Reminders

Watch the video lectures

- Add to your notes so that <u>you</u> can understand the material
- Replay/re-watch sections as necessary
- Take breaks
- Change the video speed as necessary; use shortcut keys

Read the textbook. Check the textbook for answers to your questions before posting on the Discussion Board.

BIO 230

Lecture 3 :

Prokaryotic Transcriptional Regulation Continued…

1) Recap of prokaryotic gene regulation

2) Bacteriophage Lamba

3) Synthetic Biology

4) Transcription Attenuation

Readings (Alberts *et al.* custom text)

Pages 400-405, 413-416, 876-878

Recap: Prokaryotic Gene Regulation

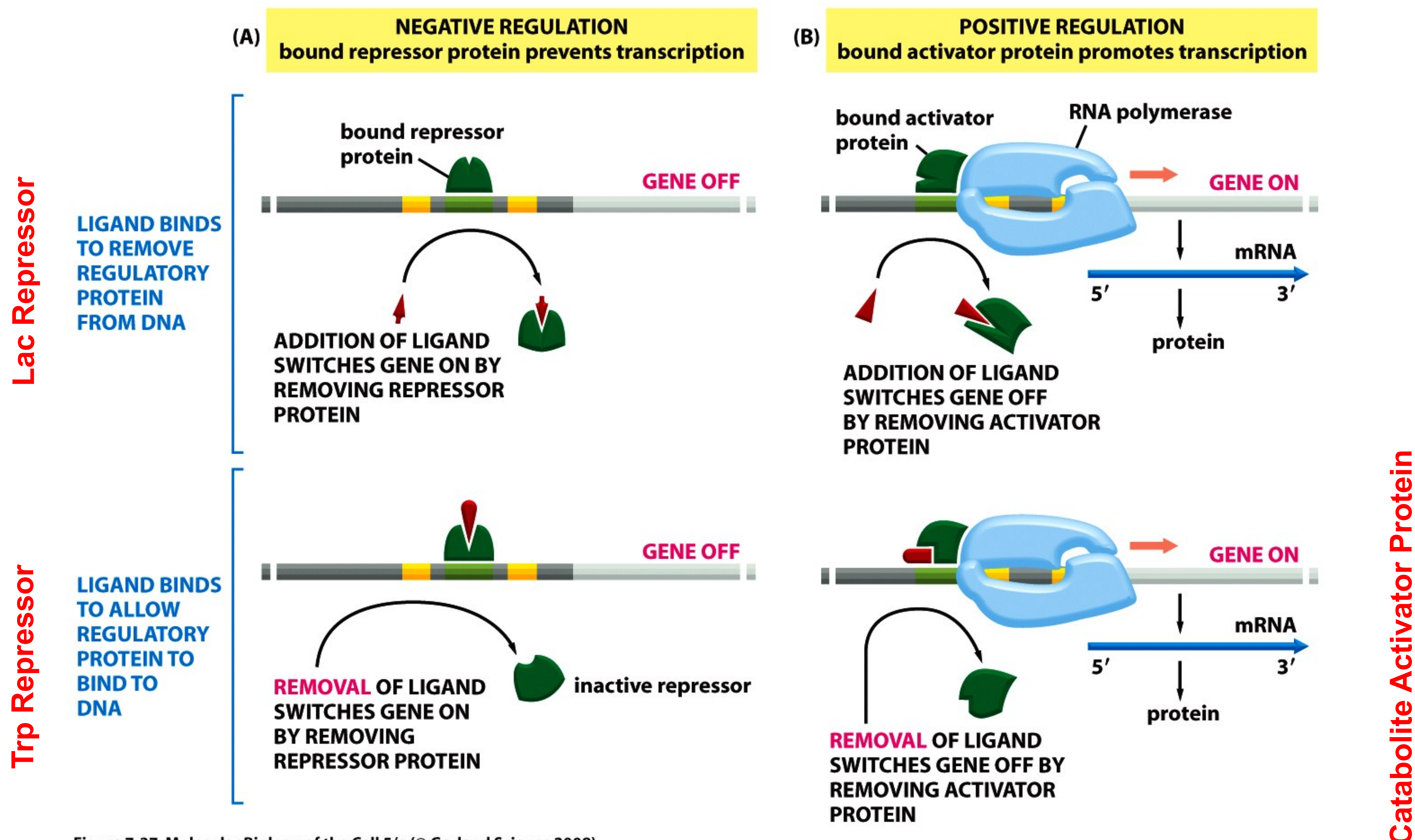
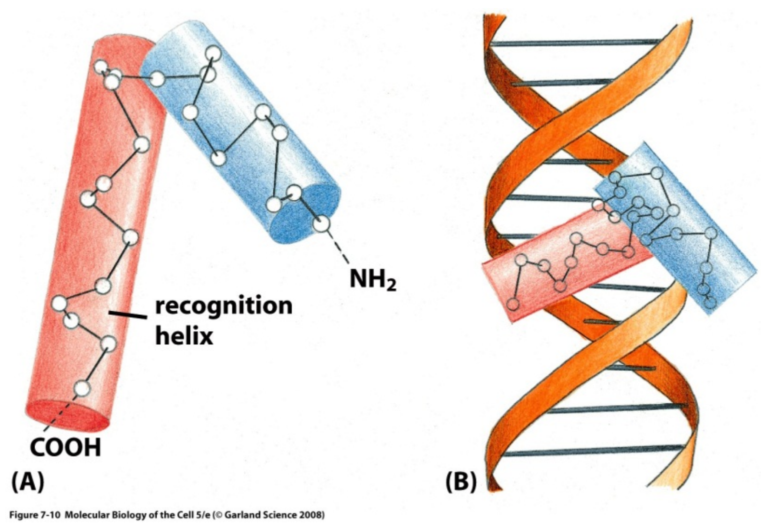


Figure 7-37 Molecular Biology of the Cell 5/e (© Garland Science 2008)

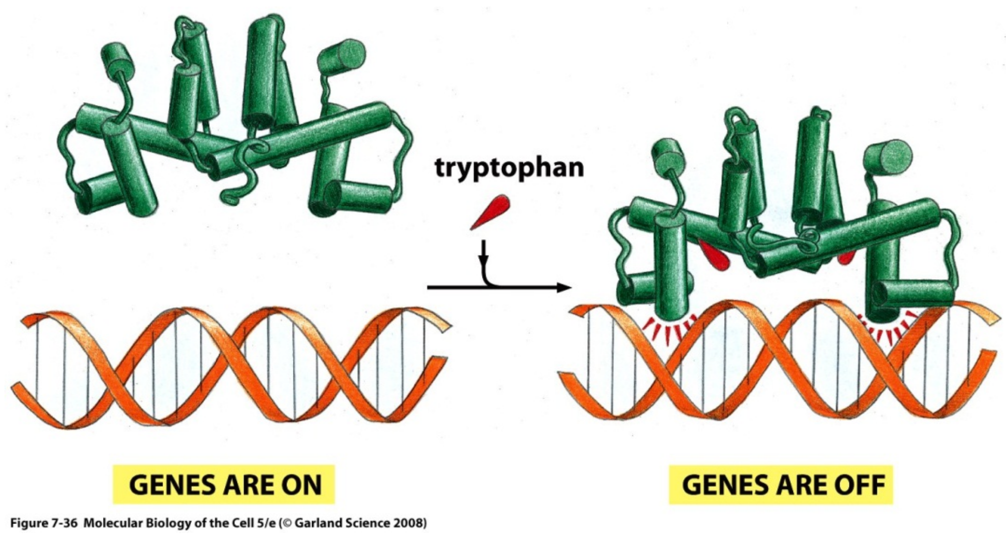
Example 1: The Tryptophan Operon

Tryptophan repressor contains a ● Helix-Turn-Helix DNA binding motif (most common DNA-binding motif)

Helix-Turn-Helix



Tryptophan Repressor



Binds in ● major groove of DNA double helix

Tryptophan binding induces
- Conformational change
- Fits in major groove

Recap: Prokaryotic Gene Regulation

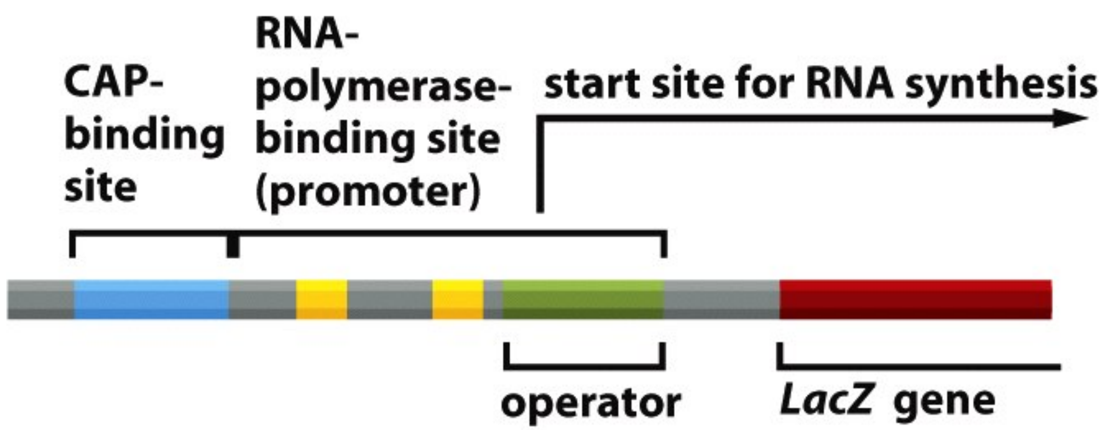
To summarize:

Negative regulation:
Competition between ● RNA polymerase and ● repressor protein for ● promoter binding

Positive regulation:
● activator protein recruits ● RNA polymerase to the promoter to activate transcription

Recap: Prokaryotic Gene Regulation

Gene regulatory elements are typically close to the transcriptional start site of prokaryotic genes



BUT regulatory elements can also be found

- Far upstream of gene
- Downstream of gene (eukaryotes)
- Within gene (introns; eukaryotes)

Recap: Prokaryotic Gene Regulation

Some regulatory elements are distant from the transcriptional start site and influence transcription - How?

- DNA looping

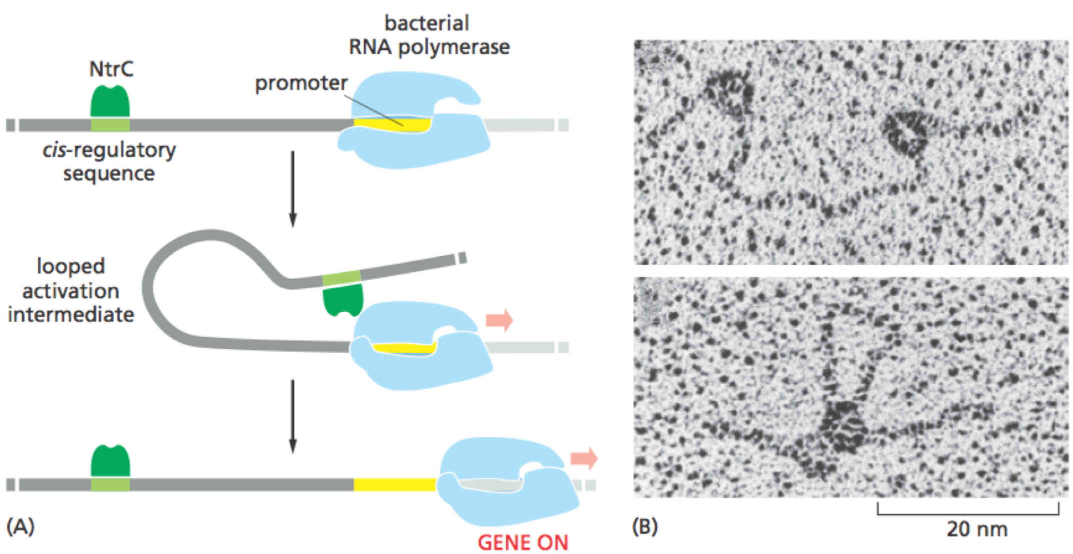


Figure 7–16 Transcriptional activation at a distance. (A) The NtrC protein is a bacterial transcription regulator that activates transcription by directly contacting RNA polymerase. (B) The interaction of NtrC and RNA polymerase, with the intervening DNA looped out, can be seen in the electron microscope. (B, courtesy of Harrison Echols and Sydney Kustu.)

(Euk. Video)

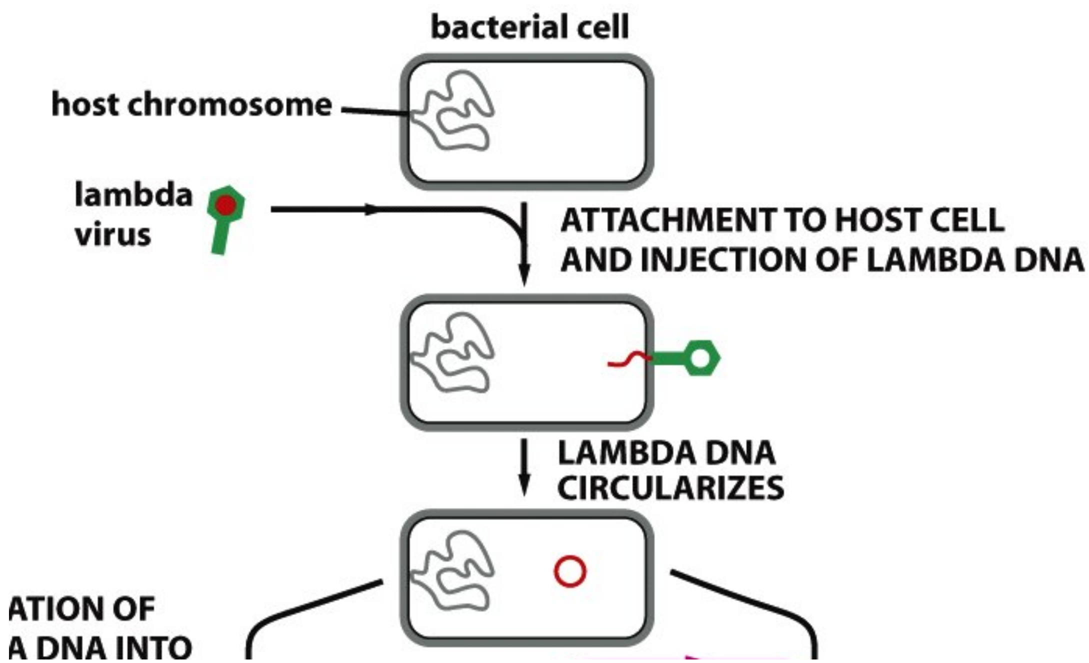
NtrC protein is a transcriptional activator

DNA looping allows NtrC to directly interact with

- RNA polymerase to activate transcription from a distance

Bacteriophage Lambda

Virus that infects bacterial cells



Positive and negative regulatory mechanisms work together to regulate the lifestyles of bacteriophage lamba

- Two proteins repress each others synthesis

Bacteriophage Lambda

Bacteriophage lambda can exist as one of two states in bacteria

Under favorable bacterial growth conditions

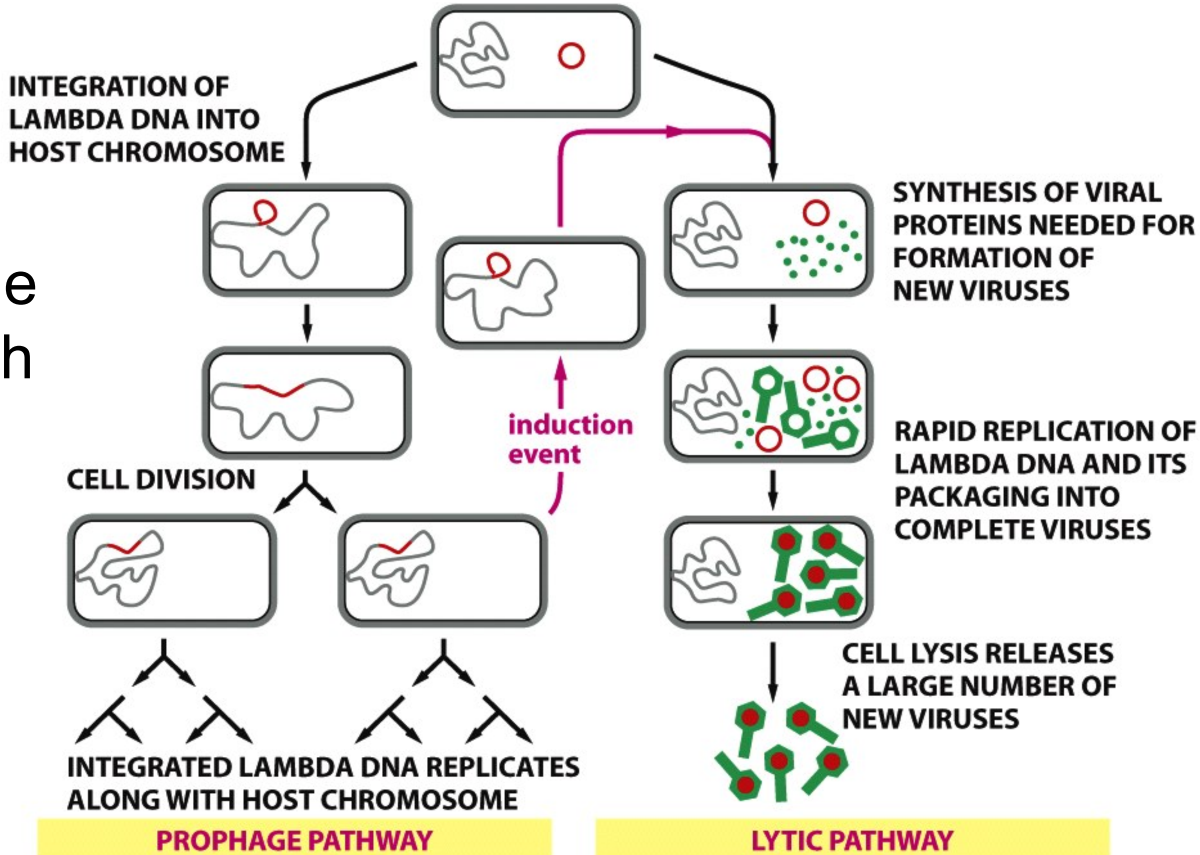


Figure 5-78 Molecular Biology of the Cell 5/e (© Garland Science 2008)

When host cell is damaged

Two gene regulatory proteins are responsible for initiating this switch

Bacteriophage Lambda

Two gene regulatory proteins are responsible for initiating the switch between prophage and lytic pathways

- lambda repressor protein (cI) and
- Cro protein

Repress each other's synthesis, giving rise to the two states.

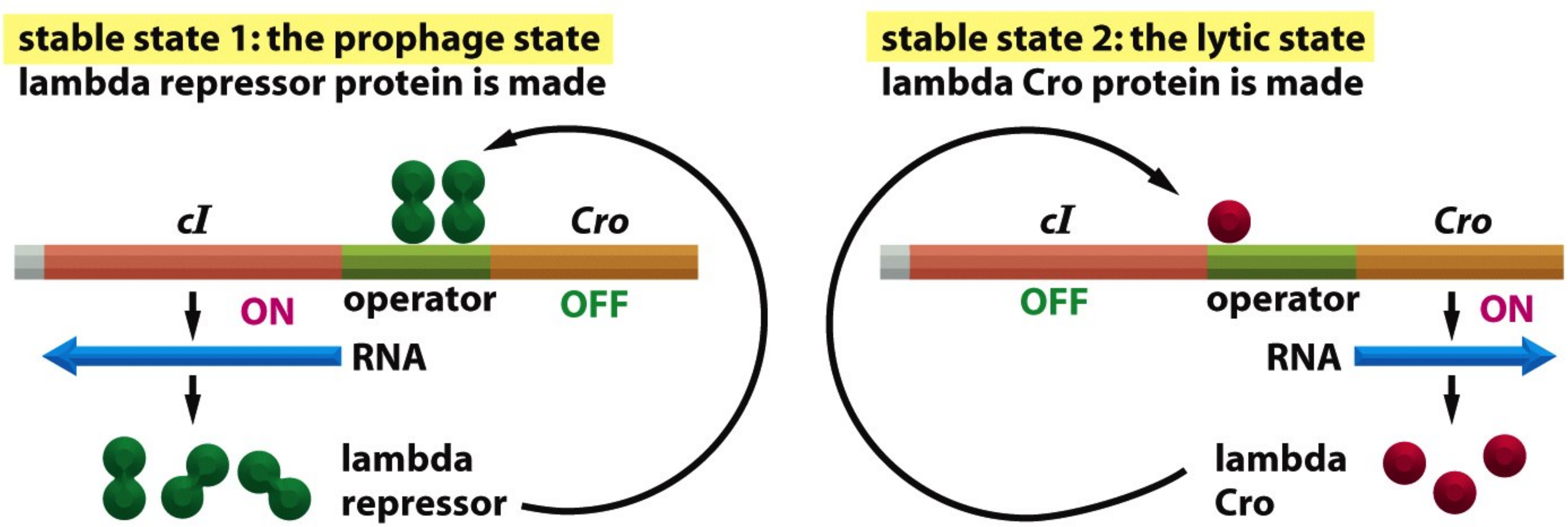


Figure 7-67 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Bacteriophage Lambda

Bacteriophage lamba: a genetic switch

State 1: Prophage

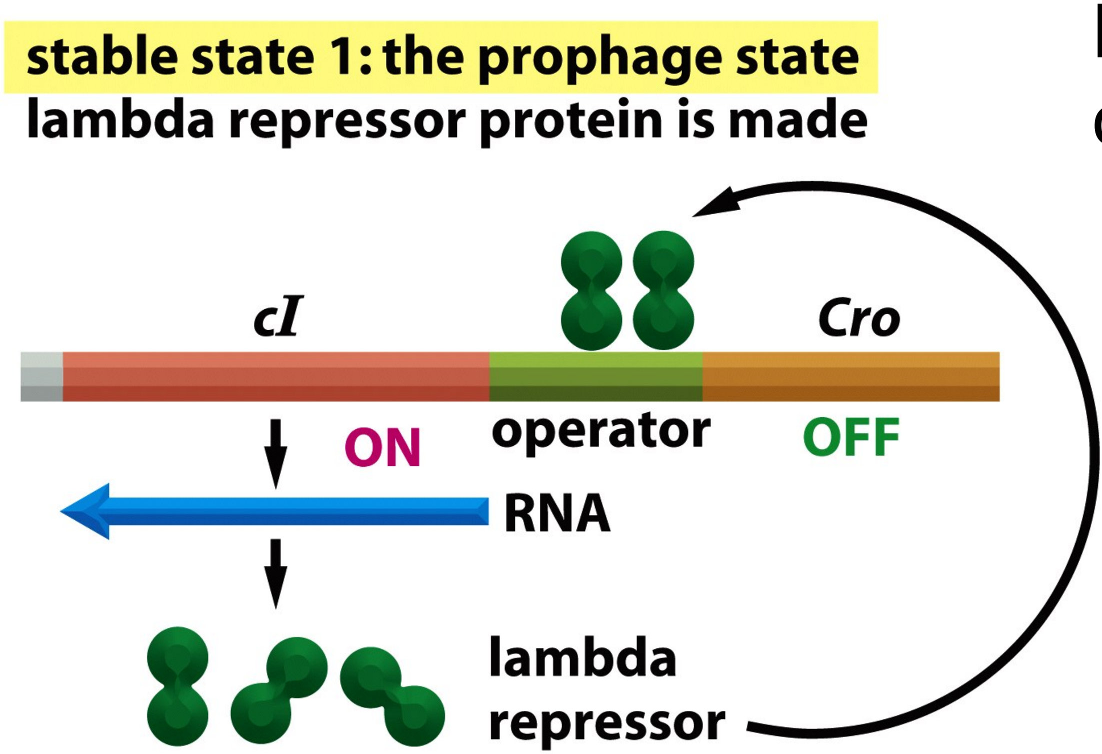


Figure 7-67 part 1 of 2 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Lambda repressor occupies the operator.

- blocks synthesis of Cro
- activates its own synthesis
- most bacteriophage DNA not transcribed

Bacteriophage Lambda

eg. bacteriophage lamba: a genetic switch

State 2: Lytic

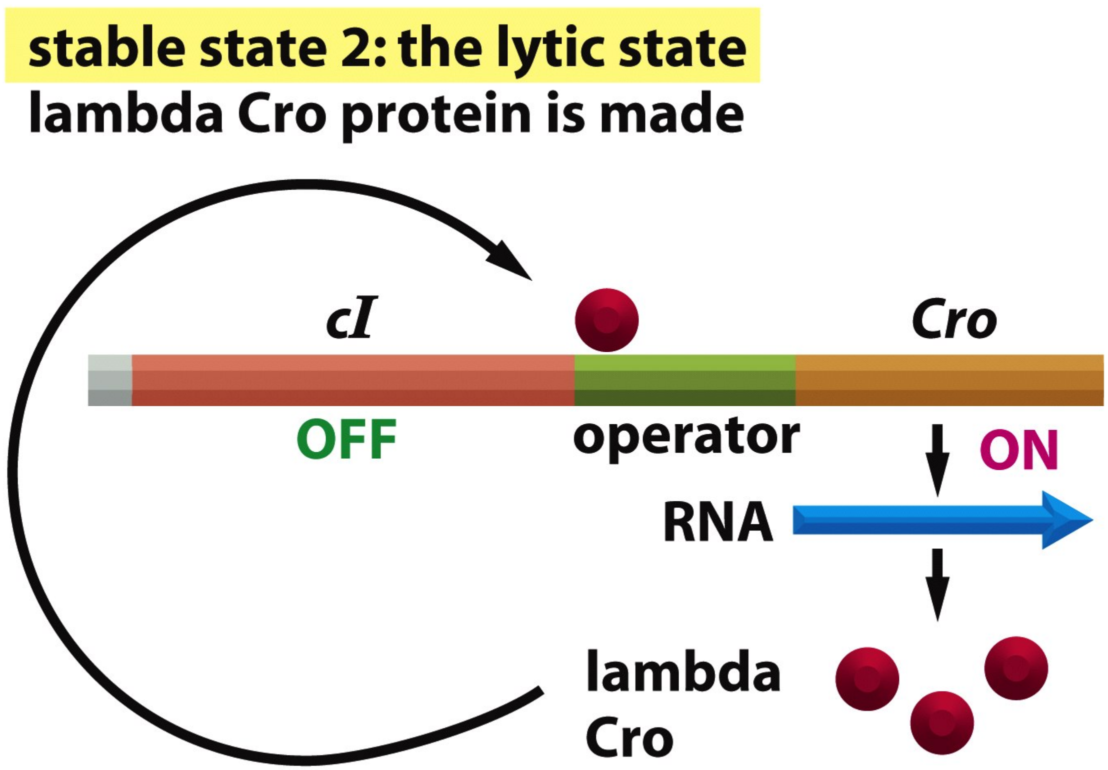


Figure 7-67 part 2 of 2 Molecular Biology of the Cell 5/e (© Garland Science 2008)

What triggers switch?

Cro occupies the operator

- blocks synthesis of repressor
- allows its own synthesis
- most bacteriophage DNA is extensively transcribed

DNA is replicated, packaged, new bacteriophage released by host cell lysis

Bacteriophage Lambda

eg. bacteriophage lamba: a genetic switch

What triggers switch between prophage and lytic states?

Host response to DNA damage
- inactivates repressor

-switch to lytic state

Under good growth conditions repressor protein turns off Cro and activates itself
- positive feedback loop

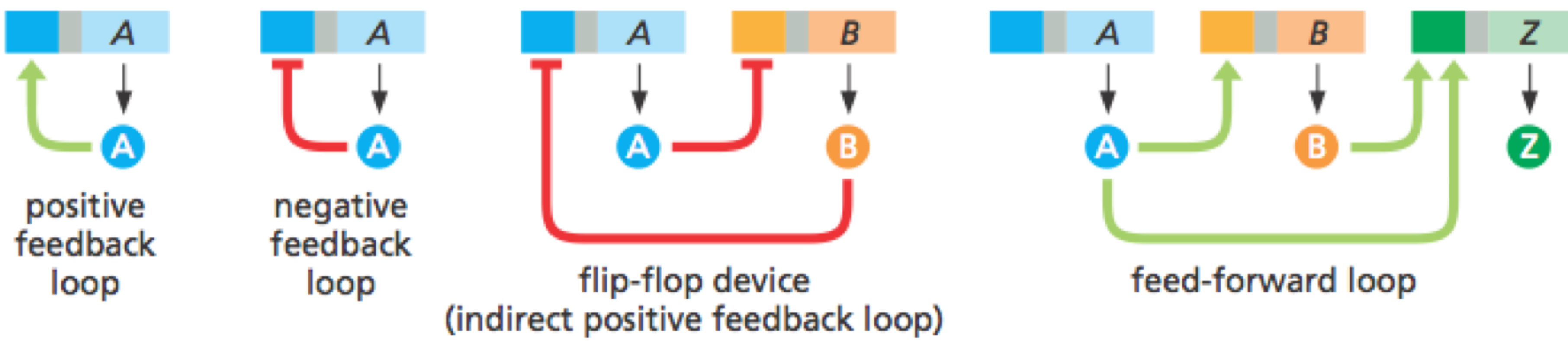
-maintains prophage state

Example of a transcriptional circuit.

Different types exist, control various biological processes

Transcriptional Circuits

Transcriptional Circuits



eg. repressor protein

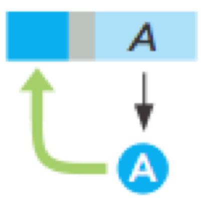
eg. Cro / Repressor switch

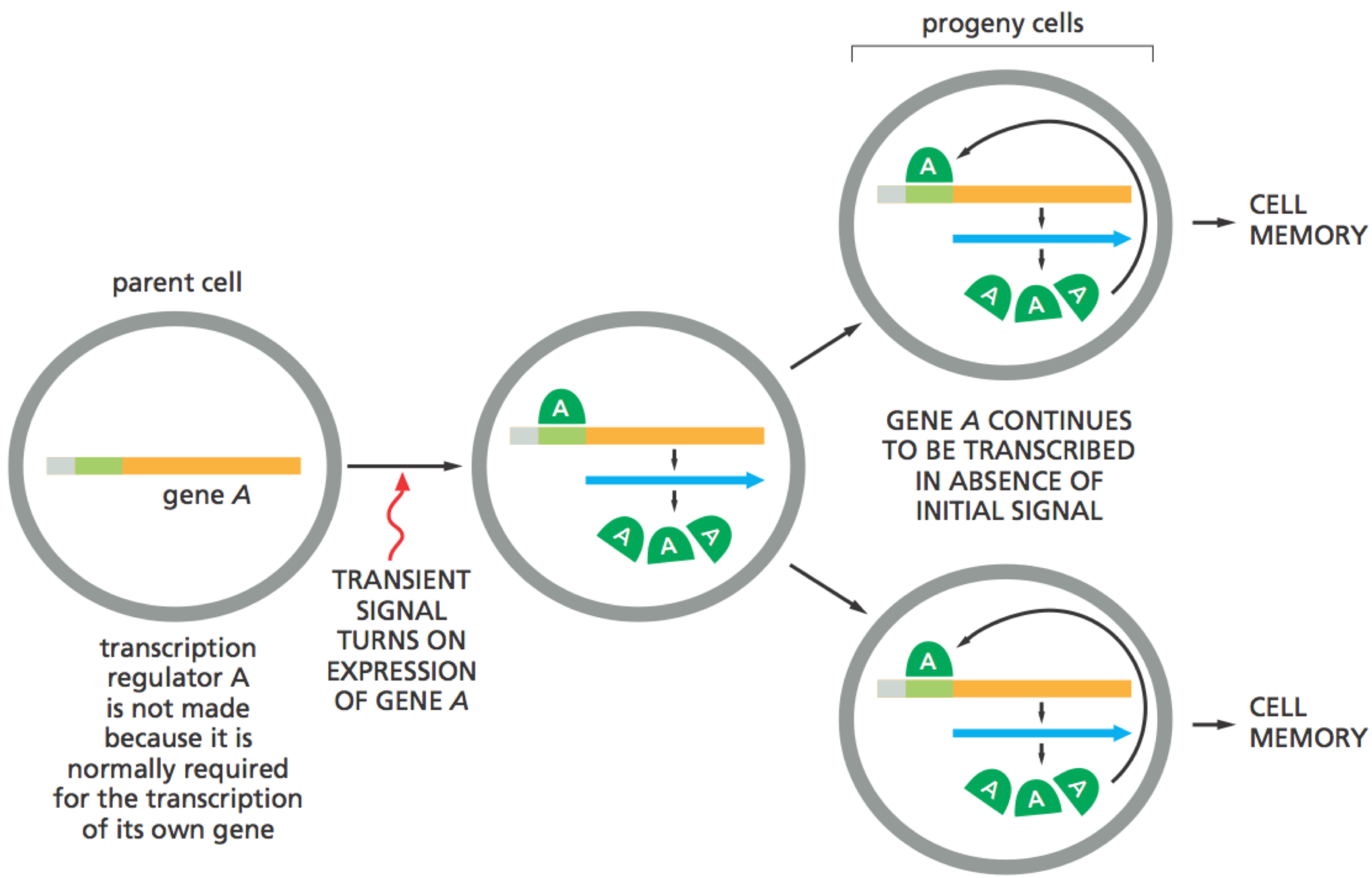
Figure 7–40 Common types of network motifs in transcription circuits. A and B represent transcription regulators, arrows indicate positive transcription control, while lines with bars depict negative transcription control. In the feed-forward loop, A and B represent transcription regulators that both activate the transcription of target gene Z (see also Figure 8–86).

Transcriptional Circuits

Transcriptional Circuits

Positive Feedback loops can be used to create cell memory

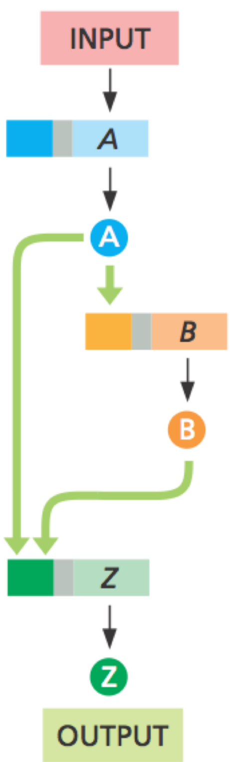




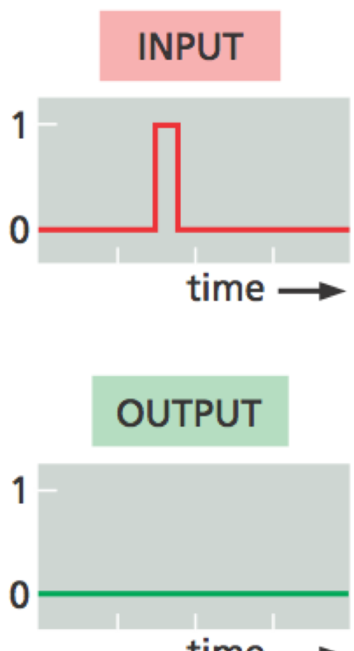
Transcriptional Circuits

Transcriptional Circuits

Feed-forward loops can measure the duration of a signal
- both A and B required for transcription of Z



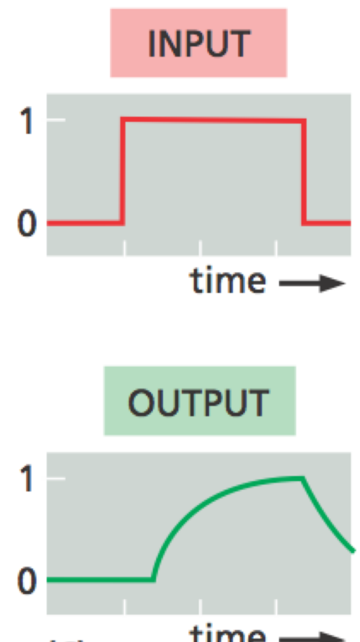
(A)



(B)

Brief input B does not accumulate

- Z not transcribed



(C)

Prolonged input B accumulates

- Z is transcribed

Transcriptional Circuits

Transcriptional Circuits

Combinations of regulatory circuits combine in eukaryotic cells to create exceedingly complex regulatory networks

Scientists can construct artificial circuits and examine their behavior in cells ● synthetic biology

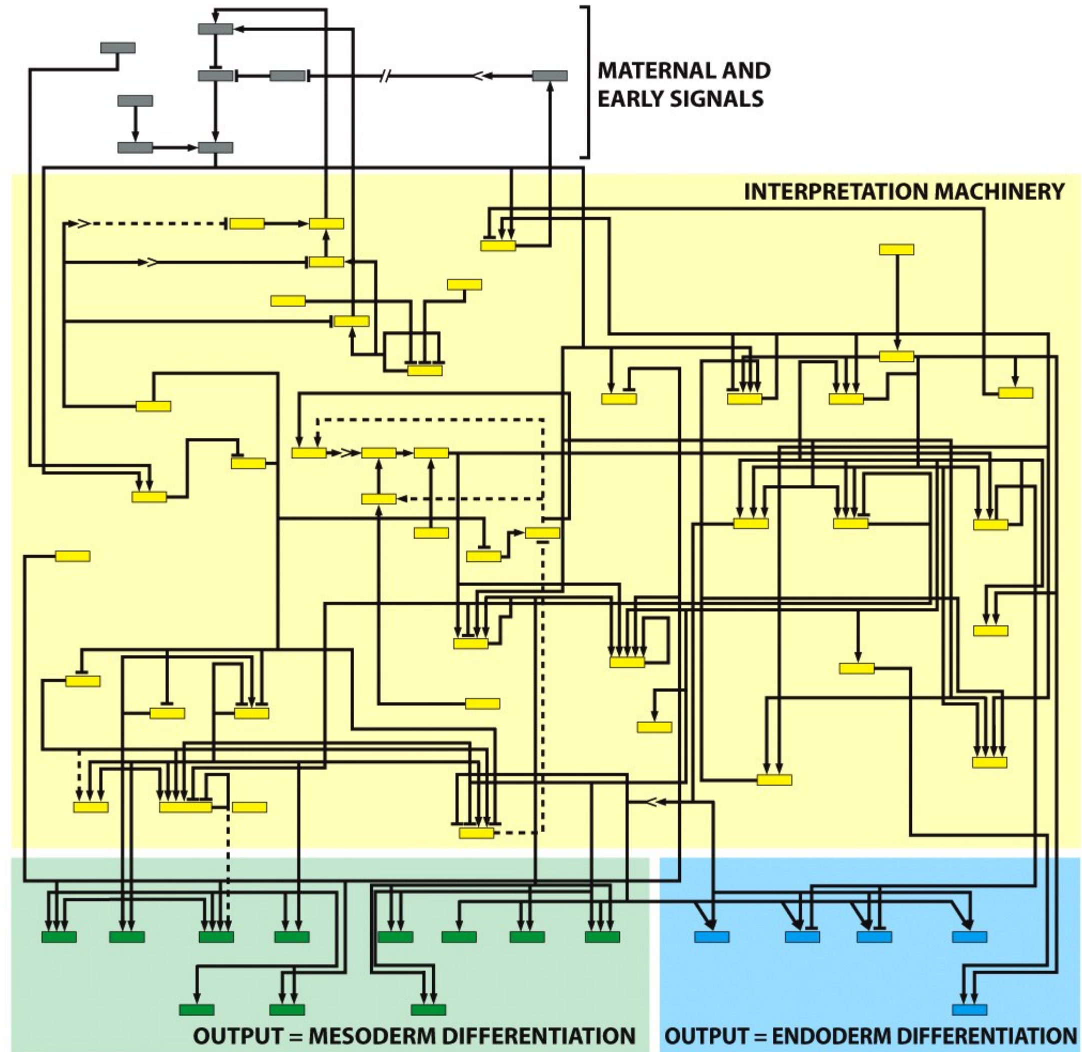


Figure 7-71 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Gene circuit of developing sea urchin embryo

Synthetic Biology

Synthetic Biology
eg. creating a simple gene oscillator using a delayed negative feedback circuit – “the repressillator”

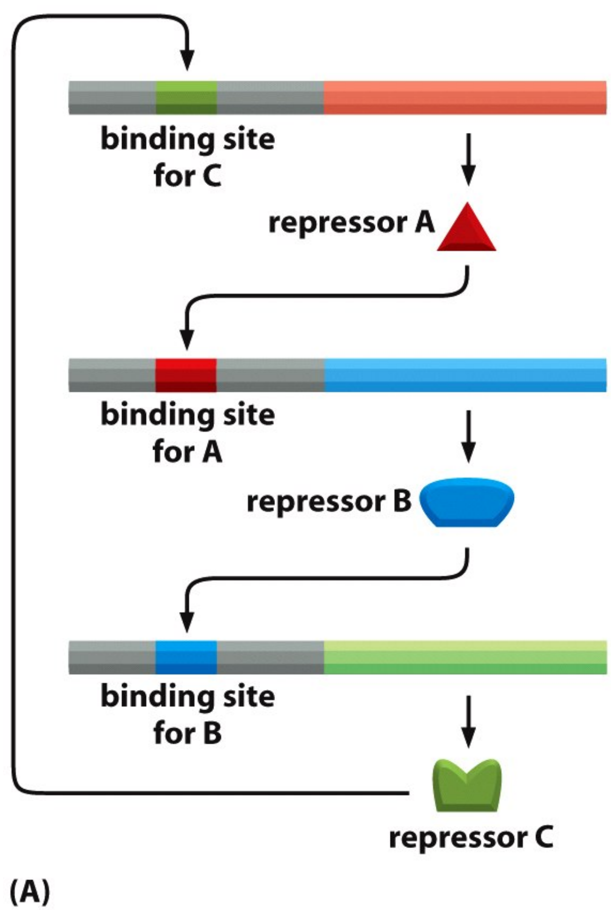


Figure 7-72 Molecular Biology of the Cell 5/e (© Garland Science 2008)

A: Lac repressor
B: Tet repressor (response to antibiotic)
C: Lambda repressor

Predicted: delayed negative feedback gives rise to oscillations

Introduced this circuit into bacterial cells and observed expression of the repressor genes

Synthetic Biology

A synthetic oscillatory network of transcriptional regulators

Michael B. Elowitz & Stanislas Leibler

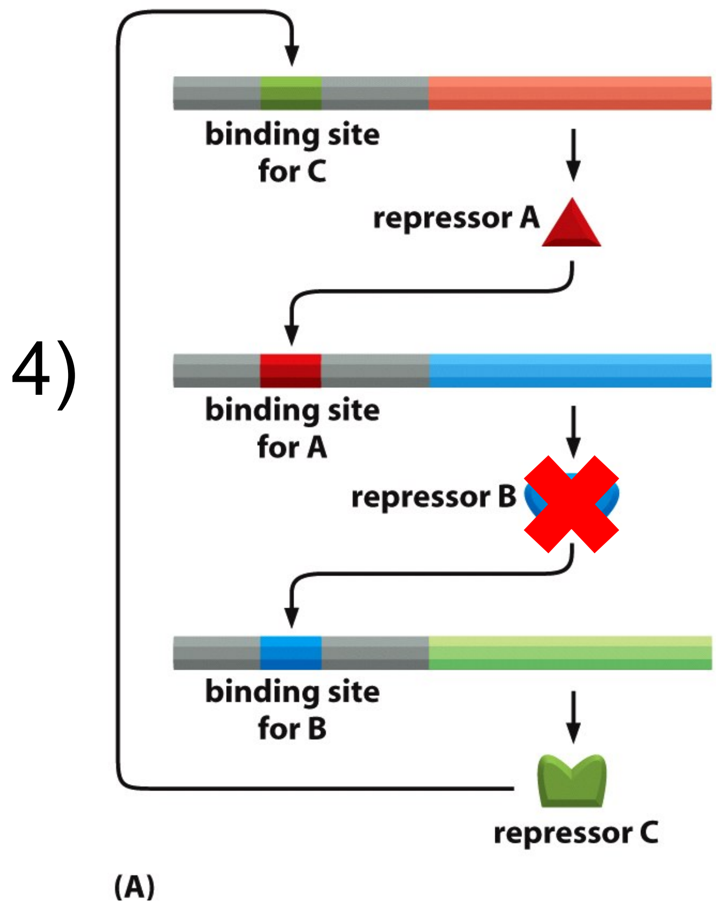
Departments of Molecular Biology and Physics, Princeton University, Princeton, New Jersey 08544, USA

Networks of interacting biomolecules carry out many essential functions in living cells[1], but the 'design principles' underlying the functioning of such intracellular networks remain poorly understood, despite intensive efforts including quantitative analysis of relatively simple systems[2]. Here we present a complementary approach to this problem: the design and construction of a synthetic network to implement a particular function. We used three transcriptional repressor systems that are not part of any natural biological clock[3–5] to build an oscillating network, termed

NATURE | VOL 403 | 20 JANUARY 2000 | www.nature.com

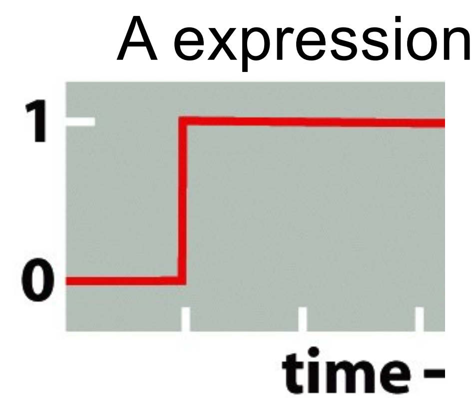
Synthetic Biology

Synthetic Biology: “the repressillator”, how does it work?



1) A expressed

2) B repressed

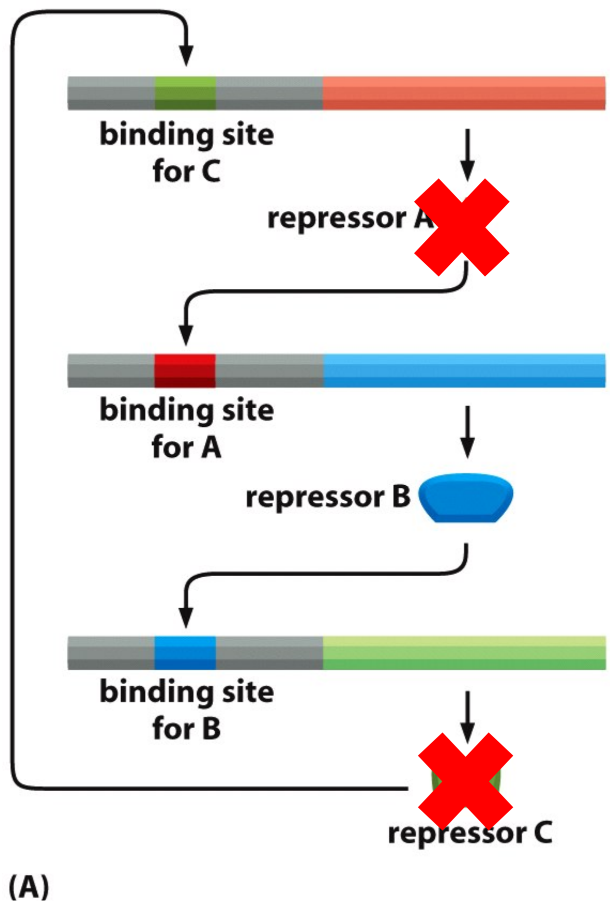


3) C expressed

4) C represses A expression

Synthetic Biology

Synthetic Biology: “the repressillator”, how does it work?

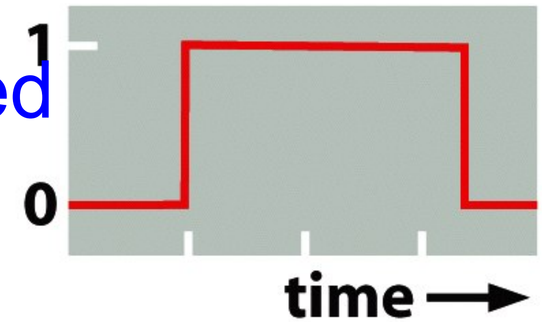


5) A repressed

6) B expressed

7) C repressed

8) Repeat 1-4



Did it work?

Synthetic Biology

Synthetic Biology
eg. creating a simple gene oscillator using a negative feedback circuit

Looking at 1 Protein (Fluorescently tagged)

Predicted

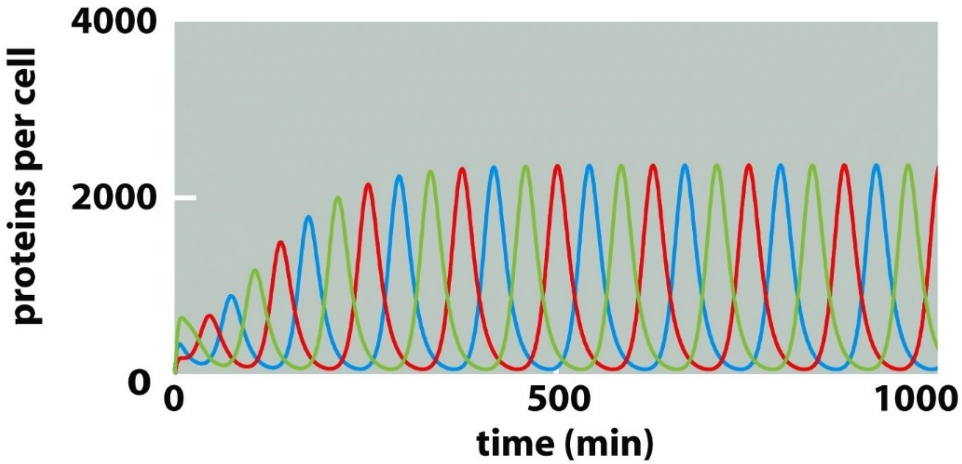


Figure 7-72b Molecular Biology of the Cell 5/e (© Garland Science 2008)

Observed

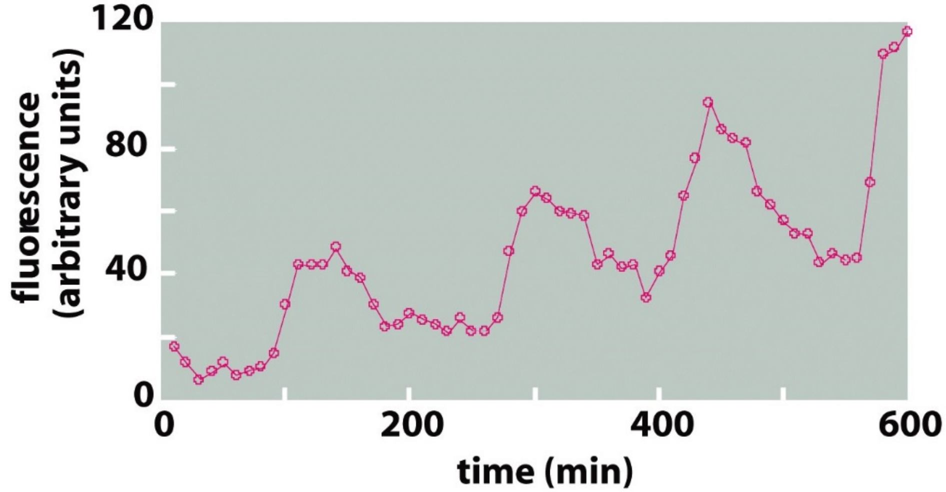


Figure 7-72c Molecular Biology of the Cell 5/e (© Garland Science 2008)

Increasing amplitude due to bacterial growth

Transcriptional Circuits

Feedback loops also circadian gene regulation
~ 24-hour cycle: eg. *Drosophila*

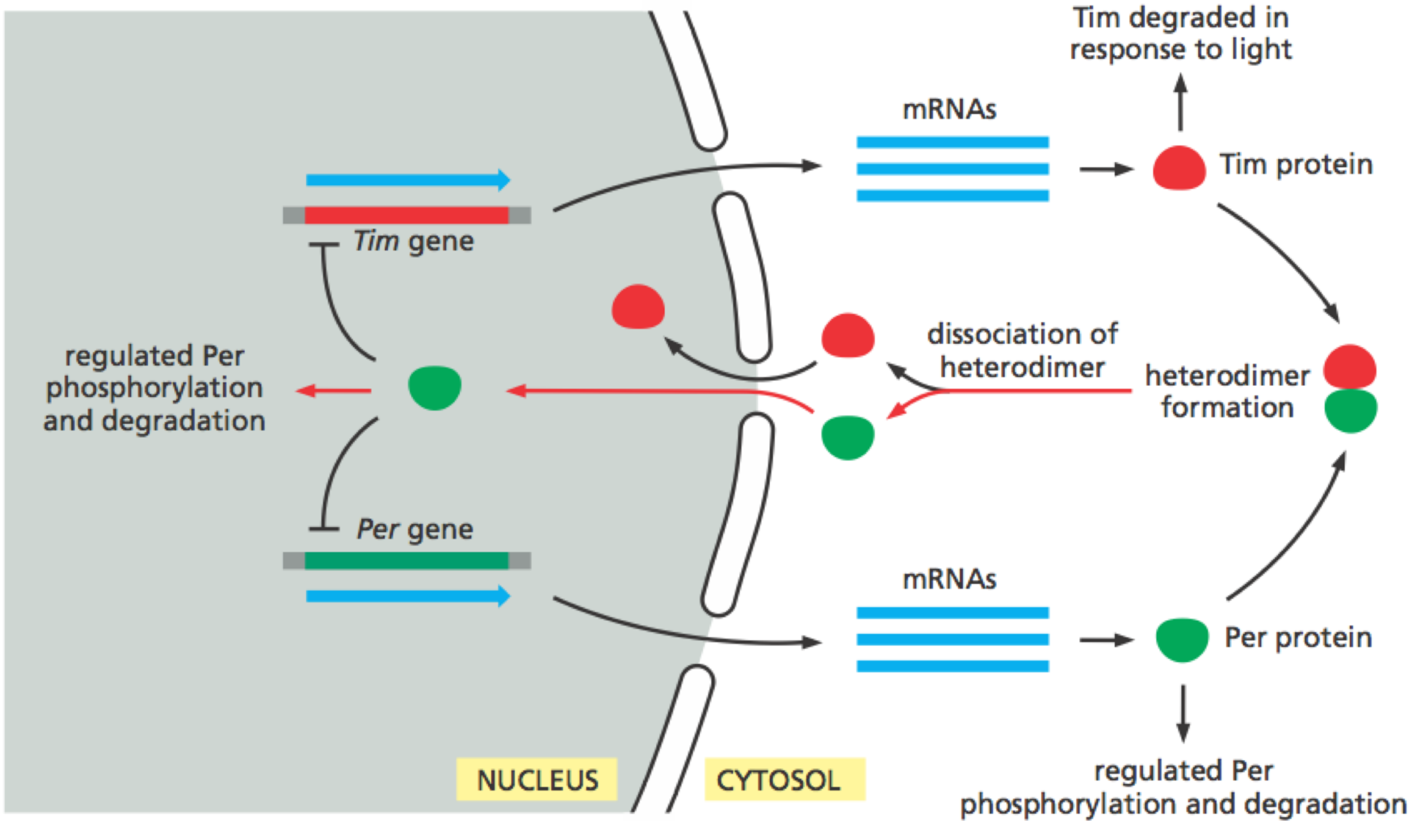


Figure 15–66

http://www.hhmi.org/biointeractive/drosophila-molecular-clock-model

- Delayed Negative Feedback Loop

Transcription Attenuation

-In both prokaryotes and eukaryotes there can be a premature termination of transcription called

- Transcription attenuation

-RNA adopts a structure that interferes with RNA polymerase

-Regulatory proteins can bind to RNA and interfere with attentuation

-Prokaryotes, plants and some fungi also use

- Riboswitches to regulate gene expression

Transcription Attenuation

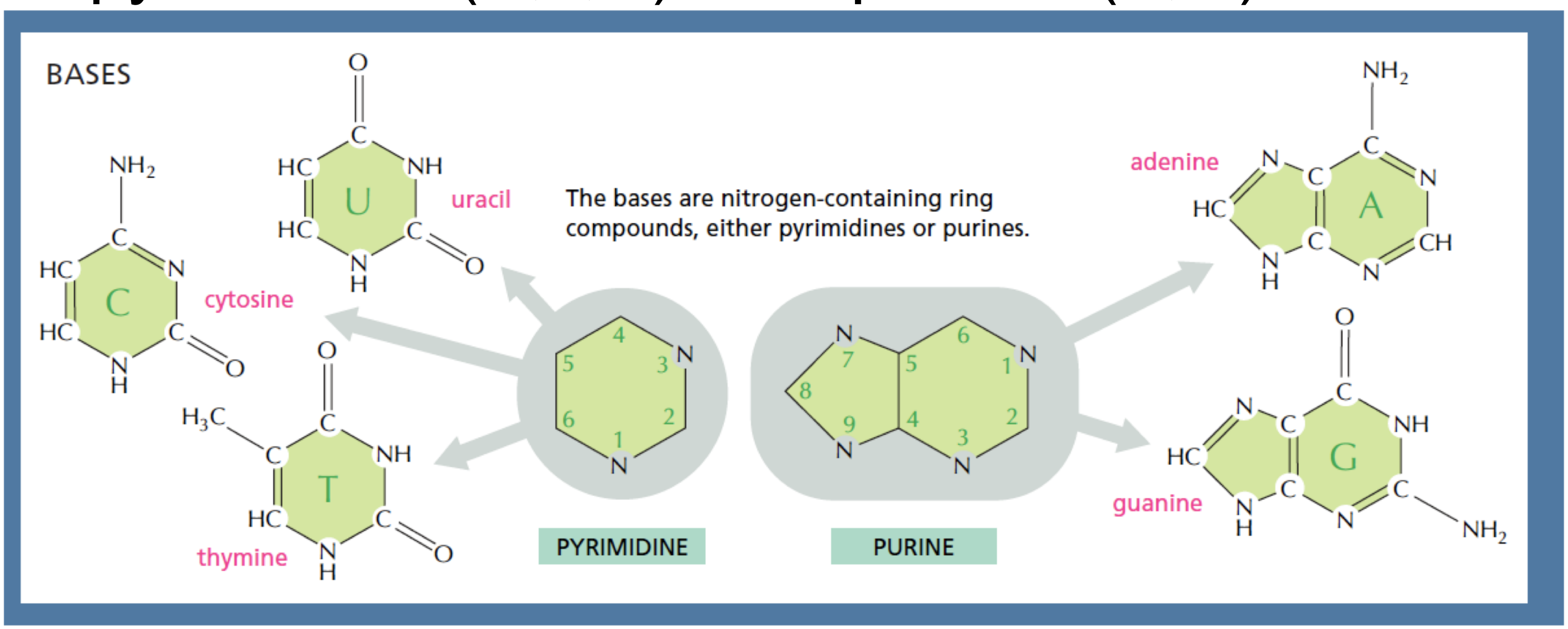
Riboswitches

Short RNA sequences that change conformation when bound by a small molecule

eg. prokaryotic riboswitch that regulates purine biosynthesis

Recall that bases making up DNA/RNA include:

pyrimidines (C,T,U) purines (A,G)



Transcription Attenuation

Riboswitches

eg. prokaryotic riboswitch that regulates purine biosynthesis

Low guanine levels

-Transcription of purine biosynthetic genes is ● on

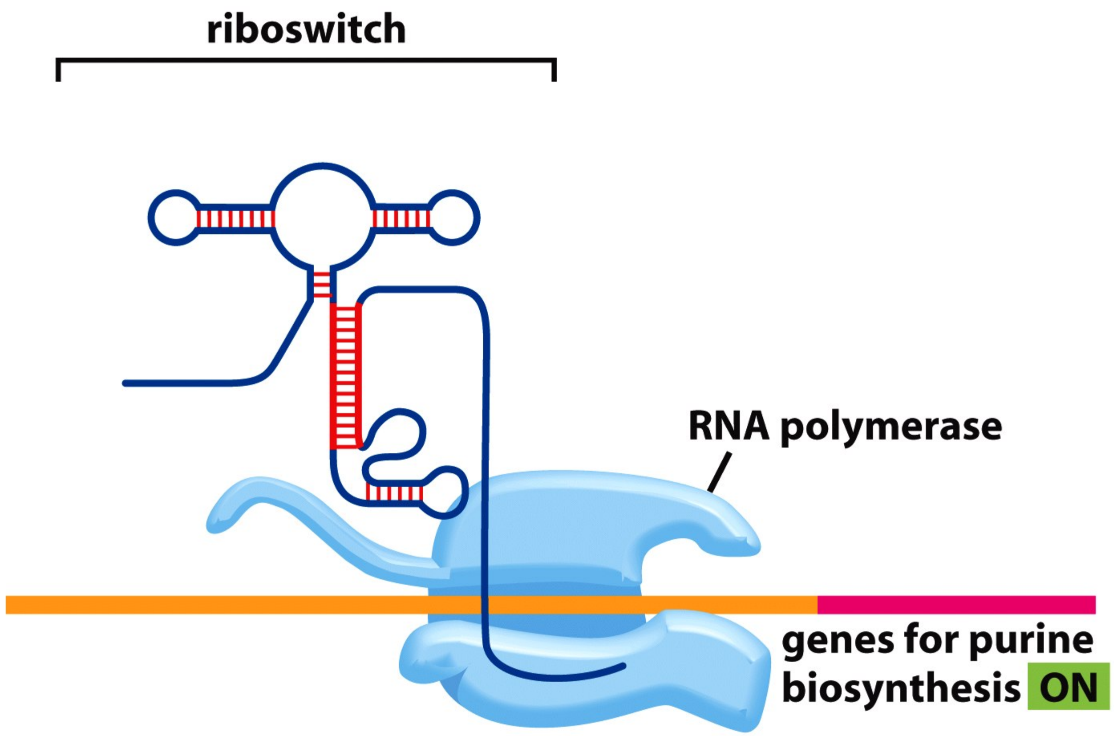


Figure 7-93a Molecular Biology of the Cell 5/e (© Garland Science 2008)

Transcription Attenuation

Riboswitches

eg. prokaryotic riboswitch that regulates purine biosynthesis

High guanine levels

-Guanine binds ● riboswitch

-Riboswitch undergoes ● conformational change

-Causes RNA polymerase to terminate transcription

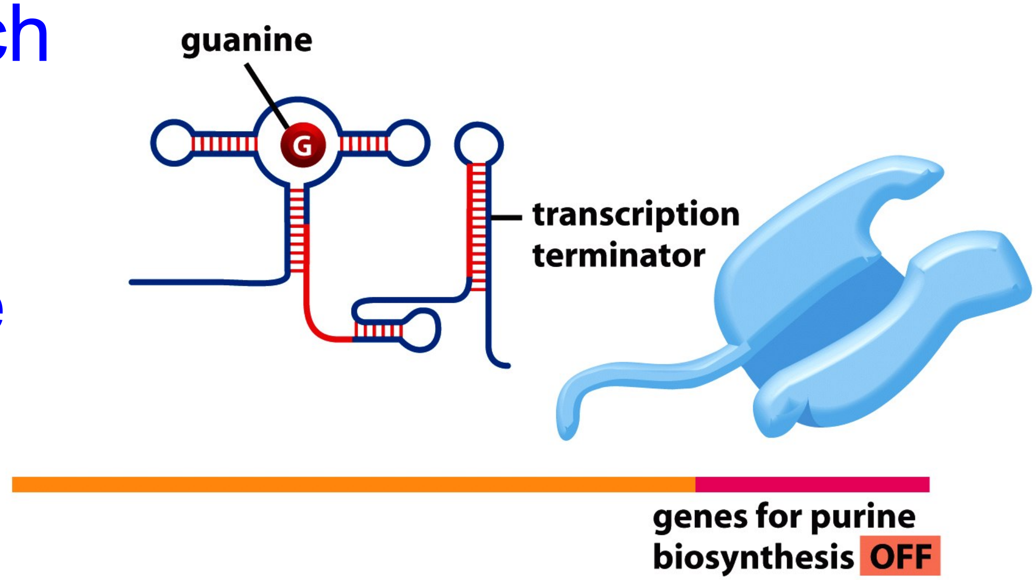


Figure 7-93b Molecular Biology of the Cell 5/e (© Garland Science 2008)

-Transcription of purine biosynthetic genes is ● off

Remember to read the textbook. Check the textbook for answers to your questions.

After reading the textbook, questions are welcome… please ask on the Discussion Board, and/or after classes.

Help one another on the Discussion Board.