Chapters 7 & 8. Weaver, 2/e. Mol Biol X107A.

Ch 7. Operons: Fine control of prokaryotic transcription.
Ch 8. Major shifts in prokaryotic transcription.

Reading note. The only required section in Weaver is Ch 7 Sect 1, on the *lac* operon. Consistent with that, Sect G, below, is not required reading.

Clark & Russell. The later parts of Ch 6 deal with gene regulation, including the *lac* operon and more. They describe the enzyme β-galactosidase on p 64.

A. Introduction

In Ch 6 we learned about the transcription process. We noted that transcription, especially initiation of transcription, is a common target of regulation. We saw how assembly of the transcription apparatus involves proteins interacting with DNA, some in a sequence-specific manner. It is also clear that other proteins interacting with DNA could affect transcription, by enhancing or inhibiting transcription steps.

In Ch 9 we will look at some aspects of how DNA and protein interact. But first, as more specific background, we will examine one regulatory system in some detail.

Highlights:

- The *lac* operon in E. coli is a paradigm of bacterial regulation.
- The ideas of positive and negative control.
- The general ideas that proteins modulate the function of DNA, and that other molecules may modulate the activity of such DNA-binding proteins.

- Regulatory systems have different degrees of specificity.

- Proteins may bend the DNA (or otherwise change its shape).

B. Terms

Induction and repression refer to the general phenomena of affecting (increasing or decreasing, respectively) the synthesis of enzymes. Examples: lactose induces synthesis of enzymes needed to utilize lactose; histidine represses synthesis of enzymes needed to make histidine. In these cases, a metabolite triggers the response; however, we shall see other situations, such as heat induction.

These terms do not imply a mechanism. The sugars lactose and arabinose both induce their respective operons in E. coli, but the mechanisms are very different.

> Caution. The term "repress" is used in two somewhat distinct ways. Tryptophan represses the synthesis of enzymes for making Trp; this usage agrees with the above point, and does not imply a mechanism. In addition, one mechanism of regulation of the Trp enzymes involves a protein binding to the DNA, preventing (repressing) gene function; this protein is called a repressor (p 197). [The small molecule (such as Trp) that interacts with a repressor protein to turn off gene function is called a co-repressor.]

Positive and negative control refer to how a regulatory protein affects the synthesis of enzymes. Examples: lactose induces the *lac* genes by inactivating a protein repressor that is otherwise preventing transcription; this is induction with negative control. Arabinose induces the *ara* genes, in part, by activating a protein activator that then promotes transcription; this is induction with positive control. (Weaver is not very clear about the positive control here; more in Sect G.)

Constitutive. We call a gene constitutive when its product is always present at about the same level. The term is particularly useful for describing mutants that have lost normal regulation.

> It is also used for genes that appear to always function at the same level naturally. However, that does not mean that such a gene is unregulated. That may be the case, or it may just be that we have not found the regulation system. Further, even if the gene does function at a constant level, that level is genetically controlled, by the strength of the promoter and the ribosome binding site. Different constitutive genes function at different rates.
>
> Weaver's multiple Glossary entries for "constitutive" are useful.

Coordinate regulation means that a group of gene products are made in constant proportion. Coordinate regulation of the genes in an operon is the norm, because the regulation is often at the level of controlling synthesis of the polycistronic mRNA. For example, removing a repressor might increase mRNA production 1000-fold, and give a 1000-fold increase in the rate of synthesis of each gene product.

This does not mean that the absolute level of expression of all genes in the operon is the same. Reasons for genes in an operon being expressed at different levels include: different stability of mRNA segments; different strength of ribosome binding site for each gene. These issues will come up later.

Unfortunately, "coordinate regulation" is now sometimes used in a looser sense of "regulated together", with no implication of constant proportion.

Feedback inhibition [not explicitly discussed]. A metabolite may affect not only the synthesis of new enzymes, but also the activity of existing enzymes. The latter provides a more rapid modulation of the pathway. A common example is that the end product of a pathway "feeds back" to inhibit the pathway, specifically by inhibiting the first step; this is called feedback inhibition.

C. Lac operon: negative control

We use the *lac* operon of E. coli as our main example of regulation in bacteria. Our primary goal is to examine individual regulatory mechanisms. In so doing, we will oversimplify the system. We also examine how genetic analysis helps us uncover regulatory mechanisms.

Lactose induces three enzymes:

- β-galactosidase, which hydrolyzes lactose to glucose + galactose. Fig 7.2. The glucose is metabolized normally. Gal is easily converted to Glc; that's a separate story.
- Permease, which transports lactose into the cell.
- Acetylase. This enzyme is not part of lactose metabolism, and its real role is unknown. However, its gene is part of the *lac* operon, and it is an easy enzyme to assay.

The genes for these enzymes are *lacZ*, *lacY*, and *lacA*, respectively. They are consecutive, in the order given: zya. (Fig 7.3)

If Lac is absent, very low "basal" levels of these enzymes are present. If Lac is added, all three are made. Thus Lac induces the Lac enzymes; the induction is coordinate.

This addresses only the role of lactose in regulating the *lac* genes. For more complexity, see Sect D, below.

Weaver notes that the actual metabolic inducer is allolactose, Fig 7.4. This complication is not important for us. Operationally, the inducer is lactose.

In the lab, one often uses a non-metabolizable analog as an inducer. IPTG (isopropylthiogalactoside; Fig 7.6) is a common one.

We can easily distinguish Lac^+ and Lac^- strains of E. coli. Only Lac^+ strains grow if Lac is the sole C-source available. Further, there are simple conditions where both kinds grow, but form different color colonies:

- pH indicator plates. Colonies that ferment Lac (or another sugar) produce acid.

- "Blue dye" plates. The lactose analog "X-gal" is used as the substrate. Hydrolysis gives a blue product, which remains localized to the colony. (Noted in Fig 4.11.)

We can find the following kinds of mutants (among others):

- Mutants that cannot grow on lactose. Lac^- phenotype. These include mutants defective in the z or y genes, or in the promoter.

- Mutants that make the Lac enzymes even if Lac is not present. They are Lac^c, constitutive for the Lac enzymes. They make normal enzymes, but are altered in the regulation.

We can create E. coli that are diploid for the *lac* genes. This allows us to test for dominance. For example:

constit $z^- a^+$

inducible $z^+ a^-$

The regulatory mutation is on one chromosome; it is shown here, without prejudice as to its nature, as "constit". We can tell which of the two chromosomes is functioning, because one can produce only the A enzyme, the other can produce only the Z enzyme. (The z and a genes are "reporter" genes.)

There are two kinds of results, depending on the Lac^c mutation. Both are shown in Fig 7.5 (along with other mutations we do not need at the moment).

- For some Lac^c mutations, the diploid constructed as above is inducible for both enzymes, just like wild type. Thus, inducibility is dominant. (Fig 7.5.a.)

- However, some Lac^c mutations show a different behavior. Z is inducible, but A is constitutive. The two chromosomes behave differently! The mutation to constitutivity affects only the chromosome it is on; it is said to act cis, or to be cis-dominant (in contrast to trans, or acting on other chromosomes, too). (Fig 7.5.b.)

The model that evolved from this work is as follows. The wild type organism makes a protein that turns off the *lac* genes. We call the protein repressor, and its gene *i* (or *lacI*), for inducibility. The repressor turns off the *lac* genes by binding near the start of the genes, at a site we call operator, or *o*. Apparently, in the presence of lactose, the repressor can no longer bind to *o*, so the *lac* genes are free to function.

> Repressor presumably functions by -- somehow -- blocking RNA polymerase. The original idea was that repressor blocked Pol binding. Weaver notes (pp

> 182-3) an alternative hypothesis, that the repressor may inhibit a later stage of the initiation process, such as promoter clearance. While this specific case may be debated further, the important point is that any mechanism that works is reasonable, and may occur in one case or another.

There are two ways this system can mutate to be constitutive. It can mutate in the *i* gene, so that no repressor is made. Or it can mutate at the *o* site, so that the repressor can no longer bind there. I^c mutations (i.e., I^-) act like ordinary Mendelian recessives, resulting from loss of a function. However, the O^c mutations are cis-acting; preventing the repressor from binding to one operator does not affect its binding to another operator.

This model, proposed by Jacob and Monod in 1961, is now known to be correct. The repressor has been isolated. In vitro, it binds to O^+ DNA, preventing transcription. Addition of lactose changes the conformation of the repressor, so that it no longer binds to the operator (example of allostery). Figs 7.6 & 7 show some of these results.

Defining the operator. Genetic: mapping the operator mutations discussed above. Footprinting: physical identification of the operator DNA and of specific points of interaction, recall methodology discussed earlier for identifying promoters, Ch 6. Recombinant DNA work: allows us to make variant operators (and repressors) at will. X-ray crystallography data on the structure of some operator-repressor complexes; Fig 7.12.

Length of operator. ~12 bases. $4^{11} = 4 \times 10^6$, the length of the E. coli genome. Roughly, this indicates that the operator sequence is the right length to identify a unique sequence in the genome.

The *lac* operon actually has three operators, Fig 7.10. We now understand how this complex array of operators achieves full repression, Fig 7.11 & 12. Go back and look at Fig 7.3a; the repressor is shown as a tetramer, but it is not obvious why. The discovery of multiple operators explains the role of the repressor tetramer.

> In terms of historical development, we are lucky that the full complexity was not apparent earlier. In fact, it is a general characteristic of the type of genetic analysis used in the early work that it tends to uncover one key player at a time. We always need to be alert that the complete system is more complex than the initial genetic works suggests. We now understand that regulatory systems often have many parts, sometimes with each part having only a subtle role. "Combinatorial control" has become a buzzword in gene regulation.

D. Lac operon: positive control; catabolite repression

Above, we saw that the Lac operon is induced by lactose. It is also repressed by Glc. This effect is termed "catabolite repression". More generally, the term refers to the preferential use of the "better" carbon source when fed a mixture of carbon sources. Glc supports faster growth of E. coli than does lactose (Fig 7.1). When E. coli is fed a mixture of Glc and Lac, it preferentially uses the Glc (Fig 7.1); the Glc prevents induction of the Lac enzymes, even

though Lac is present. (Hence the term "glucose effect" often used in the older E. coli literature.)

We now know part of the mechanism of this effect. Glucose lowers the level of cyclic AMP (Fig 7.13). (How this is done is not clear). cAMP is a co-activator of a protein called CAP, catabolite activator protein. That is, CAP is active only when bound with cAMP, and cAMP is high only when Glc is low. cAMP-CAP is a positive regulator of many "catabolite-sensitive" genes, including the *lac* genes. That is, gene function requires that this positive control protein be present on the gene. (In contrast, a negative control protein inhibits gene function, and must be removed.)

How does CAP-cAMP work? It helps RNA Pol bind; specifically, it does this by interacting with the α subunit (Fig 7.17). The lac promoter is a quite weak promoter -- without this extra activation. (It's an interesting point that weak promoters are easier to regulate.) Not all activators work the same way, but this example gets us started. (Recall Lohrke et al, 1999; Ch 6 FR.)

Another aspect of CAP function is that it bends the DNA. See next Section for more.

> [The catabolite repression system does not itself turn on genes; it merely allows them to be turned on. It is something of a "master control system", a concept often invoked with eukaryotes.]
>
> [CAP is also known as CRP = cAMP receptor protein. (p 185)]

E. DNA bending

Weaver introduces the idea of a DNA-binding protein changing the shape of the DNA it binds to. He starts with the example of CAP, and summarizes the effect in Fig 7.21. DNA looping, Fig 7.18, is a related phenomenon. There is more about this general type of effect in Ch 9.

How do we detect DNA bending? Sometimes by direct observation, e.g., Fig 7.20 or 9.24.

Another method, based on electrophoresis of the protein-DNA complex, is shown in Fig 7.19.

> Think about… Would the experiment of Fig 7.19 work if the initial DNA were linear?

F. Lac operon: summary

As you review the Lac story, look for:

- The signal(s): concentration of Lac in the medium, under control of investigator; also, concentration of Glc.
- The response: change in synthesis of Lac enzymes.

- The mechanisms
  - Initiation of transcription
  - Negative control
  - Positive control
  - cis-acting sites
  - Small molecules modulating the activity of regulatory proteins
  - Combinatorial control, with varying specificity:

    Regulatory proteins (and sites) vary in specificity. Three regulatory proteins are required for the *lac* operon to function. One, σ^{70}, is very general; it is required for most (but not all) E. coli genes. One, CAP, is required for several operons that share the feature of being induced upon glucose starvation. One, the Lac repressor, is specific for the *lac* operon. This combination of regulatory proteins, of differing specificity, allows for complex control. We refer to this as combinatorial regulation.

Condemine (2000) introduces more features of the Lac system.

G. Other systems; briefly noted

In this section I will briefly note some key features of the regulatory systems from others parts of Ch 7-8.

⇒ *You are not responsible for this Section.*

mal regulon. In the *lac* operon, above, we dealt with regulation of a group of contiguous genes, transcribed from a single promoter. However, a single regulatory protein can control at more than one site on the genome. A regulon is a group of non-contiguous genes, transcribed from separate promoters, but sharing a regulatory system. In fact, the CAP protein, discussed above, is already an example of such a system.

ara operon. A good example of DNA looping. It is also a good example of positive control, although Weaver does not really discuss this much. In Fig 7.25c, you see that the AraC protein, with Ara, now binds at sites I_1 and I_2. In fact, this binding is required for transcription. That is, the AraC protein is an activator protein, required for transcription. This aspect of AraC function was what was revealed initially by genetic analysis. Later work revealed the greater complexity of the system, with the AraC protein acting both as a repressor and an activator. See Dhiman & Schleif (2000) for more.

trp operon. This is a biosynthetic (anabolic) operon. The presence of Trp is a signal to turn off the operon. In contrast, the sugar operons above are all degradative (catabolic); presence of the namesake sugar is a signal to turn on the operon. The main novel feature of the regulatory system is the attenuation system. Here, control is mediated via premature termination of transcription, rather than initiation. Yanofsky (2000) reviews attenuation.

Ch 8. The broad theme is major changes in transcriptional patterns. Most of the transcriptional changes discussed in this chapter might be termed "developmental", in the sense that there is a programmed sequence of gene function, e.g. during a phage infection or sporulation. (By "programmed" I mean that no external stimulus is required. Of course, heat shock is an exception.) The broad "answer" is that the specificity of initiation by RNA Pol is altered -- by various detailed methods. If one thinks of this broad view, then the examples of bacterial transcriptional control discussed here do provide some precedent for how more complex (eukaryotic) systems might be regulated. However, the specifics have no particular significance.

The work discussed in the first three sections was of great historical importance; these are the cases that helped us formulate our general views of the role of Pol specificity changes.

The example of phage T7 has a practical implication. This phage makes its own, small and highly specialized, RNA Pol. Molecular biologists have exploited this specialized Pol, as Weaver notes (p 208; Fig 4.11). (Also recall Cheetham & Steitz, 1999; Ch 6 FR.)

Section 8.4, Genes with multiple promoters, opens up an interesting general topic… multiple regulatory systems for a particular gene, so that it can function under different sets of conditions. A simple approach to solving this problem is to allow more than one type of initiation event. This may involve having more than promoter, as discussed here, or more than one regulatory protein.

Of particular note in Sect 4 is σ^{54}, a somewhat unusual σ factor. I'm not sure it should be described as "defective" (p 213, bottom of left column), but learning about this σ was instrumental in forcing us to expand our views of how σ factors work. In some ways, σ^{54} is more typical of how "transcription factors" work in eukaryotes. Buck et al (2000) review σ^{54}; also see Volkman et al (2001).

Section 8.6, on phage λ, discusses a well understood set of regulatory events in this classic phage system. The biology is largely idiosyncratic, but the regulation is interesting. In a two semester course, we would spend some time on this, because we are able to describe so much of this viral life cycle in satisfying detail. Among the big issues discussed here… competition between regulatory systems; control of transcriptional termination. Shotland et al (2000) and Xu & Koudelka (2000) are examples of recent work.

Vivas & Goodrich-Blair (2001) show that a particular σ factor is needed for a bacterium to colonize one animal host -- but not another.

Several FR discuss examples of bacterial regulatory systems.

H. Further reading

General

H Yan et al, Genetics: Genetic Testing - Present and Future. Science 289:1890, 9/15/00. A "Tech.sight" feature. Discussion of genetic testing methods. One area highlighted is a new method the authors developed recently to improve analysis of heterozygotes, by examining the two alleles separately.

Classic papers

Reading some of the classic papers of molecular biology (or any field of science) can be a fun way to explore its history -- and the different style of scientific papers long ago. Studying such papers is probably an inefficient way to learn the basics, but can be a good supplement when you are so inclined.

I listed some classics in the Ch 1-3 handouts, either as collections or individual papers. Also, it used to be common to publish compilations of classic papers. The books contain papers, either complete or abridged, usually with commentary. Here are some that I have, all of which are available in the UCB library. Some may have a more recent edition than the one I list. (Sometimes you see books such as these in used book stores, or book exchanges, such as that at the El Cerrito recycling center.)

T Brock, Milestones in Microbiology, 1961.

J A Peters, Classic Papers in Genetics. 1959.

G S Stent, Papers on Bacterial Viruses. 1960.

J H Taylor, Selected Papers on Molecular Genetics. 1965.

This Chapter

> Many papers could easily be classified as Ch 7-8 or 9, since the subject matter of these chapters overlap so much.

R Kolter, Growth in studying the cessation of growth. J Bact 181:697, 2/99. Commentary. E coli is most famous for its rapid growth. However, in Nature it undoubtedly spends a good portion of its time not growing -- in what we call, perhaps inappropriately, stationary phase. Roberto Kolter has been a leader in working out how E coli manages stationary phase, including the transition to that phase. A special σ factor, now usually called σ^S, is part of the story.

I N Olekhnovich & R J Kadner, RNA polymerase α and σ^{70} subunits participate in transcription of the Escherichia coli uhpT promoter. J Bact 181:7266, 12/99. An analysis of the protein interactions that activate a promoter entirely lacking a -35 region.

C Yanofsky, Transcription attenuation: Once viewed as a novel regulatory strategy. J Bact 182:1, 1/00. Commentary. Broad overview of the role of attenuation, from one of its discoverers. Includes variations, such as the trp operon in Bacillus subtilis, where a protein is involved in the attenuation mechanism (p 200). When Trp is at high level, it binds to the protein, which can then bind to the mRNA and cause termination of mRNA synthesis. The protein is active as an 11-mer, which binds to eleven GAG's in the RNA. The TRAP protein wraps a long segment of the mRNA around itself, in a way that allows the terminator to form.

D H Green & S M Cutting, Membrane topology of the Bacillus subtilis Pro-σ^K processing complex. J Bact 182:278, 1/00. A recent paper on the role of σ factors in Bacillus sporulation (Ch 8 Sect 3).

G Condemine, Characterization of SotA and SotB, two Erwinia chrysanthemi proteins which modify isopropyl-β-D-thiogalactopyranoside and lactose induction of the Escherichia coli lac promoter. J Bacteriol 182:1340-1345, 3/00. An interesting but somewhat confusing story. He is cloning genes between the plant pathogen Erwinia and the more convenient experimental organism E coli. Certain Erwinia proteins prove to be toxic in E coli. Sorting out the reason for this leads to discovery of some genes for proteins whose main role may be to transport toxic sugars out of the cell. What makes this particularly interesting is that these efflux proteins have broad specificity, and are probably closely related to the so-called MDR (multidrug resistance transporters) that play havoc with antibiotic treatments.

Y Shotland et al, Proteolysis of bacteriophage lambda CII by Escherichia coli FtsH (HflB). J Bacteriol 182:3111-3116, 6/00. One aspect of phage λ regulation is instability of a key regulatory protein, mediated by proteolysis.

J Xu & G B Koudelka, Mutually exclusive utilization of P_R and P_{RM} promoters in bacteriophage 434 O_R. J Bacteriol 182:3165-3174, 6/00. Analysis of the competition between two mutually exclusive promoters in a lambdoid phage.

M Buck et al, The bacterial enhancer-dependent σ^{54} (σ^N) transcription factor. J Bacteriol 182:4129-4136, 8/00. Minireview. Overview of this unusual type of bacterial σ factor, which seems to have features of eukaryotic transcription. In particular, σ^{54} always requires an activator protein, which often attaches to a "distant site" and communicates with the Pol by DNA looping.

A Khvorova et al, The chromosomal location of the Bacillus subtilis sporulation gene spoIIR is important for its function. J Bacteriol 182:4425-4429, 8/00. To a first approximation, where a gene is located -- or which strand it is on -- does not affect its function. However, during Bacillus subtilis spore formation, the chromosome is transferred into the spore compartment in an ordered manner. Here they show that a particular regulatory gene must function in the prespore early in the spore formation process. Thus the gene must be near the part of the chromosome that is transferred early.

A Dhiman & R Schleif, Recognition of overlapping nucleotides by AraC and the sigma subunit of RNA polymerase. J Bacteriol 182:5076-5081, 9/00. More analysis of the AraC activator. Since the AraC activation site includes part of the -35 promoter region, the question

is whether σ binds to the -35 at all. They show that those -35 region bases not recognized by AraC are still critical, suggesting that σ does indeed bind there.

B F Volkman et al, Two-state allosteric behavior in a single-domain signaling protein. Science 291:2429, 3/23/01. (+ News, Buck and Rosen, p 2329.) This is on the NtrC protein, the activator for the E. coli σ^{54}. But what is most important is their methodology, the use of NMR to study millisecond-microsecond range fluctuations in protein structure during the activation process. Actually, their methods extend down to the picosecond range, though the slower ones turned out to be most important.

H-J Choi et al, Structural basis of the redox switch in the OxyR transcription factor. Cell 105(1):103-113, 4/6/01. The OxyR regulon is part of the cellular defense mechanisms, against oxygen toxicity. Specifically, the OxyR factor is activated by H_2O_2, and then activates transcription of protective genes. How does the protein respond to oxidation? H_2O_2 causes formation of an intramolecular disulfide bond in the OxyR protein; the details of how this happens are still open for debate. The oxidized and reduced forms of OxyR have different structures -- and different DNA-binding properties.

B Hayes, Computing comes to life. Amer Sci 89:204, 5/01. A computer scientist tries to model regulatory systems.

Y-H Dong et al, Quenching quorum-sensing-dependent bacterial infection by an N-acyl homoserine lactonase. Nature 411:813, 6/14/01. (+ News, Leadbetter, p 748.) An article on a fascinating phenomenon in bacteria: their behavior as groups. The first observation involved luminescent bacteria: they glow only at high population density. Why? They secrete the inducer [of the luminescence genes] into the medium. Only at high population density is there enough inducer in the medium to re-enter the cells and induce. The same basic phenomenon has now been found widely in bacteria, and is referred to as quorum sensing. It is probably involved in some bacterial pathogenesis processes. For example, quorum sensing has been implicated in the development of biofilms by Pseudomonas -- a phenomenon that might be relevant in cystic fibrosis. Here, they explore the possibility of blocking pathogenesis by interfering with the chemical signaling that is behind quorum sensing. In a test model, they show that tobacco plants expressing an enzyme that degrades the quorum-sensing chemical does protect the plant.

A Kuroda et al, Role of inorganic polyphosphate in promoting ribosomal protein degradation by the Lon protease in E. coli. Science 293:705, 7/27/01. (+ News, Gottesman & Maurizi, p 614.) An interesting story of how E. coli responds to amino acid starvation. Synthesis of polyphosphate is increased, and that complexes with a key protease and promotes degradation of (free) ribosomal proteins. A co-author is Stanford's Arthur Kornberg, who is most famous for discovering the first DNA polymerase; he has spent recent years exploring the role of polyphosphate.

E I Vivas & H Goodrich-Blair, Xenorhabdus nematophilus as a model for host-bacterium interactions: rpoS is necessary for mutualism with nematodes. J Bacteriol 183:4687-4693, 8/01. They show that mutant bacteria lacking a particular σ factor are unable to colonize the nematode gut. However, the same mutant bacteria display unchanged pathogenicity towards an insect host.

S M Hoffer et al, Autoamplification of a two-component regulatory system results in "learning" behavior. J Bacteriol 183:4914-4917, 8/01. From their abstract…"… Bacteria respond faster or more extensively to a signal when a similar signal has been perceived in the past." They show that this is so with their system, and explain it as a feature of the regulatory loop.

I. Homework

From Weaver, only questions Ch 7 #1-13 are relevant to assigned material. (We won't discuss the Weaver questions, unless you bring them up.)

1. For each of the following partial diploids, tell whether the Z and A enzymes will be made constitutively, inducibly (= normal), or not at all:

a. $i^+z^+a^-/i^+z^-a^+$ b. $i^+z^+a^-/i^-z^-a^+$

c. $i^+z^+a^-/i^+o^cz^-a^+$ d. $i^+z^+a^-/i^+p^-z^-a^+$ (p = promoter for zya operon)

2. The situation in #1 ignores one *lac* gene. In order for the inducer to actually induce, it must enter the cell (Table 7.1). Entry of lactose requires $LacY^+$ phenotype, i.e., active permease. Taking this additional consideration into account, how would the following partial diploids behave:

a. $i^+z^+y^+a^-/i^+p^-z^-y^-a^+$ b. $i^+z^+y^-a^-/i^+p^-z^-y^+a^+$

3. There is yet another consideration that would affect the above results, the role of "catabolite repression" (CR). Assuming that the cell is wild type for all CR-related functions, how does that affect the above questions?

4. The partial diploids discussed in #1 are commonly made by having one set of genes on an F plasmid. Generally, it does not matter which of the genotypes is on the main chromosome, which is on the plasmid; the number of copies of F in the cell is about the same as the number of chromosome copies. But what if we use a "high copy number" plasmid, one present at perhaps 50 copies per cell? The promoter for the repressor gene is very weak, and only a very few repressor molecules are normally made. As a result, the following diploid will behave differently depending on which set of genes is on the plasmid: $i^+z^+a^-/i^-z^-a^+$. What will be the phenotype of that diploid in the two cases?

5. Look at Fig 7.6. What concentration of repressor gives half-maximal binding to the operator?

6. We have seen that proteins can bend DNA. But bending DNA takes energy. Where does the energy for DNA bending come from?

J. Partial answers

1. a. Both Z and A enzymes would be made inducibly; in fact, the phenotype of this cell would appear quite normal. Repressor is made, one chromosome can provide Z, one can provide A. The genotype indicated shows no operator mutations, so the repressor will operate normally.

b. Same phenotype as part a, because i^+ is dominant.

c. Z will be inducible, A constitutive. Genes cis to the o^c mutation will be constitutive.

d. The p^- mutation inactivates that piece of DNA, cis. Thus Z will be inducible, A non-inducible (non-functional).

2. The only difference between the two cases is the arrangement of the y^+ and y^- alleles with respect to the defective promoter. In a the cell is, phenotypically, $LacY^+$; in b it is $LacY^-$. Thus the cell in b can't be induced, because there will be no permease to bring in the inducer; none of the Lac Z, Y, or A proteins will be made. The cell in part a will induce normally, but can't make acetylase because the only a^+ gene is behind the defective promoter; it is like #1d.

3. Glucose should be absent in the above questions, in order to ensure that cAMP is high, allowing cAMP-CAP to play its positive role. Remember that the cAMP-CAP activation is a necessary but not sufficient condition for Lac gene function.

4. If the i^+ allele is on the chromosome, then we will have a very small number of repressor molecules, but a very large number of operators (because of the high copy number plasmid). Thus, even though the partial diploid would appear to be $repressor^+$, there won't be enough repressor to go around, and the *lac* genes will function. Since all operators are equivalent, both the chromosomal and plasmid genes will function; the cell will effectively be constitutive for the Lac enzymes.

Induction of genes by "titrating" the repressor, by having excess copies of the operator, is called escape synthesis. It may also occur during the replication of phages carrying bacterial genes, if they are regulated by repressors that are at low concentration.

5. About 0.03 μg/mL.

6. Think about the overall event. How would you write an equation to describe it? More about this in Ch 9, but I think it is useful to try this now.