Bi8 Lecture 19

Review and Practice Questions

March 8th 2016

Common Misconceptions

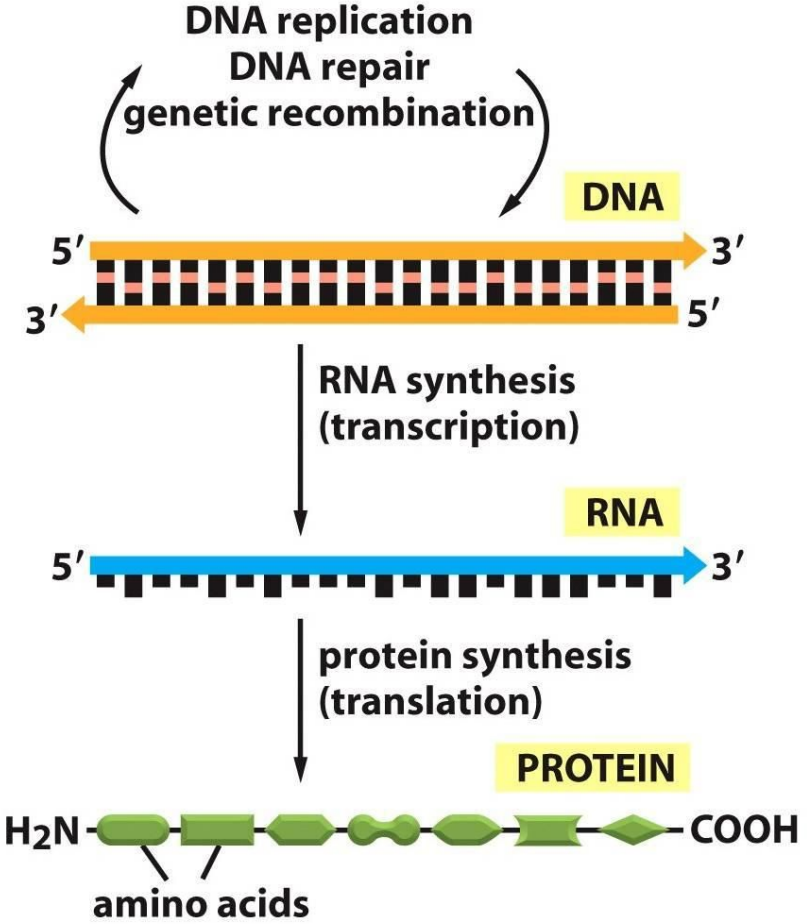
Where Words Matter

Common Misconceptions

- DNA vs RNA vs Protein
  - *Su(H)* vs Su(H)

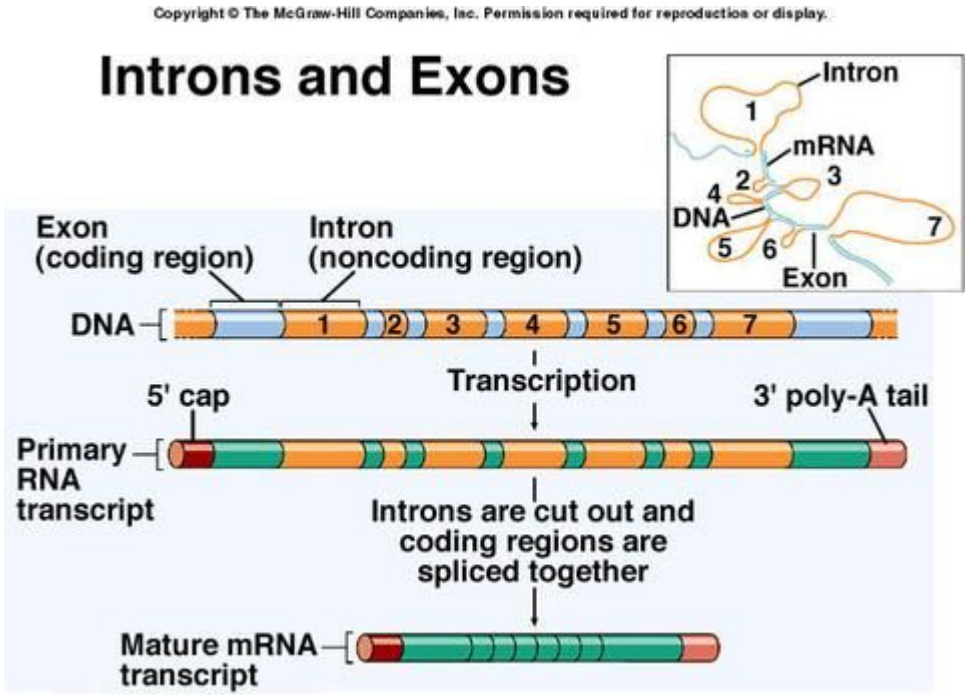
- Replication
- Transcription
- Transcribed vs Transcripted
- Translation
- Transduction

- Pathways
- Upstream/Downstream



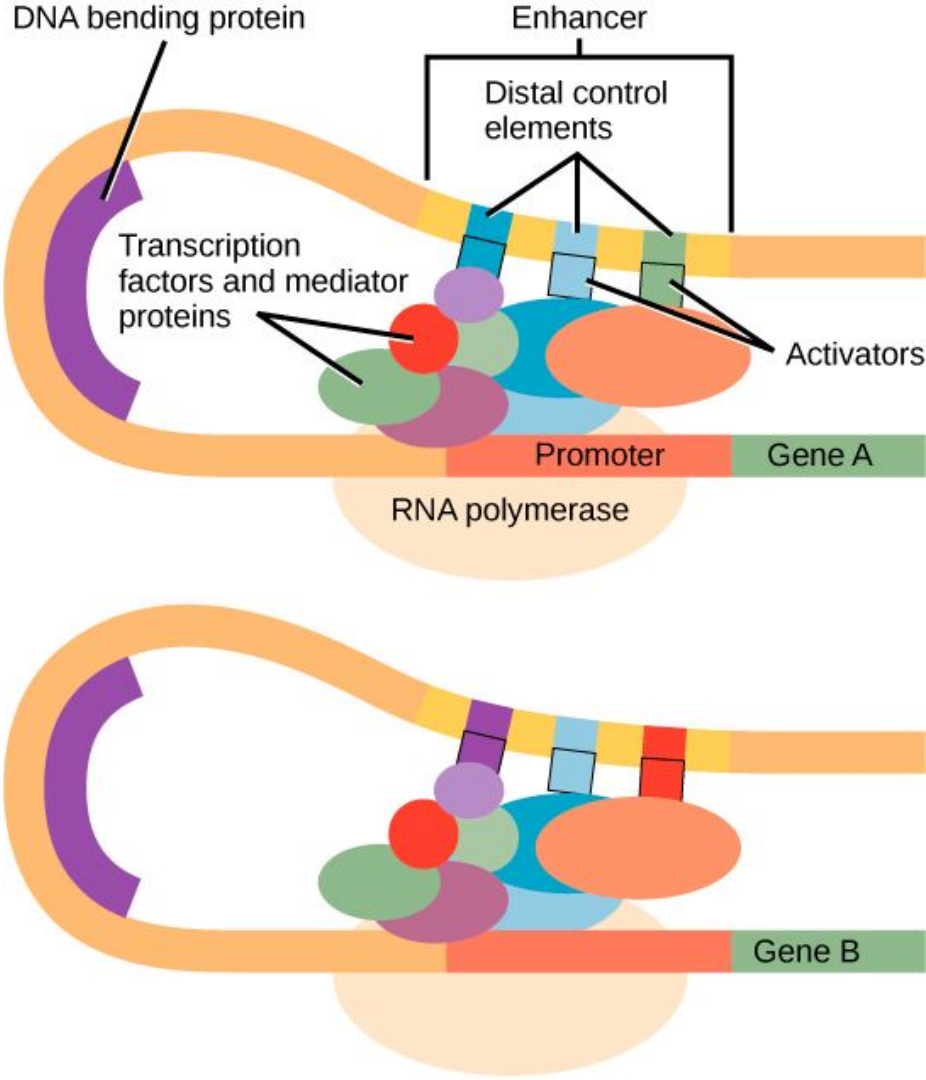
Common Misconceptions

- Exons
  - Code for protein
- Introns
  - Not useless

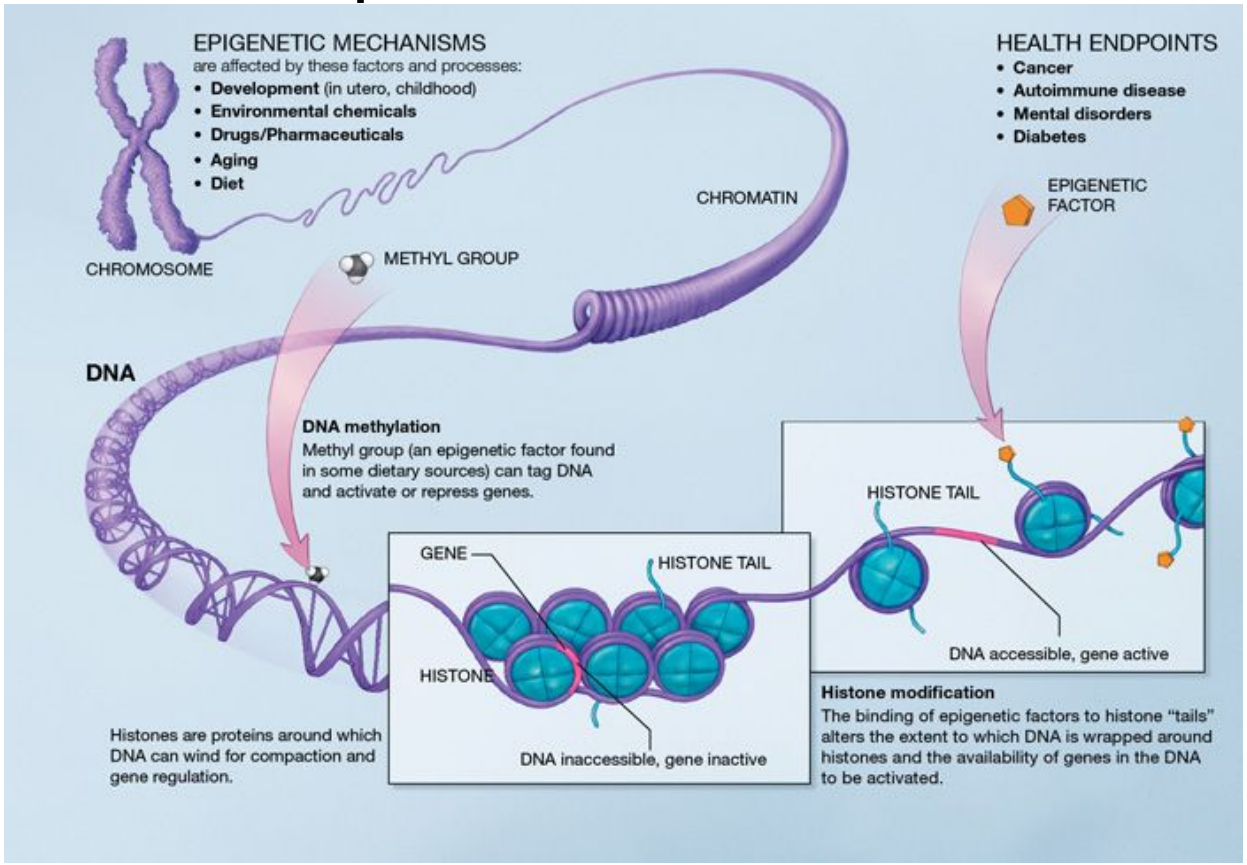


Common Misconceptions

- Genes
- Transcription Factors
- Enhancers
- Promoters



Common Misconceptions



Protein Binding Techniques

- EMSA
  - Easy (mix things together and run on gel)
  - Not super informative
    - Don’t actually see the protein, just see that something is bound
    - Doesn’t tell you where the protein binds
    - Doesn’t tell you if more than one protein binds
    - Requires strong interactions
- DNA Footprinting Assay
  - Technically challenging
  - Overcomes most problems with EMSA
- ChIP-Seq
  - Combines binding and sequencing - great for finding binding sites on DNA

Other Techniques

- In Situ Hybridization
  - Visualization of mRNA patterns in situ
- Reporter assays
  - Visualization of proteins in vivo
- Screens
  - Testing wide array of conditions
  - Phenotyping

Practice Questions

Sample Question 1

7. The TCGF gene codes for a factor that is turned on in immune cells by antigen stimulation. The TCGF protein is secreted and used as a growth factor, both to support the cell that expresses it and to support other neighboring immune cells as long as a local immune reaction is going on. Cells are finicky about the conditions for activating TCGF expression; the gene is normally completely silent and only turned on when the cells' surface receptors are activated. Like the IFNb enhancer, the TCGF enhancer has sites for NF-kB and for two other factors, AP-1 and NFAT. All of these factors are transcriptional activators, not repressors. Each of these three factors is activated by environmental signals, and each is mobilized by a biochemically different activating signaling pathway. None of them regulate each other.

Sample Question 1

7. The TCGF gene codes for a factor that is turned on in immune cells by antigen stimulation. The TCGF protein is secreted and used as a growth factor, both to support the cell that expresses it and to support other neighboring immune cells as long as a local immune reaction is going on. Cells are finicky about the conditions for activating TCGF expression; the gene is normally completely silent and only turned on when the cells' surface receptors are activated. Like the IFNb enhancer, the TCGF enhancer has sites for NF-kB and for two other factors, AP-1 and NFAT. All of these factors are transcriptional activators, not repressors. Each of these three factors is activated by environmental signals, and each is mobilized by a biochemically different activating signaling pathway. None of them regulate each other.

Sample Question 1

(a) What are the two extreme alternative ways that this combination of factors might enable a TCGF-like enhancer to respond to environmental signaling by these three pathways? What would these alternatives mean for the conditions in which the cell expresses TCGF?

Sample Question 1

(b) Before testing the alternatives you consider, you notice two strange things about the structure of the TCGF enhancer. First, there are multiple sites for each of the three transcription factors in the same enhancer; second, looked at individually, each of these sites is bound pretty weakly by the factor. How might these site characteristics contribute to the control of the TCGF gene? What kind of logic would this promote?

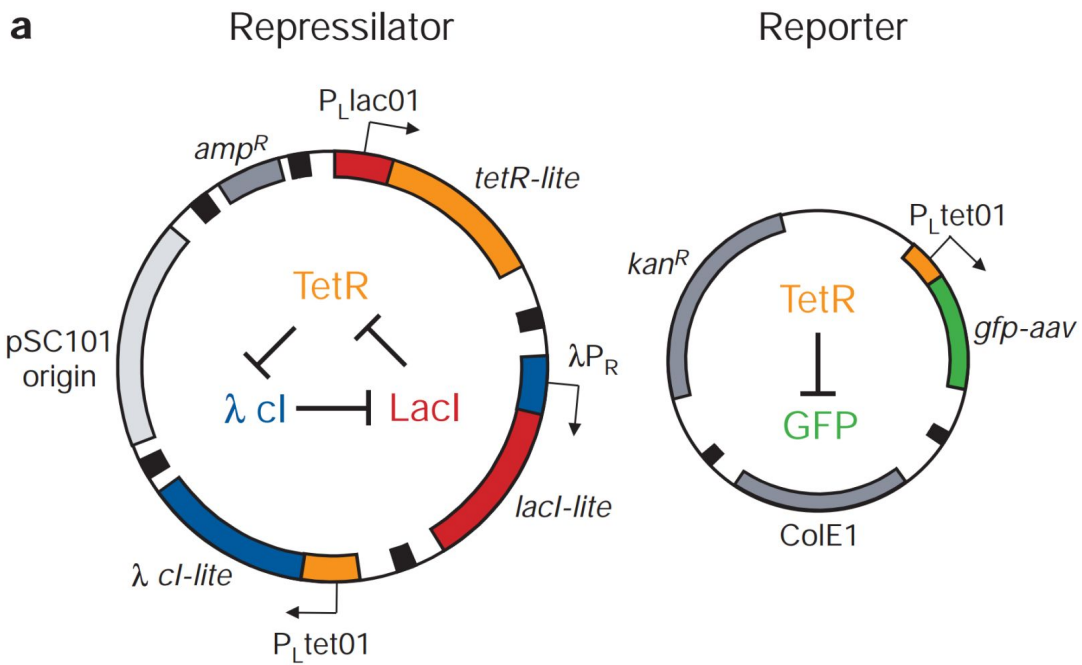
Sample Question 1

(c) What are some mechanisms that TFs use to bind cooperatively?

Sample Question 1

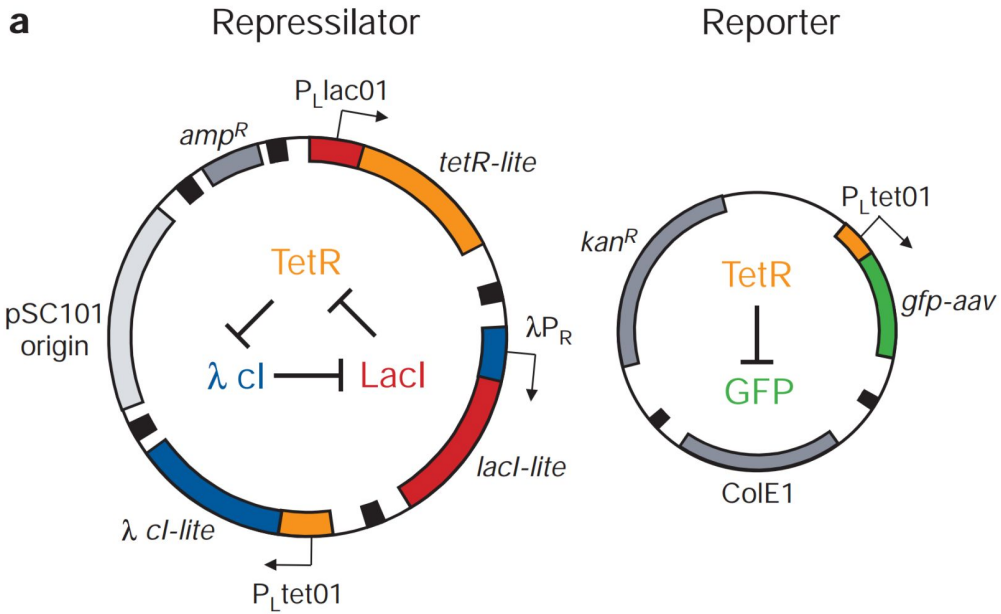
(d) If you mutate the sites in the enhancer to become stronger binding sites for each of the three factors, what is likely to happen to control of TCGF?

Sample Question 2



Sample Question 2

a) **Why would <u>GFP</u> ever be expressed in this system?** Under what condition would GFP be expressed, and why would the researcher want GFP to be expressed?



Sample Question 2

b) How do you expect that the three proteins interact with the promoters of the other genes to repress their expression? How would their ability to repress be affected by their concentration?

Thinking more generally may help:

How do most repressors work?

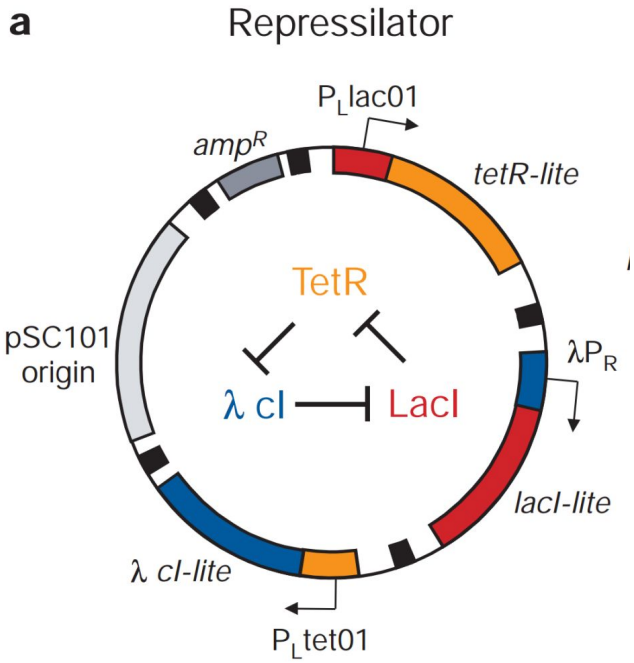
How does concentration generally affect the efficacy of transcription factors?

Sample Question 2

c) **Explain how this set up would affect expression of the three proteins in the repressilator itself.**

Pick a starting point:

What happens when TetR is around?



Sample Question 2

c) **Explain how this set up would affect expression of the three proteins in the repressilator itself.**

Pick a starting point:

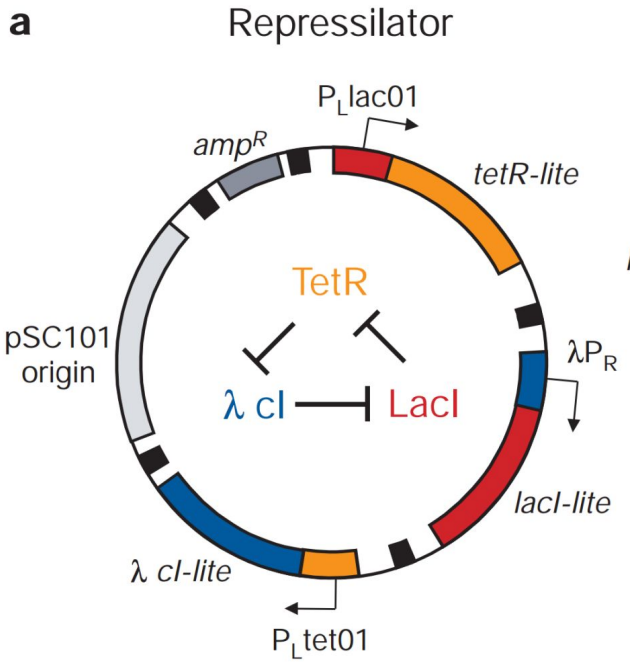
What happens when TetR is around?

TetR represses λ cI, LacI comes on.

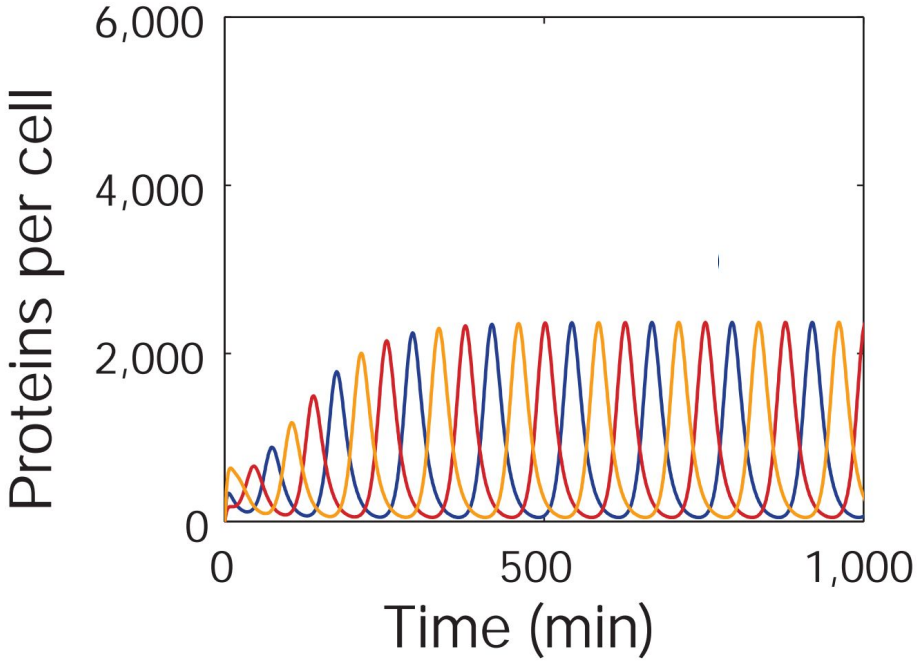
LacI represses TetR, λ cI comes on.

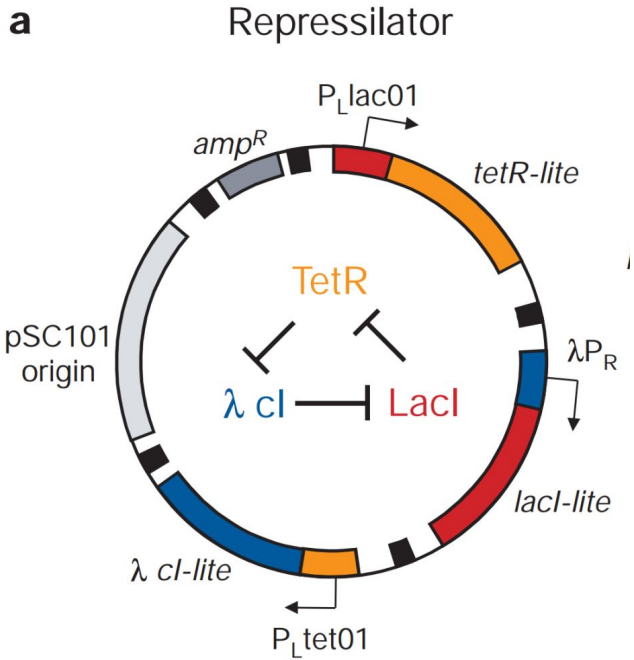
λ cI represses LacI, TetR comes on.

. . .



Sample Question 2 - Simulation Data



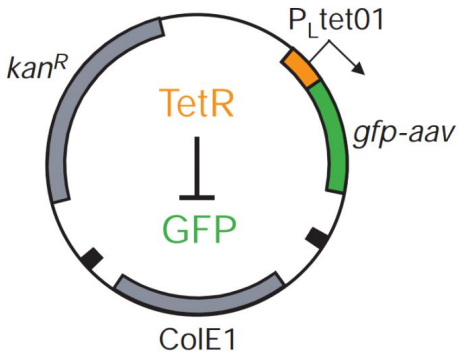


Sample Question 2

d) **How would you expect GFP expression to <u>change over time</u>? What portion of the time would you expect GFP to be “on”? How would your answers change if TetR activated GFP expression instead?**

Remember when GFP would be on?

Reporter



Sample Question 2

d) **How would you expect GFP expression to <u>change over time</u>? What portion of the time would you expect GFP to be “on”? How would your answers change if TetR activated GFP expression instead?**

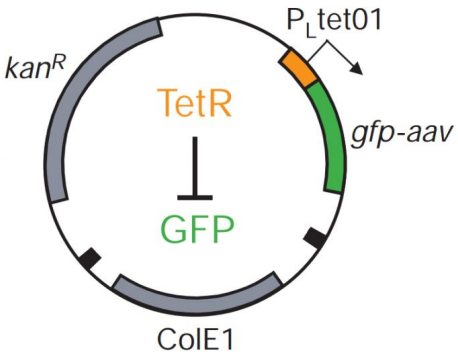
Remember when GFP would be on?

GFP on when TetR off: ⅔ of the time

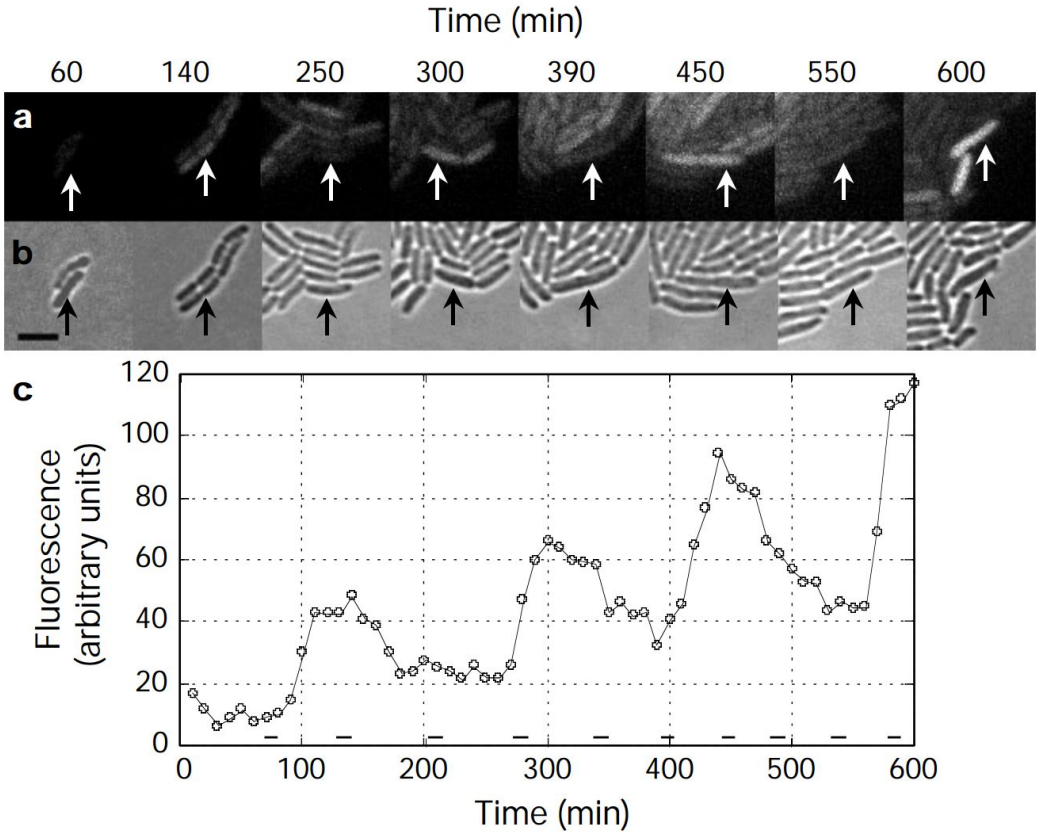
For “opposite” reporter:

GFP on when TerT on: ⅓ of the time

Reporter



Sample Question 2 - Data



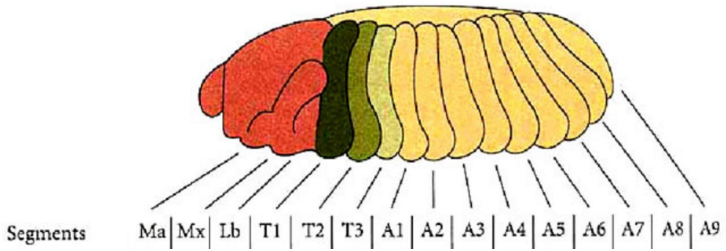
Sample Question 2

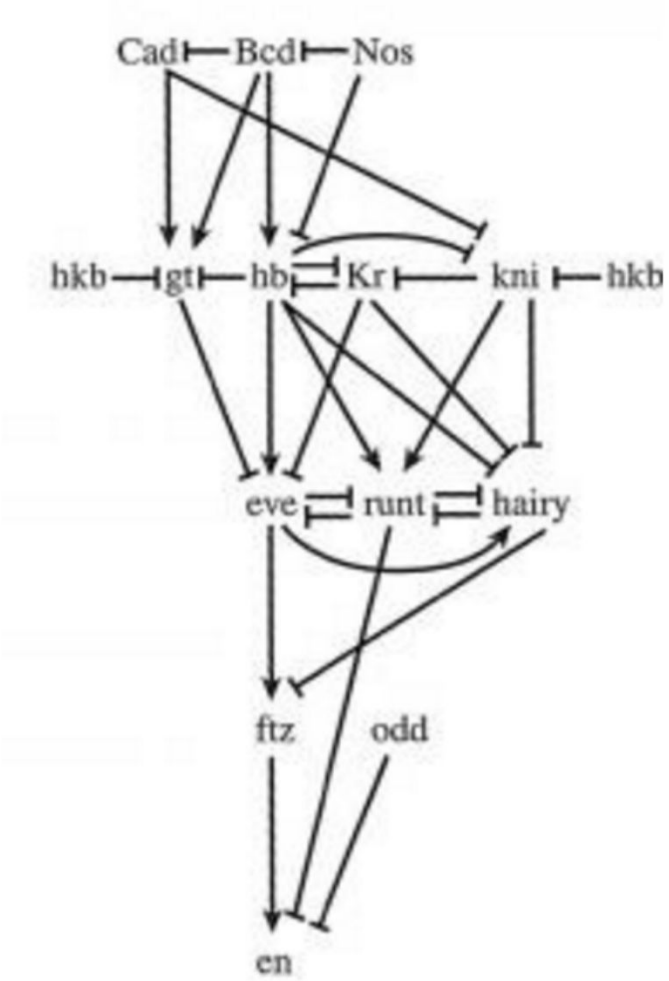
e) **While this is a synthetic system, give an example of why a natural organism may use a similar oscillatory system.**

Think of a situation where an organism would need expression of gene only periodically.

Sample Question 3

A very important process during embryo development is segmentation, which involves the establishment of a series of repetitive segments in animal body plan. The figure on the right shows the gene regulatory network of segmentation in Drosohpila (fruit fly) embryo.

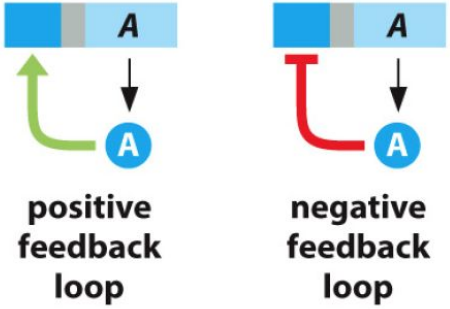


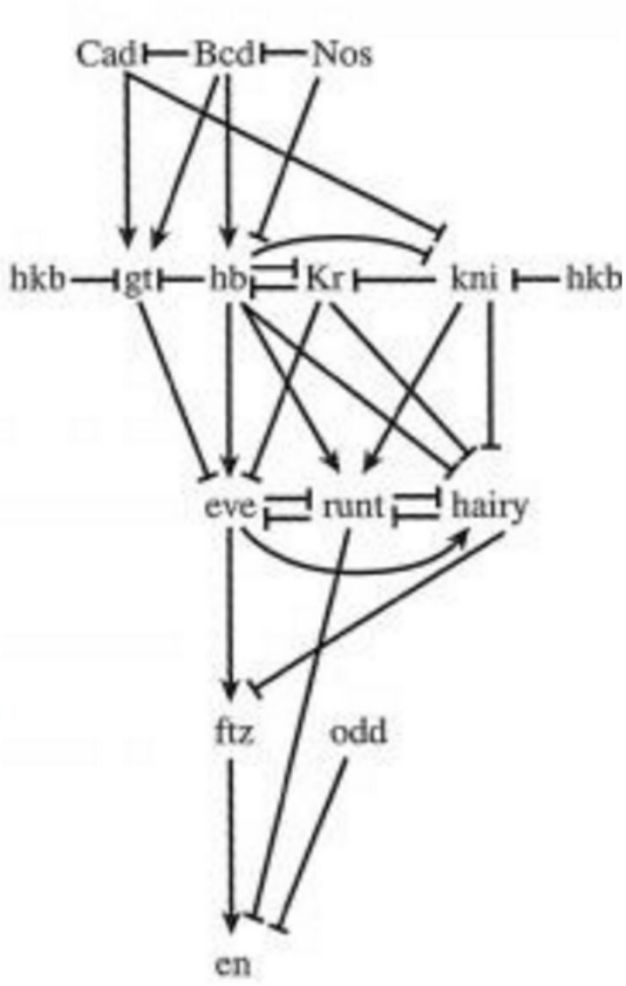


Sample Question 3

(a) According to the diagram, please identify at least five different three-element control systems, and point out which belongs to feedback loop, incoherent feedforward loop and coherent feedforward loop.

Feedback loop: the product (usually a protein) of the the output gene can reversely control the input gene.



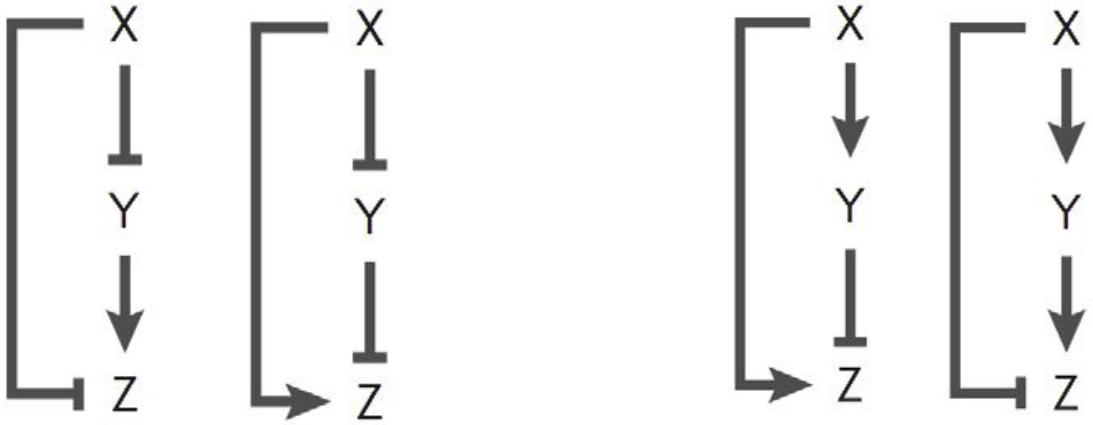


Sample Question 3

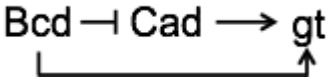
coherent/incoherent feedforward loop: Usually input gene has two ways (direct and indirect) to control the expression of output gene.

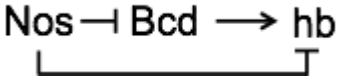
Coherent feedforward loop -- the purpose of the two regulatory ways is consistent

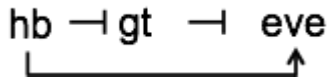
Incoherent feedforward loop -- the purpose of the two regulatory ways is opposite

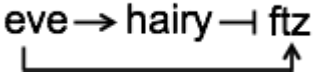


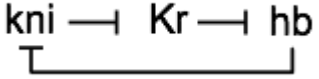
Sample Question 3



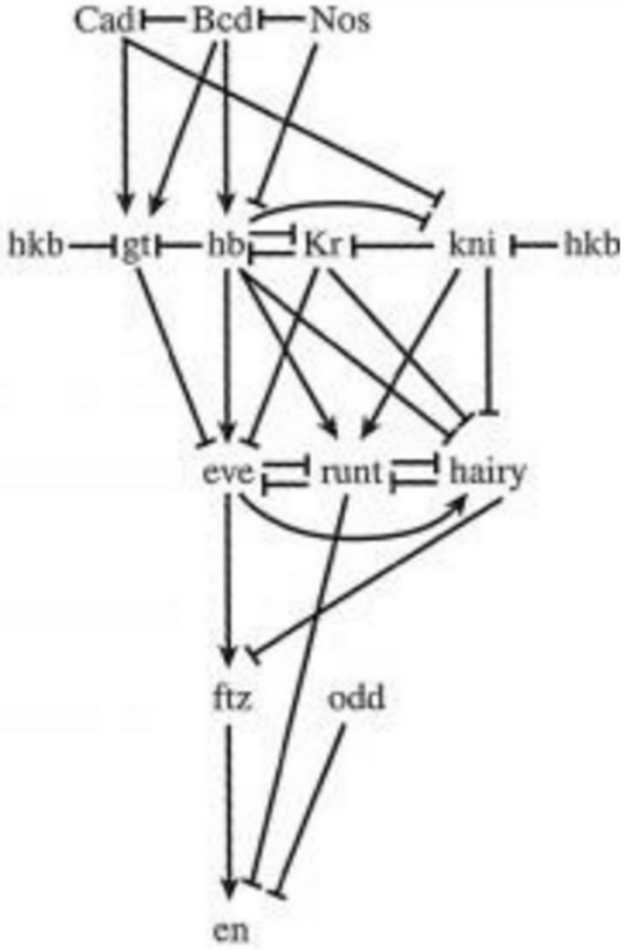






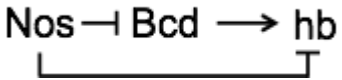


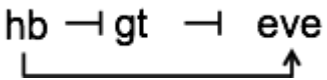
Negative feedback loop



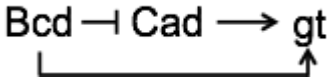
Sample Question 3

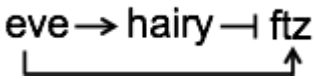
The robustness of spatiotemporal gene expression control ensures normal development. Considering the conditions: overexpress input gene two-fold. Describe how these control systems will respond to this condition, and which control system(s) is/are relatively robust.



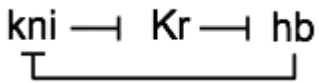


dramatically increase or decrease output gene expression

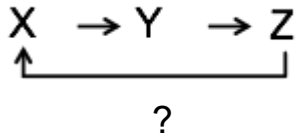


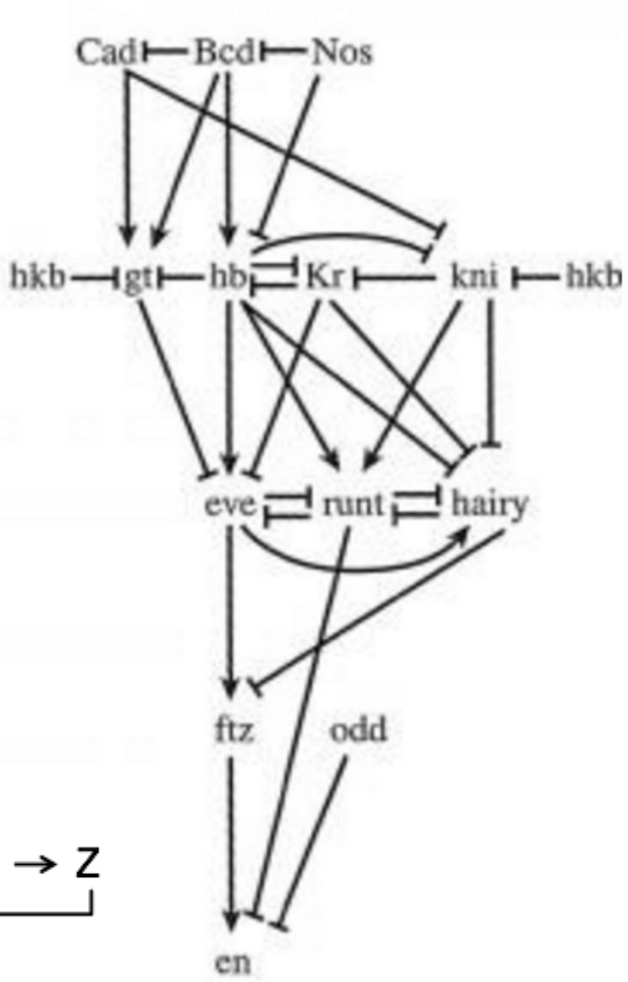


increase output gene expression for a short period, and after the intermediate gene is expressed, it will reach a new balance again.



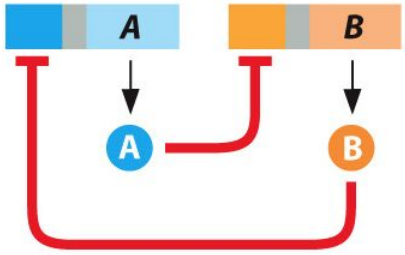
Increase hb expression for a short period. After hb is expressed, it quickly reach the original balance again.



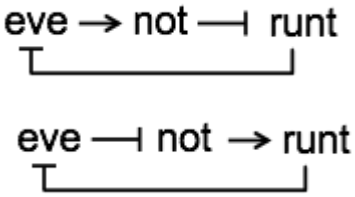


Sample Question 3

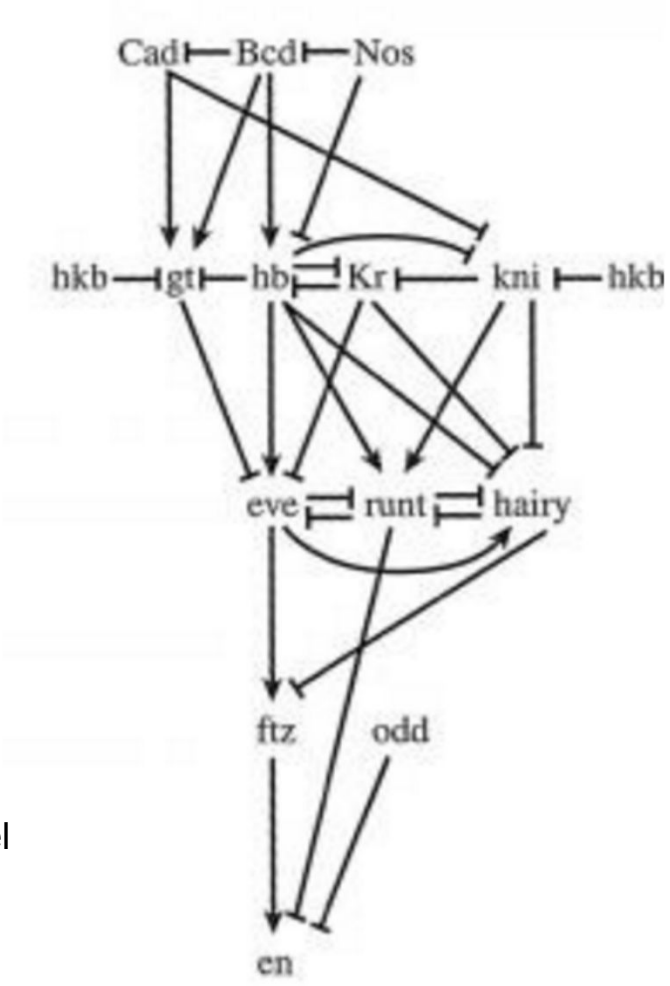
(c)“eve” and “runt” form a flip-flop device. Suppose you discovered another gene called “not”, which seems to be under control of “eve”, but regulate “runt” expression. If “eve” and “runt” keep this repression state against each other, how will you fit “not” in, forming a three-element control system? Please describe two simple models. How to detect which model is the real one existent in the organism?



flip-flop device
(indirect positive feedback loop)



Overexpress “eve”, and monitor the expression level of “not”



Sample Question 3

Overexpress a gene:

Transfect cells with exogenous plasmid carrying the desired gene

Use CRISPR-Cas9 tech to introduce an additional copy of gene in genome

Knockdown gene expression:

CRISPR-Cas9 tech

microRNA/siRNA tech

Detect if protein “A” acts as an TF to gene “B” :

ChIP-seq, ChIP-qPCR

Protein-binding microarray

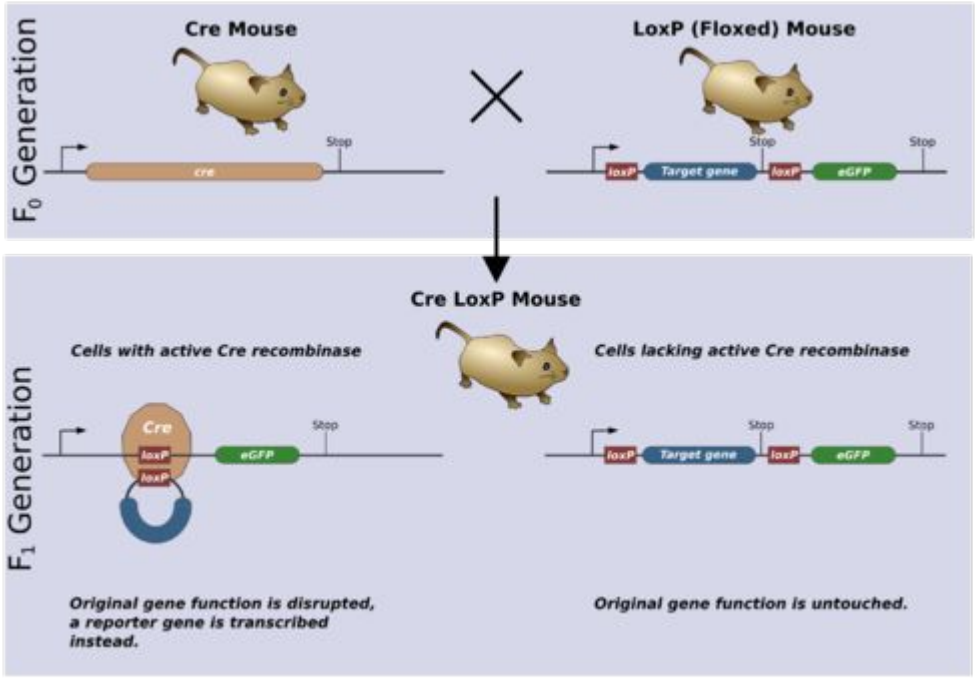
Electrophoretic Mobility Shift Assay

Sample Question 4

You are trying to understand if the gene bil3 is expressed during development of the liver. You would like to prove this through cell lineage tracing-- aka following the fate of cells expressing the gene over time. In your very up-to-date lab, with tons of money and people working for you, making small changes in the mouse genome is relatively doable.

a) How could you use Cre, LoxP, and GFP to test the involvement of bil3 in developing liver? Suggest the issues that might go into designing your ideal strategy. Knowledge of other genes in development and in the liver are not necessary to answer this question.

Sample Question 4



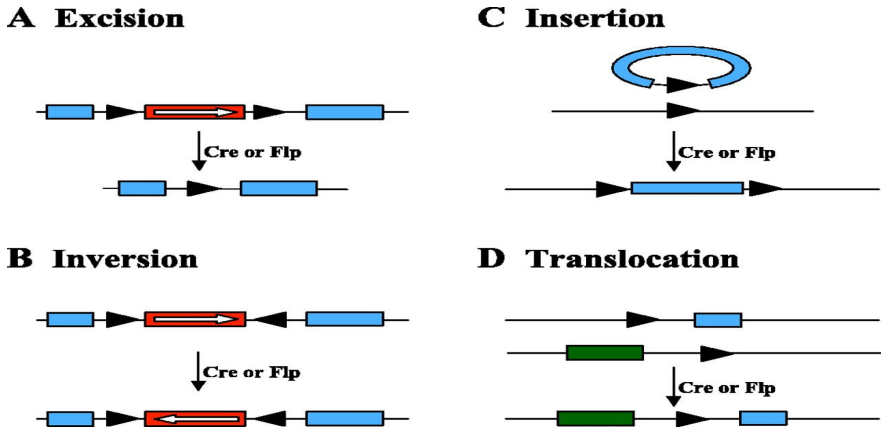
- We want to visualize expression over time
- Importantly, we want to see this *in vivo*
- Cre-Lox causes more or less irreversible genetic modification
  - Once Cre is expressed, loxP recombination removes gene between
  - Takeaway: the moment a gene is expressed once in the organism, all subsequent expression is visualized

Sample Question 4

Caveats to the Cre-LoxP approach

- Bil3 may not be entirely localized to a certain tissue subset
- Bil3 expression cannot be quantified precisely using visualization
- Expression of reporter gene itself is just that-- cannot tell what the role of Bil3 is precisely

Other uses



Sample Question 4

b) What would you expect to see under UV light in the adult organism that has been successfully modified by your cre-lox experiment? What are the possibilities?

- GFP fluoresces under UV→ expect to see GFP SOMEWHERE
- What if Bil3 was a general factor needed in all developing cells?
- Would you see more or less cells with Bil3 if it was highly specific to cell type/expressed at higher levels of differentiation?
- Would you expect to see localization?

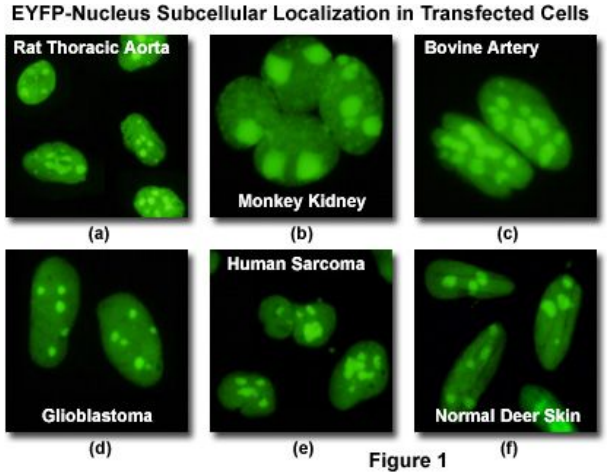


Figure 1

Sample Question 4

c) You make a mutant mouse strain according to (a). Surprisingly, your first mutant mouse doesn't show ANY fluorescence at all by midpoint of development. Why might this be? What kinds of tests could you do to see why?

- Obvious: Bil3 not expressed
  - If loxP sites incorrect, *Bil3* sequence missing
- Less obvious: GFP addition gone wrong
  - Where was it inserted?
  - GFP might have been placed in intron
- Timing
  - Too early in development for it to be expressed
  - How might the organism modulate when it's expressed? EPIGENETICS

Sample Question 4

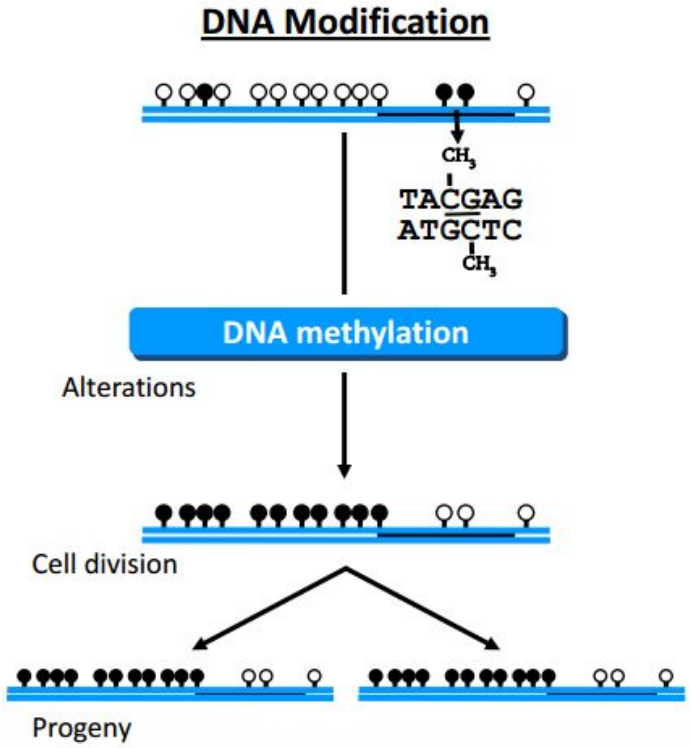
Epigenetic reason:

Histone wrapping

- Limits accessibility to DNA
- Test: DNase sensitivity
- Modified by methylation, acetylation, and phosphorylation

Methylation--CpG islands

- 300-3000bp, >50% GC content, 5’ regulatory
- Methylation status:Generally non-methylated in coding regions allowing gene expression, methylated in non-coding regions
- Genomic distribution:70% of promoter regions have CpG islands
- Test: C to U conversion: Bisulfite modification: C’s are converted to U’s, Methylated C’s are NOT converted



Sample Question 4

d) You make another mutant that you verify expresses fluorescence, designing it so that it should disrupt bil3. (Why?) But when you look at the adult mutant mouse, you also find that your mouse's liver is just fine. What does this imply about the necessity of this gene if it DOES somehow play a role in liver development? Can you comment on its sufficiency with the results of your experiment? Why or why not?