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The Glucose Transpoter Enzyme $IIBC^{Glc}$ Recruits the Global Repressor Mlc

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The bacterial phosphoenolpyruvate:sugar phosphotransferase system (PTS) regulates a variety of physiological processes in addition to the catalysis of sugar uptake. Many genes are repressed or induced in the the presence of glucose. While the mechanism for carbon catabolite repression by glucose is well documented, that for glucose induction is not established. Recently, glucose induction of several genes has been shown to be mediated by the global repressor Mlc in *Escherichia coli*. We identify the general mechanism for glucose induction of gene expression in *E. coli*, showing a novel type of regulatory circuit for gene expression which is mediated by the phosphorylation state-dependent interaction of a membran-bound protein with a repressor. The dephospho-form of enzyme $IIBC^{Glc}$, but not its phospho-form, interacts directly with Mlc and induces transcription of Mlc-regulated genes by displacing Mlc from its target sequences. Glucose dephosphorylates the membrane-bound transporter enzyme $IIBC^{Glc}$, which in turn recruits Mlc to derepress the glucose-dependent expression of the Mlc regulon.

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A Mechanism of Redox-dependent Modulation of RsrA, an Anti-sigma Factor in *Streptomyces coelicolor*

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SigR (s^R) is a sigma factor responsible for inducing the thioredoxin system in response to oxidative stress in *Streptomyces coelicolor*. RsrA specifically binds to s^R and inhibits s^R-directed transcription under reducing conditions. Exposure to H_2O_2 or thiol-specific oxidant diamide dissociates s^R-RsrA complex. RsrA contains 7 cysteine residues in 105 total amino acid residues. Using single and multiple cysteine substitutuin mutagenesis into serines, we found out that 4 cysteines at 11, 41, 44, and 62st position are necessary for the activity of RsrA, as judged by s^R binding assay as well as inhibition of s^R-directed transcription *in vitro*. The mutation at each of these positions caused loss of s^R-binding ability, suggesting that the free thiol groups, not just the absence of disulfide bonds between them, are necessary for RsrA function. Since the essential thiols include those in the conserved HXXXCXXC motif, a putative metal binding site, we measured the content of metals in both wild type and triple-substitution mutant purified from *E. coli* containing *S. coelicolor rsrA* gene in pET15b, by atomic absorption and ICP-emission spectral analyses. Metals such as Ca, Mg, and Zn were detected in varying amounts depending on the batch of preparation. The content of Zn was systematically lowered under oxidized condition, suggesting that it depends on the thiol-disulfide status of the cysteines in RsrA and Zn may be involved in redox-dependent modulation of RsrA. However, the RsrA which was renatured in the absence of any metals from the guanidine-HCl treated sample resumed the ability to bind s^R, implying that metals are not required for the binding activity of RsrA. SPR analysis using BIACORE also demonstrated that oxidized RsrA resumed s^R-binding activity in the

absence of any metals.

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Purification of the *E.coli* expressed p60 Protein from Listeria welshimeriII by Amylose Resin Based Affinity Chromatography

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The Listeria welshimeriII is an animal and human pathogen and its p60 protein is a major extracellular protein, which is encoded in iap(invasion associated protein) gene. These proteins are believed to be involved in the invasion of these bacteria into their host cells. To produce p60 in *E.coli*, the iap gene was recombinantly cloned and overexpressed. A purification protocol was developed for MBP(maltose binding protein)-p60 fusion protein by amylose-resin based affinity chromatograpy. The purified MBP-p60 was detected either as denaturated or neutralized form using a specific p60 monoclonal antibody. The method might be an easy alternative to common purification protocols of p60 from Listeria spp. for antibody production.

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Studies on Mechanism and Binding Proteins of Human HtrA2, Serine Protease

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Human HtrA 2 (omi) is a recently described novel member of the mammalian serine proteases family homologous to the Escherichia coli Htr A gene that are essential for bacterial survival at high temperatures. Although the physiological function of this new family is unclear, the current understanding is that as well as being involved in the degradation of aberrantly folded proteins during the conditions of cellular stress, they may posses a chaperon-like role under nomal conditions. For understanding the mechanism of the HtrA 2 protein, we isolated HtrA 2 binding protein using the yeast two-hybrid assay. The pLex-HtrA 2, containging the gene for N-terminal region of the HtrA 2 inserted into PEG202 vector, was used as a bait plasmid to identify interacting protein gene from a human HeLa cDNA library. Final 16 X-gal positive colonies were selected from 1×107 colonies, isolated the plasmid containing the library cDNA and sequenced. The selected sequence represented the human karyopherin alpha 2, human embryonic ectoderm development protein (HEED), NADH dehydrogenase (ubiquinone) 1.

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HIV-1 Nef Decreased the Transcription Activity of the CREB-2

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Nef is a 27kDa myristoylated phosphoprotein expressed early in infection by HIV-1. To find the HIV-1 Nef interacting protein from T Jurkat cell, yeast two hybrid system was performed. The sequence determination and homology search of the isolated cDNA showed that one of the selected colonies encode the human cAMP