CHAPTER I

GENERAL INTRODUCTION

Histidyl-Aspartyl (His-Asp) Phosphorelay Systems

To survive, bacteria monitor and adapt to changes in their growth environment (toxic chemicals, nitrate starvation, osmolarity, nutrition, etc.) through a family of signal transduction proteins, namely, sensor kinases and their cognate response regulators. The former are histidine kinases that sense some environmental parameter and the latter mediate changes in gene expression or cell behavior. This so-called two component system is unique in transferring the signal by the reversible phosphorelay of a phosphate between the histidine residue of a sensor kinase and the aspartic acid residue in a response regulator. In addition to the classic two component system which originated in 1986 (Nixon *et al.*, 1986), systems have been identified in multi-component pathway such as seven components involving chemotaxis pathway, and multistep phosphorelay (His-Asp-His-Asp) pathway in sporulation and anaerobic regulations. Currently, the system is described as the histidyl-aspartyl (His-Asp) phosphorelay system to note the unique mode of protein phosphorylation and dephosphorylation cascade among these adaptive signal transduction processes (Inouye, 1996). Once believed to be predominant in prokaryotes, the system has been identified also in eukaryotes including *Saccharommyces cerevisiae, Arabidopsis thaliana,* and *Neurospora crassa* (reviewed in Swanson *et al.*, 1994). These evidences suggest the His-Asp phosphorelay system is a universal mode of signal transduction both in prokaryotes and eukaryotes.

Bacterial Histidine Kinases

Histidine kinases are found in various bacterial sensory systems and characterized by a conserved C-terminal catalytic domain of 240 amino acids (Figure I-1). This catalytic domain contains five conserved features: a histidine residue which is the autophosphorylation site, an asparagine residue, a phenylalanine residue, and two-glycine-rich G1 (DXGXG) and G2 (GXGXG) boxes that are thought to be involved in ATP binding. Among histidine kinases, CheA is one exception since the autophosphorylation site (His-48) is located at the upstream of the catalytic domain. In addition, there found hybrid kinases exemplified by ArcB, that contain a sensor kinase domain and a response regulator domain at the C-terminal.

The autophosphorylation in histidine kinases are considered to occur in *trans* manner between the two identical subunits of a dimeric protein. Evidences have been reported for several histidine

second transmembrane domain (residues 163-179), and the cytoplasmic segment (residues 180-450). The C-terminal cytoplasmic segment is composed of a linker domain (residues 180-222) and the kinase/phosphatase domain (residues 223-450) (Forst *et al.*, 1987). From genetic analysis it has been demonstrated that EnvZ functions as a homodimer in which one subunit transphosphorylates the His-243 residue of the other using ATP (Yang and Inouye, 1991; Yang *et al.*, 1993). As a result of this auto-transphosphorylation reaction, high energy phosphoryl group is formed at the His-243 residue which is subsequently transferred to Asp-55 of OmpR. The phospho-OmpR thus generated binds to the upstream promoter regions of the porin genes, *ompF* and *ompC*, regulating their transcription (Mizuno and Mizushima, 1987; Slauch and Silhavy, 1989). EnvZ also functions as phosphatase, dephosphorylating phospho-OmpR, to inactivate its transcription enhancing activity. The removal of the high-energy phosphoryl group from phospho-OmpR may occur either by direct hydrolysis or by reverse transfer of the phosphoryl group to His-243 (Dutta and Inouye, 1996). Signal transduction through histidine kinases is unique in this ability of reversible transfer of the high-energy phosphoryl group between the conserved histidine and aspartate and thus termed the His-Asp phosphorelay system (Inouye, 1996).

The kinase/phosphatase domain of EnvZ has been isolated as a soluble polypeptide, with or without the linker domain (Forst and Roberts, 1994). This domain can be further dissected into two functional fragments, domain A (residues 223-289) and domain B (residues 290-450) (Park *et al.*, 1998). Domain A forms a stable homodimer and contains the auto-transphosphorylation site, His-243. The association constant (Ka) for EnvZ dimerization is estimated approximately 10^5 M^{-1} (Hidaka *et al.*, 1997). On the other hand, domain B exists as a monomer in solution, containing structural motifs conserved among histidine kinases (the N-box, the F-box, the G1 and G2 boxes). Neither domain A nor domain B alone can exhibit enzymatic activity. However, making the mixture of both domains in the presence of ATP, they can reconstitute the kinase activity: His-243 in domain A can be phosphorylated and this phosphoryl group is transferred to Asp-55 in OmpR (Park *et al.*, 1998).

The structural independence and functional complementation of domains A and B allowed the structure determination of each domain. In this study, the nuclear magnetic resonance (NMR)-derived solution structure of domain B is elucidated in chapter II. The structure revealed a unique kinase fold that is similar to DNA gyrase B subunit and heat shock protein 90 (Hsp90). Here, in

chapter III, the three-dimensional structure in solution of EnvZ domain A using multidimensional NMR spectroscopy is also investigated. This structure reveals that EnvZ domain A forms a four-helix bundle within the homodimer, providing the structural basis for the dimerization of the histidine kinase. The interaction of EnvZ and OmpR is also investigated in chapter IV, that suggests an importance of electrostatic interactions between the two proteins. Implications for the phospho transfer mechanism of the entire cytoplasmic kinase domain will be discussed.

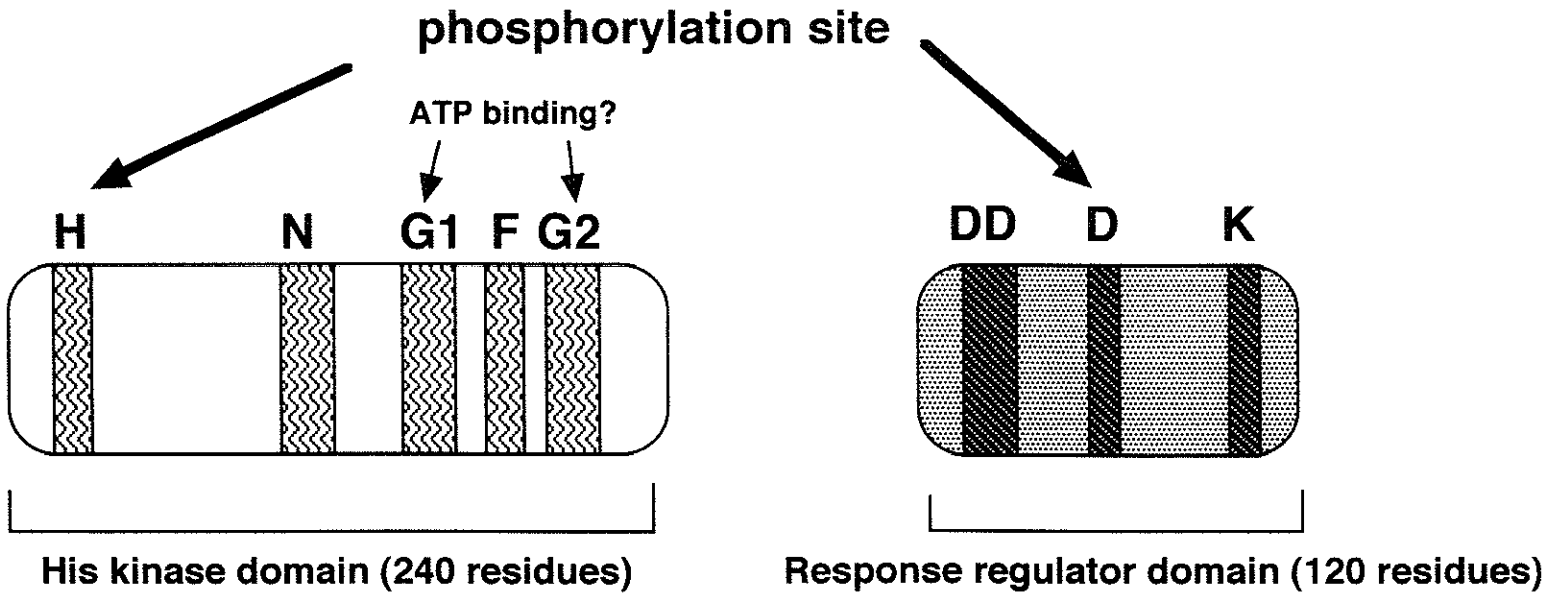

Figure I-1. Conserved sequence motifs of histidine kinase (left) and response regulator protein (right).

Characteristic sequence motifs in the most conserved domains of histidine kinase family and response regulators are indicated by boxes and labeled with the prominent amino acid residue.

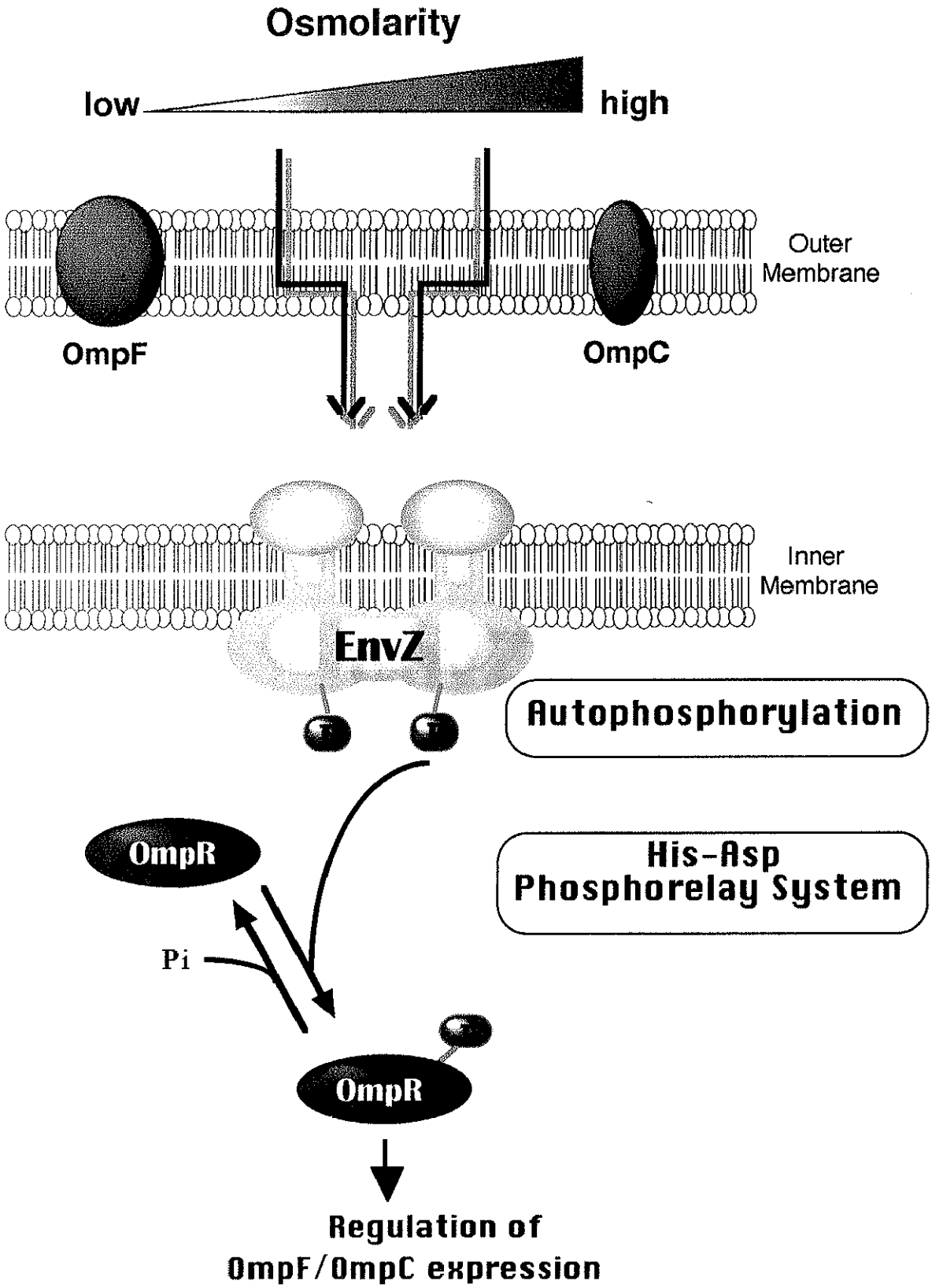

Figure I-2. Osmoregulation system in *E.coli*.

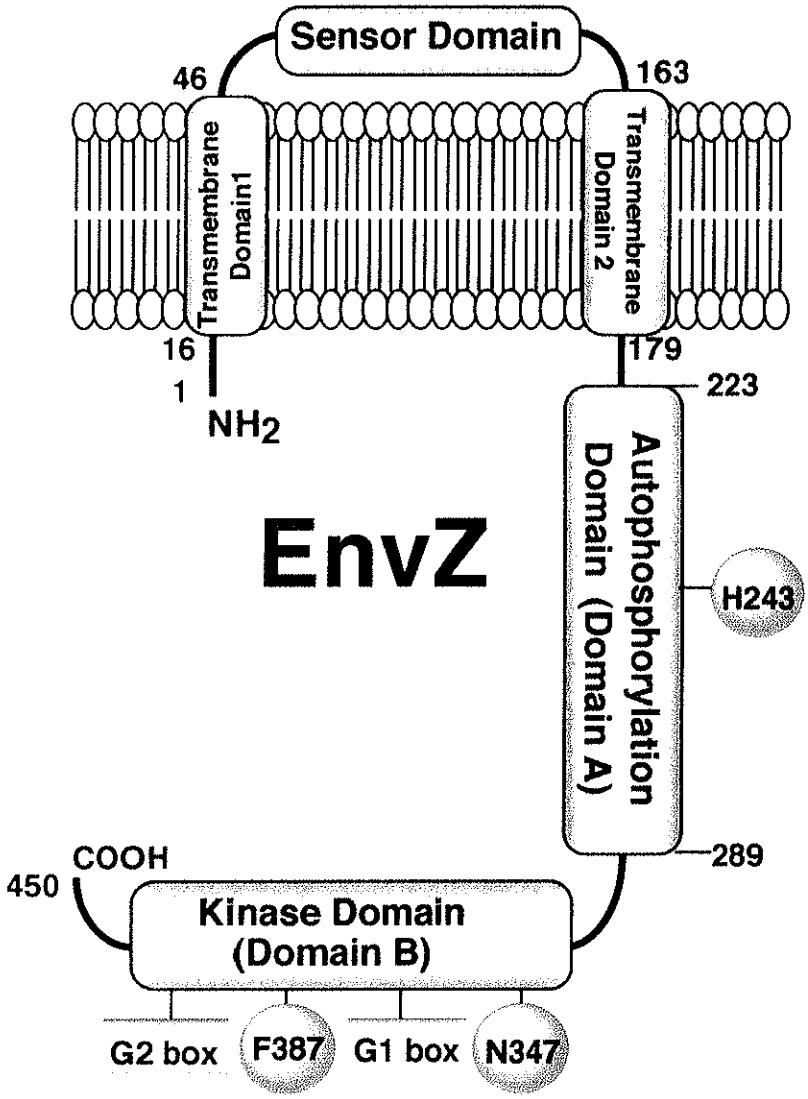

Figure I-3. Domain organization of EnvZ.

Conserved sequence motifs in the catalytic cytoplasmic region are labeled in circles and boxes.