BICH/GENE 431 KNOWLEDGE OBJECTIVES

<u>Chapter 16 – Transcriptional Regulation in Prokaryotes</u>

Basic concepts: constitutive expression (unregulated), basal level, negative control with repressor, positive control with activator

Recruitment mechanism by activator

Activation by allostery – RNAP not recruited, but stimulates rate of open complex formation

Action at a distance – DNA looping; role of architectural proteins to bend DNA

E. coli lac operon:

- Jacob and Monod, Paris, 1950s, 60s
- functions of lacZ, lacY, lacA genes in operon
- role of operator, outcome of mutations there
- role of repressor, outcome of mutations, works as tetramer and can bind two operators
- effect of inducer (allolactose or IPTG)
- CAP (or CRP) activator protein – effect of cAMP and role of glucose on cAMP levels
- CAP works at many more promoters too, combinatorial control of transcription
- understand transcriptional outcomes in presence/absence of glucose, lactose
- target for CAP is RNAP alpha CTD
- understand significance of activator bypass experiments
- helix-turn-helix DNA binding motif; recognition helix in major groove forms H bonds with edges of base pairs; presence of HTH in CAP, lac repressor (lambda repressor, Cro repressor)

Alternative sigma factors: heat shock, nitrogen metabolism; cascade of sigma factors to control timing of gene transcription in Salmonella phage SPO1

Mechanism of activation by NtrC in E. coli: nitrogen metabolism (glnA promoter), sigma54, activation by stimulating open complex, role of ATP in this process, action at a distance

Basic mechanism of MerR activator in E. coli: merT promoter, twisting of DNA by MerR plus Hg+2

Mechanism of activation by AraC activator at araBAD promoter in E. coli

- basic function of this operon and regulation by arabinose and glucose
- CAP function too
- know how AraC binds differently +/- arabinose

Attenuation at E. coli trp operon (read Box 18-1 in Chapter 18, pp. 639-640)

- biosynthetic genes for trp encoded in operon
- controlled by trp repressor and by attenuation
- understand how mechanism works with leader, rho-independent terminator, coupled to translation of leader, alternative RNA hairpin formation, sensing of trp conc. by codons in leader
- other examples of attenuation are leu and his biosynthetic operons

Bacteriophage lambda lysis vs. lysogeny

Understand transcriptional regulation at P_R, P_L, P_{RM} by lambda repressor (CI) and Cro represson

- multiple operators at P_R, P_L and relative affinities by lambda repressor, Cro

- oligomerization of lambda repressor
- helix-turn-helix motifs for lambda repressor, Cro
- understand positive autoregulation by CI at P_{RM} promoter
- understand negative autoregulation by CI at P_{RM} promoter

Lysogenic induction

- RecA induces autoproteolysis of lambda repressor; what is biological significance?
- Understand course of transcriptional regulatory events leading to induction

Decision between lysis/lysogeny after initial infection by lambda

- roles of CII, CIII proteins
- function of P_{RE} and activation by CII
- relative ratio of Cro:CII proteins controls this decision
- role of E. coli FtsH protease on CII protein levels
- role of relative ratio of phage particles to bacterial host cells in this choice
- effect of E. coli growth conditions (i.e., presence of glucose) on FtsH activity, CII levels and decision between lysogeny vs. lysis

CII activates other promoters besides P_{RE}: P_I promoter to produce integrase protein; P_{AQ} promoter controls amount of antitermination factor Q