whose phosphorylation on tyrosine was dependent on the availability of combined nitrogen suggests that IphP, or an as yet undetected PTP, may be involved in the regulation of nitrogen acquisition.

A tyrosine-specific phosphatase, PtpA, has been purified from the mycelium- and spore-forming bacterium *Streptomyces coelicolor* A3[2] (64). PtpA has been shown to be a member of the "small, acid PTP", or SA-PTP, subfamily of the protein-tyrosine phosphatases. Genes sharing sequence similarities with PtpA and other eukaryotic SA-PTPs have been observed in *Bacillus subtilus*, *Klebsiella pneumoniae*, *Pseudomonas solanocearium*, and *Erwinia amylovora* (65); however, with the exception of PtpA, it has not been demonstrated that any of the predicted protein products of these genes possess PTP activity. PtpA can be inhibited *in vitro* by several inhibitors of protein-tyrosine

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[60]Menzel, R. and J. Roth. (1981). Enzymatic properties of the purified PutA protein from *Salmonella typhimurium. J. Biol. Chem.* **256**: 9762-66.

[61]Ostrovsky de Spicer, P., K. O'Brien, and S. Maloy. (1991). Regulation of proline utilization in Salmonella typhimurium: a membrane-associated dehydrogenase binds DNA *in vitro. J. Bacteriol.* **173**: 211-19.

[62]Ostrovsky, P. C., and S. Maloy. (1995). Protein phosphorylation on serine, threonine, and tyrosine residues modulates membrane-protein interactions and transcriptional regulation in *Salmonella typhimurium. Genes & Dev*. **9**: 2034-41.

[63]Potts, M., H. Sun, K. Mockaitis, P. J. Kennelly, D. Reed, and N. K. Tonks. (1993). A protein tyrosine/serine phosphatase encoded by the genome of the cyanobacterium *Nostoc commune* UTEX 584. *J. Biol. Chem.* **268**: 7632-35.

[64]Li, Y., and W. R. Strohl. (1996). Cloning, purification, and properties of a phosphotyrosine protein phosphatase from *Streptomyces coelicolor* A3(2). *J. Bacteriol.* **178**: 136-42.

[65]Li, Y., and W. R. Strohl. (1996). Cloning, purification, and properties of a phosphotyrosine protein phosphatase from *Streptomyces coelicolor* A3(2). *J. Bacteriol.* **178**: 136-42.

phosphatases and growth of *S. coelicolor* on minimal medium is reduced in the presence of these inhibitors. Because *ptpA* is located proximal to genes involved in the metabolism of sulfur amino acids, Li and coworkers suggest that PtpA may be involved in the regulation of sulfur amino acid metabolism (66).

The Cyanobacteria

Shown in Figure 1.2 is a schematic diagram of a typical cyanobacterial vegetative cell. Cyanobacteria have been found in almost every ecosystem examined to date and play a crucial role in maintaining the integrity of the atmosphere by replenishing molecular oxygen and removing carbon dioxide. Representatives of this diverse group of bacteria can exhibit unicellular or multi-cellular (filamentous) growth, inhabit deserts or fresh and marine waters, exist as free-living cells or in symbiosis with other organisms (67)(68), withstand repeated cycles of dessication and rehydration (69)(70), display circadian rhythms (71)(72), undergo

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[66]Li, Y., and W. R. Strohl. (1996). Cloning, purification, and properties of a phosphotyrosine protein phosphatase from *Streptomyces coelicolor* A3(2). *J. Bacteriol*. **178**: 136-42.

[67]Rippka, R., J. Deruelles, J. B. Waterbury, M. Herdman, and R. Y. Stanier. (1979). Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *J. Gen. Microbiol*. **111**: 1-61.

[68]Stanier, R. Y. and G. Cohen-Bazire. (1977). Phototrophic prokaryotes: the cyanobacteria. *Ann. Rev. Microbiol*. **31**: 225-74.

[69]Potts, M., and M. A. Bowman. (1985). Sensativity of *Nostoc commune* UTEX 584 (cyanobacteria) to water stress. *Arch. Microbiol*. **141**: 51-6.

[70]Scherer, S., A. Ernst, T. W. Chen, and P. Boger. (1984). Rewetting of drought-resistant blue-green algae: time-course of water uptake and reappearance of respiration, photosynthesis, and nitrogen fixation. *Oecologia* **62**: 418-23.

[71]Huang, T. C., J. Tu, T. J. Chow, and T. H. Chen. (1990). Circadian rhythm of the prokaryote *Synechococcus* sp. RF-1. *Plant Physiol*. **92**: 531-33.

[72]Kondo, T., T. Mori, N. V. Lebedeva, S. Aoki, M. Ishiura, and S. S. Golden. (1997). Circadian rhythms in rapidly dividing cyanobacteria. *Science* **275**: 224-27.

environmentally-induced cell differentiation (73)(74), and fix dinitrogen (75). All cyanobacteria are capable of fixing carbon (i.e., CO_2) by plant-like, oxygenic photosynthesis (76). Not surprisingly, in light of the aforementioned attributes, the cyanobacteria possess the largest genomes of any bacterial group (77).

Given the apparent unversality of regulatory protein phosphorylation, the study of phosphorylation in any bacterial species should provide clues for understanding this mode of regulation in all organisms, including the Eucarya. However, the cyanobacteria are uniquely suited among prokaryotes for this task, as they possess physiological properties otherwise unique to either eukaryotes (e.g., oxygenic photosynthesis) or prokaryotes (e.g., nitrogen fixation), the proper functions of which are believed to rely, at least in part, on protein phosphorylation (see below).

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[73]Stewart, W. D. P. (1980). Some aspects of structure and function in N_2-fixing cyanobacteria. *Annu. Rev. Microbiol*. **34**: 497-536.

[74]Stanier, R. Y. and G. Cohen-Bazire. (1977). Phototrophic prokaryotes: the cyanobacteria. *Ann. Rev. Microbiol*. **31**: 225-74.

[75]Rippka, R., J. Deruelles, J. B. Waterbury, M. Herdman, and R. Y. Stanier. (1979). Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *J. Gen. Microbiol*. **111**: 1-61.

[76]Houmard, J. (1994). Gene transcription in filamentous cyanobacteria. *Microbiol*. **140**: 433-41.

[77]Tandeau de Marsac, N. and J. Houmard. (1993). Adaptation of cyanobacteria to environmental stimuli: new steps towards molecular mechanisms. *FEMS Microbiol. Rev.* **104**: 119-90.
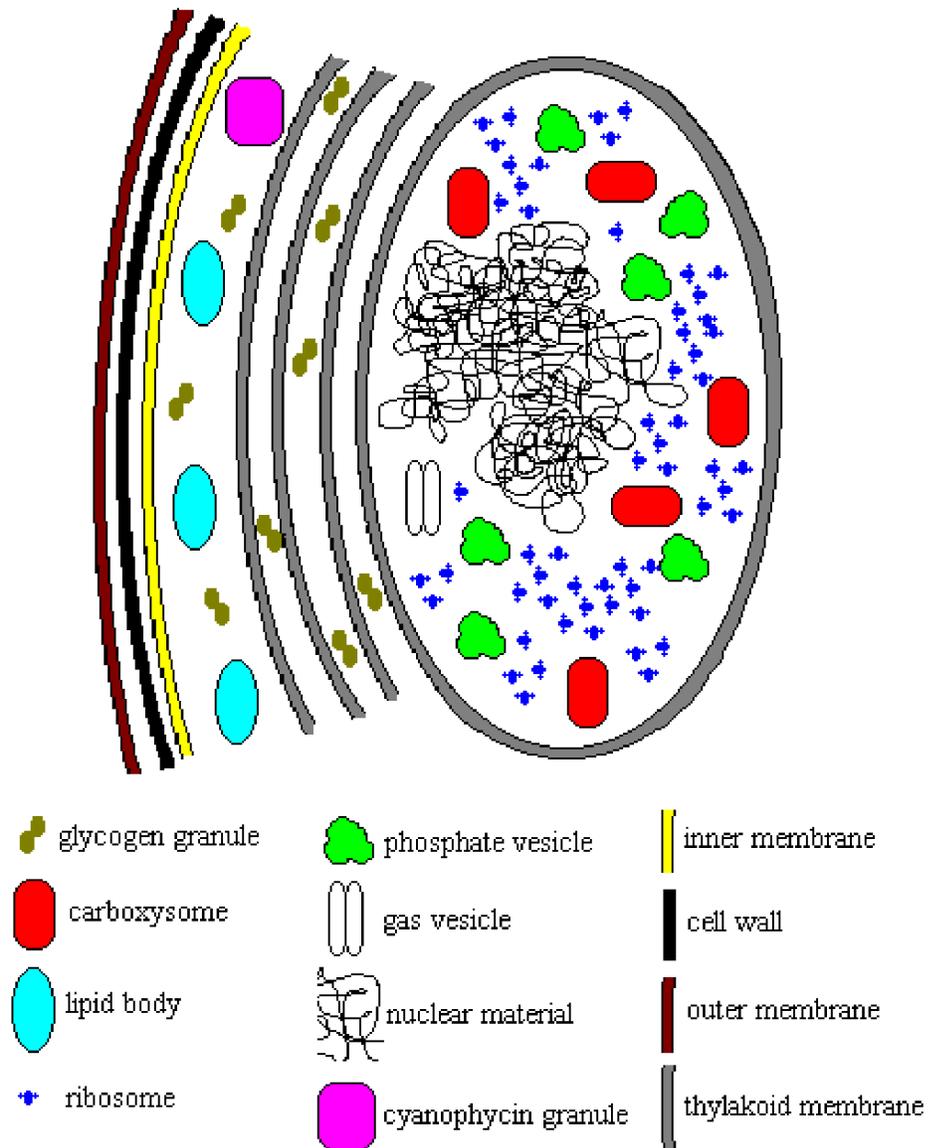
Figure 1.2
A schematic diagram of a typical cyanobacterial vegetative cell. Adapted from   Allen, M. M. (1984). Cyanobacterial cell inclusions. *Ann. Rev. Microbiol.* **38**: 1-25.

Protein Phosphorylation in the Cyanbacteria

Numerous reports have appeared describing the use of regulatory protein phosphorylation by the cyanobacteria (78). Evidence first emerged in 1985 when Allen and coworkers observed a correlation between the phosphorylation states of membrane proteins and the distribution of excitation energy (79). Since that time, protein phosphorylation has been implicated in the cyanobacterial response to carbon and nitrogen availability (80)(81), light quality (82)(83), and salt stress (84), as well as the regulation of carbon and nitrogen

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[78]Mann, N. H. (1994). Protein phosphorylation in cyanobacteria. *Microbiol*. **140**: 3207-15.

[79]Allen, J. F., C. E. Sanders, and N. G. Holms. (1985). Correlation of membrane protein phosphorylation with excitation energy distribution in the cyanobacterium *Synechococcus* 6301. *FEBS Lett*. **193**: 271-75.

[80]Bloye, S. A., N. J. Silman, N. H. Mann, and N. G. Carr. (1992). Bicarbonate concentration by *Synechocystis* PCC 6803: modulation of protein phosphorylation and inorganic carbon transport by glucose. *Plant Physiol*. **99**: 601-6.

[81]Forchhammer, K. and N. Tandeau de Marsac. (1994). The P_{II} protein in the cyanobacterium *Synechococcus* sp. strain PCC 7942 is modified by serine phosphorylation and signals the cellular N-status. *J. Bacteriol*. **176**: 84-91.

[82]Allen, J. F., C. E. Sanders, and N. G. Holms. (1985). Correlation of membrane protein phosphorylation with excitation energy distribution in the cyanobacterium *Synechococcus* 6301. *FEBS Lett*. **193**: 271-75.

[83]Sobczyk, A., G. Schyns, N. Tandeau de Marsac, and J. Houmard. (1993). Transduction of the light signal during complementary chromatic adaptation in the cyanobacterium *Calothrix* sp. PCC 7601: DNA-binding proteins and modulation by phosphorylation. *EMBO J*. **12**: 997-1004.

[84]Hagemann, M., D. Gollback. J. Biggins, and N. Erdmann. (1993). Salt-dependent protein phosphorylation in the cyanobacterium *Synechocystis* sp. PCC 6803. *FEMS Microbiol. Lett*. **113**: 205-10.

metabolism (85) (86), photosynthesis (87), and chemotaxis (88). To date, research has focused primarily on the role of protein phosphorylation in response to light and the regulation of the photosynthetic apparatus.

Protein Phosphorylation and Light Quality

Most cyanobacteria are obligate photoautotrophs. This property has been attributed to an incomplete tricarboxylic acid cycle and the lack of sugar permeases in cyanobacterial membranes (89)(90). Photosynthesis occurs within the thylakoid membrane, which contains two photosystems, PS-I and PS-II (Fig. 1.3A). Light energy is harvested by large, thylakoid membrane-associated structures called phycobilisomes that are functionally analagous to the light-harvesting complex (LHC) of chloroplasts in plants. Chlorophyll *a* is employed in the reaction centers of both PS-I and PS-II; however, chlorophyll *b* is not synthesized by cyanobacteria. Instead, the phycobilisomes of all cyanobacteria contain at least three unique light-harvesting proteins called phycobiliproteins (PBPs): phycocyanin, allophycocyanin, and

[85]Mann, N. H. (1994). Protein phosphorylation in cyanobacteria. *Microbiol*. **140**: 3207-15.

[86]Liang, J., L. Scappino, and R. Hasselkorn. (1992). The *patA* gene product, which contains a region similiar to CheY of *Escherichia coli*, controls heterocyst pattern formation in the cyanobacterium *Anabaena* PCC 7120. *Proc. Natl. Acad. Sci. USA* **89**: 5655-59.

[87]Allen, J. F. (1992). Protein phosphorylation in regulation of photosynthesis. *Biochim. Biophys. Acta*. **1098**: 275-335.

[88]Mann, N. H. (1994). Protein phosphorylation in cyanobacteria. *Microbiol*. **140**: 3207-15.

[89]Tandeau de Marsac, N. and J. Houmard. (1993). Adaptation of cyanobacteria to environmental stimuli: new steps towards molecular mechanisms. *FEMS Microbiol. Rev*. **104**: 119-90.

[90]Stanier R. Y. and G. Cohen-Bazire. (1977). Photrophic prokaryotes: the cyanobacteria. *Annu. Rev. Microbiol*. **31**: 225-74.
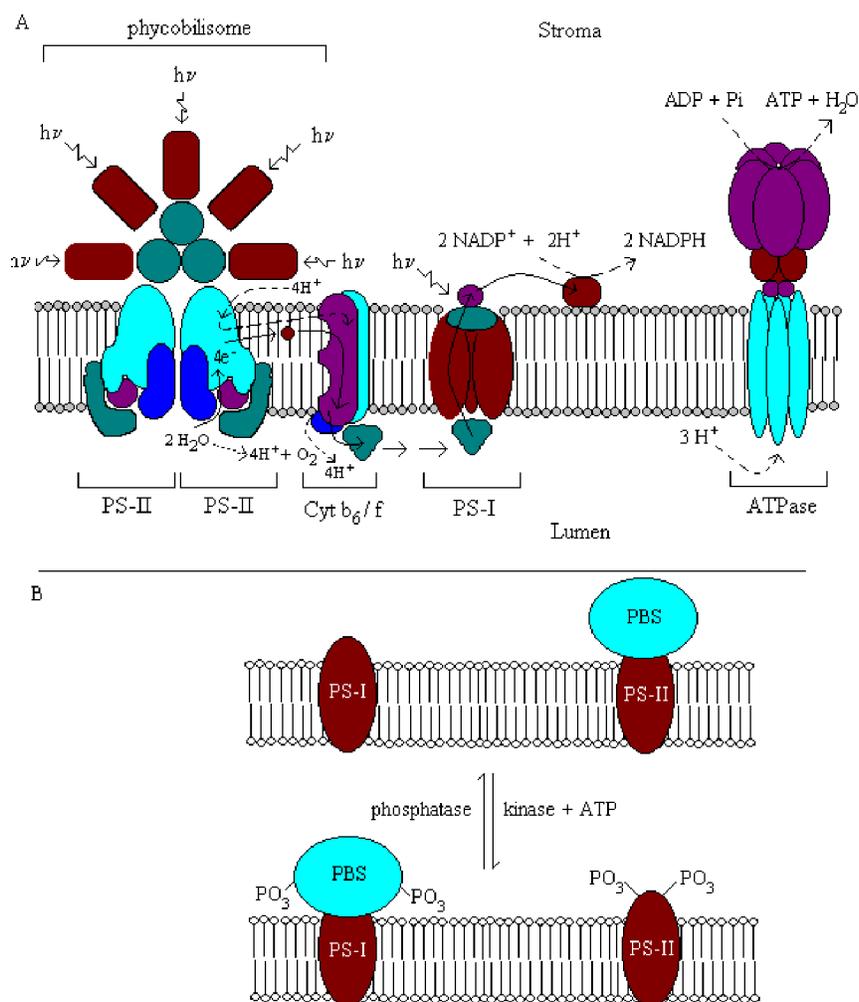
Figure 1.3

The photosynthetic apparatus of a typical cyanobacterium. Panel A (top), A schematic diagram of the thylakoid membrane. The light-harvesting phycobilisomes, shown here in association with two PS-II complexes, capture and transfer light energy to the photosystems with nearly 100% efficiency. The energy supplied by the phycobilisome is used by PS-II to lyse water, generating molecular oxygen, protons, and electrons. The protons are deposited into the lumen and contribute to the proton gradient, which drives ATP production by ATPase; the electrons are passed to PS-I and eventually to NADP+ on the stromal side of the membrane. Diagram adapted from Tandeau de Marsac, N, and J. Houmard. (1993). F*EMS Microbiol. Rev.* **104:** 119-90. Panel B (bottom), a model depicting the role of protein phosphorylation in a state transition. Adapted from Allen, J.F., C.E. Sanders, and N.G. Holmes. (1985). *FEBS Letts.* **193:** 271-75.

allophycocyanin B (91). Two additional PBPs, phycoerythrin and phycoerthrocyanin, are present in most cyanobacteria as well. The importance of these proteins is underscored by their abundance; during photosynthetic growth PBPs may account for as much as 50% of total cellular protein. Light energy captured by the phycobilisome is transferred to PS-II, which catalyzes the lysis of 2 molecules of water to produce O_2, $4e^-$, and $4H^+$. The protons are released into the lumen where they contribute to the proton gradient that drives ATP production. The electrons are passed to PS-I and eventually are used to reduce $NADP^+$ to NADPH. The NADPH and ATP are subsequently employed in the Calvin Cycle to assimilate carbon.

Cyanobacteria, like other photosynthetic organisms, can adapt to short-term changes in the spectral quality of incident light so as to maximize light-harvesting efficiency (93). This adaptation response, known as state transition, involves thepreferential shunting of excitation energy to either PS-II (State 1) or PS-I (State 2). It has been suggested that transition to State 2 is redox-controlled and involves the dissociation of phycobilisome-PS-II complexes and the formation of phycobilisome-PS-I complexes (94) (Fig. 1.3B). It was proposed that in *Synechococcus* 6301 the reversible phosphorylation of a 15kDa, thylakoid membrane protein induces the dissociation of the phycobilisome-PS-II complex (95). An 18.5kDa phycobiliprotein, β-phycocyanin, has also been shown to become phosphorylated (on a tyrosine residue) in response to illumination with PS-II-light, but not with PS-I-light. It was suggested that phosphorylation produces a photochromic form of the pigment

[91]Stanier R. Y. and G. Cohen-Bazire. (1977). Photrophic prokaryotes: the cyanobacteria. *Annu. Rev. Microbiol.* **31**: 225-74.

[93]Williams, W. P., and J. F. Allen. (1987). State 1/state 2 changes in higher plants and algae. *Photosynthesis Res.* **13**: 19-45.

[94]Allen, J. F. (1992). Protein phosphorylation in regulation of photosynthesis. *Biochim. Biophys. Acta.* **1098**: 275-335.

[95]Harrison, M. A., N. F. Tsinoremas, and J. F. Allen. (1991). Cyanobacterial thylakoid membrane proteins are reversibly phosphorylated under plastoquinone-reducing conditions in vitro. *FEBS Lett.* **282**: 295-99.

(96)(97). In addition, the protein could be subsequently dephosphorylated by removal to the dark, suggesting to the investigators the presence of a dark-activated PTP (98).

Other "dark-active" PTP activities have been described more recently in *Prochlorothrix hollandica* and *Synechococcus* PCC 7942 (99). In both cases, a protein of ~88kDa was found to be tyrosine phosphorylated when cultures were grown under conditions of high light intensity, but was not phosphorylated when the cultures were shifted to low intensity lighting conditions. However, it remains unclear as to whether a PTP activity is actually stimulated by dark conditions (as suggested by the authors), or whether, for example, a protein kinase activity is stimulated. Although preliminary steps have been taken to identify and characterize the phosphoproteins involved, no attempts have been made thus far to characterize either the PTK or PTP activities presumably responsible for their phosphorylation / dephosphorylation.

Protein Phosphorylation and Carbon Metabolism

Correlative evidence exists implicating protein phosphorylation in the uptake and utilization of CO_2 in *Synechocystis* PCC 6803. Bloye and coworkers (100) were able to demonstrate the accumulation of several phosphoproteins when cultures were shifted from low to high bicarbonate concentrations. However, it has been suggested that the observed alterations in the patterns of phosphorylation may be the consequence of a nutritionally-induced state transition under conditions of limited carbon availability (101).

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[96]Mann, N. H. (1994). Protein phosphorylation in cyanobacteria. *Microbiol*. **140**: 3207-15.

[97]Allen, J. F. (1992). Protein phosphorylation in regulation of photosynthesis. *Biochim. Biophys. Acta*. **1098**: 275-335.

[98]Allen, J. F. (1992). Protein phosphorylation in regulation of photosynthesis. *Biochim. Biophys. Acta*. **1098**: 275-335.

[99]Warner, K. M, and G. S. Bullerjahn. (1994). Light-dependent tyrosine phosphorylation in the cyanobacterium *Prochlorothrix hollandica. Plant Physiol*. **105**: 629-33.

[100]Bloye, S. A., N. J. Silman, N. H. Mann, and N. G. Carr. (1992). Bicarbonate concentration by *Synechocystis* PCC 6803: modulation of protein phosphorylation and inorganic carbon transport by glucose. *Plant Physiol*. **99**: 601-6.

[101]Mann, N. H. (1994). Protein phosphorylation in cyanobacteria. *Microbiol*. **140**: 3207-15.

Protein Phosphorylation and Nitrogen Metabolism

In addition to fixing carbon dioxide, many species of cyanobacteria assimilate inorganic nitrogen. Several strains are capable of fixing CO_2 (by oxygenic photosynthesis) while simultaneously fixing N_2. Like all nitrogen-fixing organisms, however, the cyanobacteria employ an extremely O_2-sensitive nitrogenase complex to incorporate dinitrogen. This paradoxical feat is accomplished in heterocystous cyanobacteria by the terminal differentiation of certain vegetative cells of the trichome into structurally and biochemically distinct cells called *heterocysts,* which are able to fix nitrogen, but are incapable of photosynthesis. The specialized heterocysts are surrounded by a thick envelope that presumably aids in maintaining an anaerobic intracellular environment. Thus, oxygenic photosynthesis and the oxygen-sensitive process of nitrogen fixation are spatially separated. In addition to the heterocystous cyanobacteria, some non-heterocystous strains, both filamentous and unicellular, can fix carbon and nitrogen concurrently. Very little is known, however, regarding the mechanisms employed by these bacteria for protecting nitrogenase from molecular oxygen. Temporal, rather than spatial, separation of nitrogen fixation and photosynthesis is generally believed to be the method of protection in most strains, with nitrogen fixation occuring predominantly in the dark (102). However, a nitrogen-fixing cycle has recently been described in the unicellular N_2-fixing species *Synechococcus* sp. RF-1 that may function independently of a light cycle, since cells grown in continuous light in nitrate-free medium still develop a circadian N_2-fixing rhythm (103).

Protein phosphorylation has been implicated in the cyanobacterial response to nitrogen starvation (104)(105). In 1993, Zhang provided the first direct evidence for the involvement of protein phosphorylation in the modulation of nitrogen metabolism when he

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[102]Gallon, J. R., and A. E. Chaplin. (1988). Recent studies on N_2 fixation by non-heterocystous cyanobacteria. In: Nitrogen Fixation: Hundred years after (Bothe, H., F. J. deBruijn, and W. E. Newton, Eds). pp. 183-88.

[103]Huang, T. C., J. Tu, T. J. Chow, and T. H. Chen. (1990). Setting of the circadian N_2-fixing rhthym of the prokaryote *Synechococcus* sp. RF-1. *Plant Physiol.* **92**: 531-3.

[104]Zhang, C. C. (1993). A gene encoding a protein related to eukaryotic protein kinases from the filamentous heterocystous cyanobacterium Anabaena PCC 7120. *Proc. Natl. Acad. Sci. USA* **90**: 11840-4.

[105]Mann, N. H. (1994). Protein phosphorylation in cyanobacteria. *Microbiol.* **140**: 3207-15.

reported the existence of a family of eukaryotic-like protein kinases in *Anabaena* PCC 7120 (106). The expression of one of the genes, *pknA*, was regulated during heterocyst development and appeared to participate in heterocyst pattern formation. The deduced amino acid sequence of the gene product exhibited significant sequence homology with the catalytic domain of the *Myxoccocus xanthus* protein serine / threonine kinase and eukaryotic protein serine / threonine kinases.

In 1993 it also was discovered that an indole-phosphate hydrolase from *Nostoc commune* UTEX 584 (107)(108) possessed the HAT sequence characteristic of eukaryotic protein tyrosine phosphatases (109). When the enzyme, designated IphP, was expressed in *E. coli* the recombinant protein showed DSP activity toward phosphoprotein substrates. Since IphP was encoded within the genome of *N. commune* UTEX 584, it represented the first DSP to be discovered whose origin was unambiguously bacterial. As mentioned previously, the physiological role of this DSP is unknown, but the concurrent discovery of an 85kDa phosphotyrosyl-phosphoprotein in cells grown in the presence of combined nitrogen, but not in nitrogen-starved cells, suggests that tyrosine phosphorylation may play a role in nitrogen metabolism in *N. commune* UTEX 584.

Further evidence that protein phosphorylation plays a role in nitrogen metabolism was provided by Forchhammer and Tandeau de Marsac in 1994 (110). In 1989, Sanders and coworkers described the effect of light regimes on *in vivo* protein phosphorylation in

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[106]Zhang, C-C. (1993). A gene encoding a protein related to eukaryotic protein kinases from the filamentous heterocystous cyanobacterium *Anabaena* PCC 7120. *Proc. Natl. Acad. Sci. USA* **90**: 11840-44.

[107]Xie, W-Q, B. A. Whitton, J. W. Simon, K. Jager, D. Reed, and M. Potts. (1989). *Nostoc commune* UTEX 584 gene expressing indole phosphate hydrolase activity in *Escherichia coli*. *J. Bacteriol*. **171**: 708-13.

[108]Whitton, B. A., M. Potts, J. W. Simon, and S. L. J. Grainger. (1990). Phosphatase activity of the blue-green alga (cyanobacterium) *Nostoc commune* UTEX 584. *Phycologia* **29**: 139-45.

[109]Potts, M., H. Sun, D. Mockaitis, P. J. Kennelly, D. Reed, and N. K. Tonks. (1993). A protein-tyrosine / serine phosphatase encoded by the genome of the cyanobacterium *Nostoc commune* UTEX 584. *J. Biol. Chem*. **268**: 7632-35.

[110]Forchhammer, K., and N. Tandeau de Marsac. (1994). The PII protein in the cyanobacterium *Synechococcus* sp. strain PCC 7942 is modified by serine phosphorylation and signals the cellular N-status. *J. Bacteriol*. **176**: 84-91.

Synechoccocus 6301. They observed that a 13kDa soluble protein was ^{32}P-labelled exclusively under PS-II light (111). It was discovered in 1991 that the 13kDa protein shared sequence similarities with the *Escherichia coli glnB* gene product, P_{II} (112). In *E. coli* and other enteric bacteria, the P_{II} protein is uridylylated on a tyrosine residue. The modified P_{II} protein in those organisms stimulates the deadenylylating activity of glutamine synthetase adenylyl transferase, leading to the activation of glutamine synthetase and subsequent glutamine production. The process is reversed by the demodification of P_{II} by P_{II}-uridylyltransferase / uridylyl removing enzyme which is itself regulated allosterically by α-ketogluterate. It was assumed initially that P_{II} modification in *Synechococcus* 6301 was the same as for other bacteria, i.e., modification by uridylylation. However, in 1994, Forchhammer and Tandeau de Marsac determined that P_{II} was not tyrosine-uridylylated in cyanobacteria, but in fact was phosphorylated on a serine residue. Moreover, they determined that phosphorylation of the P_{II} protein signals the cellular N-status and that P_{II} modification was only indirectly affected by the light regime (113).

[111]Sanders. C. E., M. Anastasios, and J. F. Allen. (1989). In vivo phosphorylation of proteins in the cyanobacterium *Synechoccocus* 6301 after chromatic acclimation to photosystem I or photosystem II light. *Biochim. Biophys. Acta*. **976**: 168-72.

[112]Tsinoremas, N. F., A. M. Castets, M. A. Harrison, J. F. Allen, and N. Tandeau de Marsac. (1991). Photosynthetic electron transport controls nitrogen assimilation in cyanobacteria by means of postranslational modification of the *glnB* gene product. *Proc. Natl. Acad. Sci. USA* **88**: 4565-69.

[113]Forchhammer, K., and N. Tandeau de Marsac. (1994). The P_{II} protein in the cyanobacterium *Synechococcus* sp. strain PCC 7942 is modified by serine phosphorylation and signals the cellular N-status. *J. Bacteriol*. **176**: 84-91.

Thesis Objectives:

Protein tyrosine phosphorylation represents a significant means for regulating key physiological phenomena in eukaryotic cells, such as the response to hormones and the consummation of the cell division cycle [114][115]. For many years this modification event was believed to be restricted to the members of this phylogenetic domain [116]. However, recent discoveries, such as that of a genomically encoded HAT protein phosphatase (IphP) in the cyanobacterium *Nostoc commune* UTEX 584, have raised new questions as to the origin of protein tyrosine phosphorylation and the extent to which it is utilized in nature. In these studies, we asked whether the neccesary components of a tyrosine phosphorylation network existed in *Anabaena* PCC 7120, a cyanobacterium amenable to genetic manipulation. In addition, the catalytic capabilities and the substrate recognition properties of IphP were examined in order to surmise the most plausible role of this phosphatase *in vivo*.

Specific objectives for the characterization of IphP were to
1. investigate the *in vitro* selectivity and efficacy of IphP by challenging the enzyme with a broad range of potential substrates;
2. determine if IphP could dephosphorylate cyanobacterial phosphoproteins *in vitro*.

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[114]Yarden, Y. and A. Ullrich. (1988). Growth factor receptor tyrosine kinases. *Annu. Rev. Biochem*. **57**: 443-78.

[115]Fischer, E. H., H. Charbonneau, and N. K. Tonks. (1991). Protein tyrosine phosphatases: a diverse family of intracellular and transmembrane enzymes. *Science* **253**: 401-6.

[116]Kennelly, P. J. and M. Potts. (1996). Fancy meeting you here! a fresh look at "prokaryotic" protein phosphorylation. *J. Bacteriol*. **178**: 4759-64.

Specific objectives for the elucidation of a tyrosine phosphorylation network in *Anabaena* were to

1. radiolabel phosphoproteins in extracts of *Anabaena* PCC 7120 employing endogenous protein kinases;
2. examine radiolabelled phosphoproteins for ^{32}P-phosphotyrosine content by phosphoamino acid analysis and selective dephosphorylation by a phosphotryosine-specific protein phosphatase;
3. characterize the major soluble protein tyrosine phosphatase activity from *Anabaena* PCC 7120.