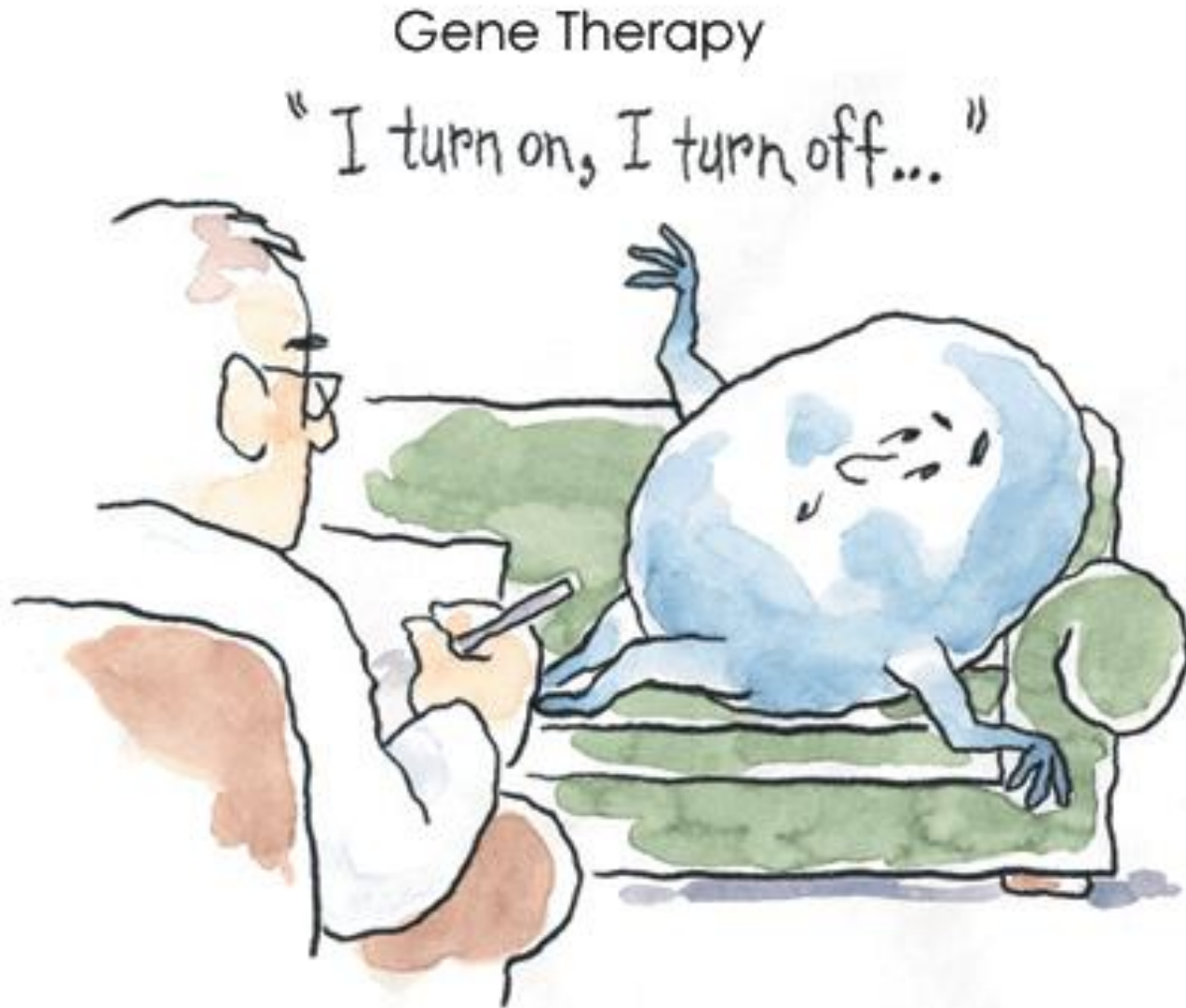


# Module 5 – Regulation of Prokaryotic Gene Expression



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BIO/BCH 3170  
Molecular Biology

# Resources – Recommended Reading

## Nature Scitable Articles

- **Lecture 15** – [Gene Expression Regulates Cell Differentiation](#)  
– [Transcription Factors and Transcriptional Control in Eukaryotic Cells](#)
- **Lecture 16** – [Operons and Prokaryotic Gene Regulation](#)
- **Lecture 17** – none

## Prefer a textbook?

- Molecular Biology of the Cell – **Chapter 7**
- Lecture 17 – Molecular Biology of the Gene - **Ch. 18 p636 - 652**

# Resources – Helpful Videos

- [Amoeba Sisters - Gene Regulation and the Order of the Operon](#)

# Optional Resources to Explore

Additional Reading –

[Do transcription factors actually bind DNA? DNA footprinting and Gel Shift Assays](#)

[Negative Transcription Regulation in Prokaryotes](#)

[Positive Transcriptional Control The Glucose Effect](#)

Fun Stuff –

- Interpretive Dance - [The intricate dance of transcription factors and polymerase](#)
- **Why should you care about phages?** – [Science Couple Phages Out Superbug](#) (podcast, 34:32)

# Regulation of Gene Expression

## L15 - General Introduction

# Learning Outcomes

By the end of this lesson, you should be able to:

- Discuss the importance of the regulation of gene expression and appreciate that it occurs at many stages
- Appreciate and discuss how the regulation of gene expression occurs independently within each cell
- Describe the importance of the regulation of the initiation of transcription and acknowledge its limitations
- Distinguish between **cis** and **trans regulation** of transcription
- Appreciate that the genome encodes for many transcription factors
  - Describe generally how TFs recognize specific DNA sequences
  - Describe the structure of several classes of TFs and identify how each interacts with DNA
    - By the final exam you should know at least 1 specific example for each class
  - Explain how **dimerization of TFs** can contribute to the diversity of regulatory mechanisms
- Distinguish positive control from negative control of gene expression.
- Describe positive and negative feedback, feed forward and flip flop circuits, explaining how these could be useful in biological systems (we will see specific examples in future)
  - Draw simple circuits and feedback loops

# All Somatic Cells Contain the Same Genetic Material

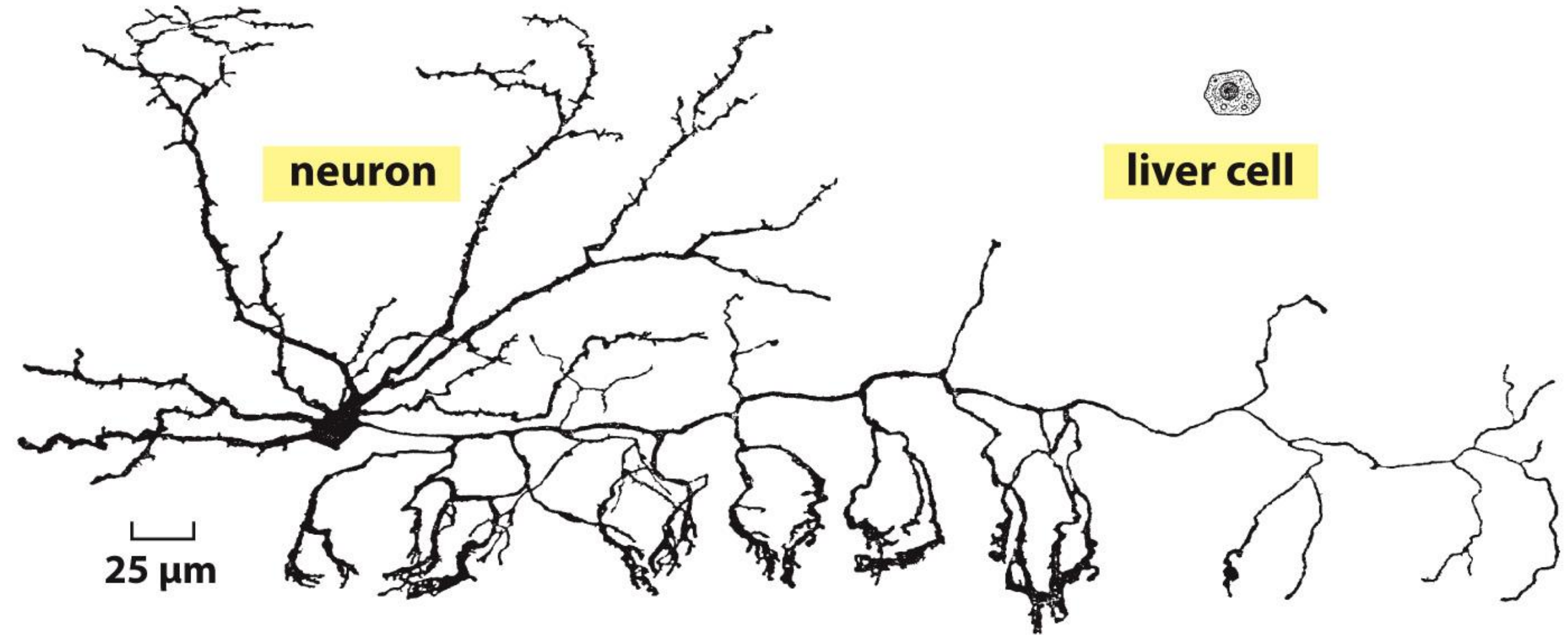


Figure 7-1 Molecular Biology of the Cell 6e (© Garland Science 2015)

# All Somatic Cells Contain the Same Genetic Material

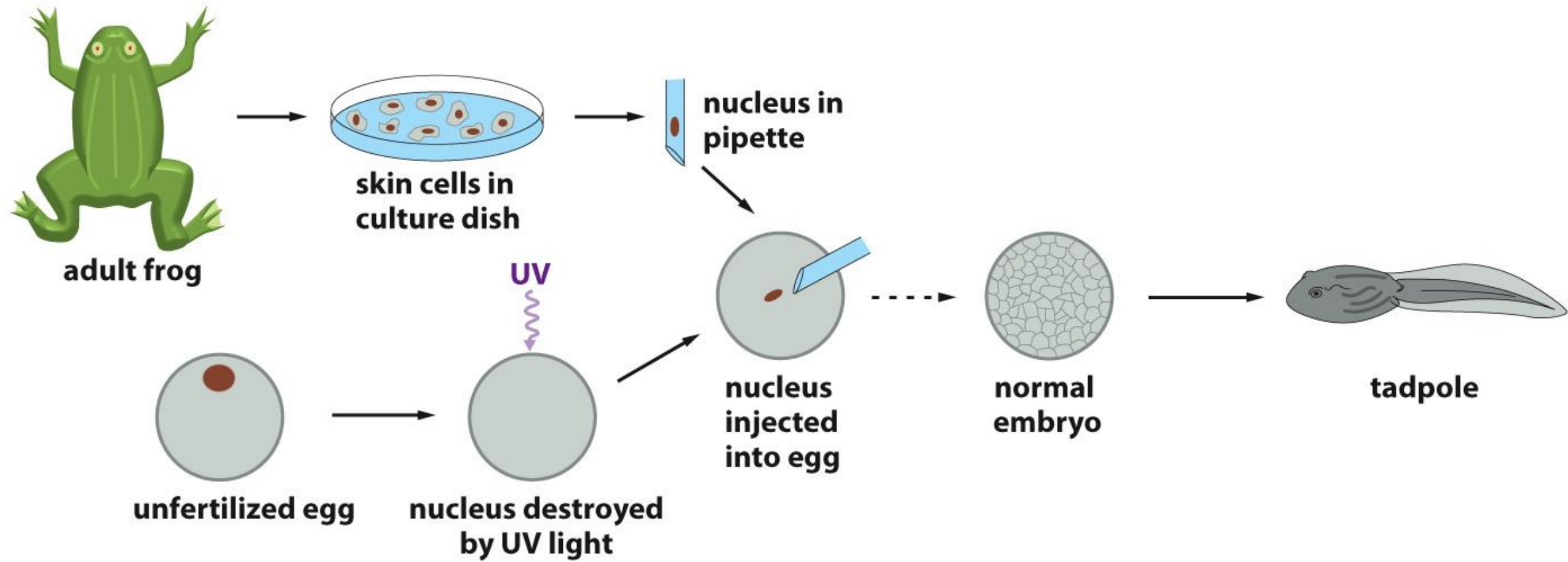


Figure 7-2a Molecular Biology of the Cell 6e (© Garland Science 2015)

# All Somatic Cells Contain the Same Genetic Material

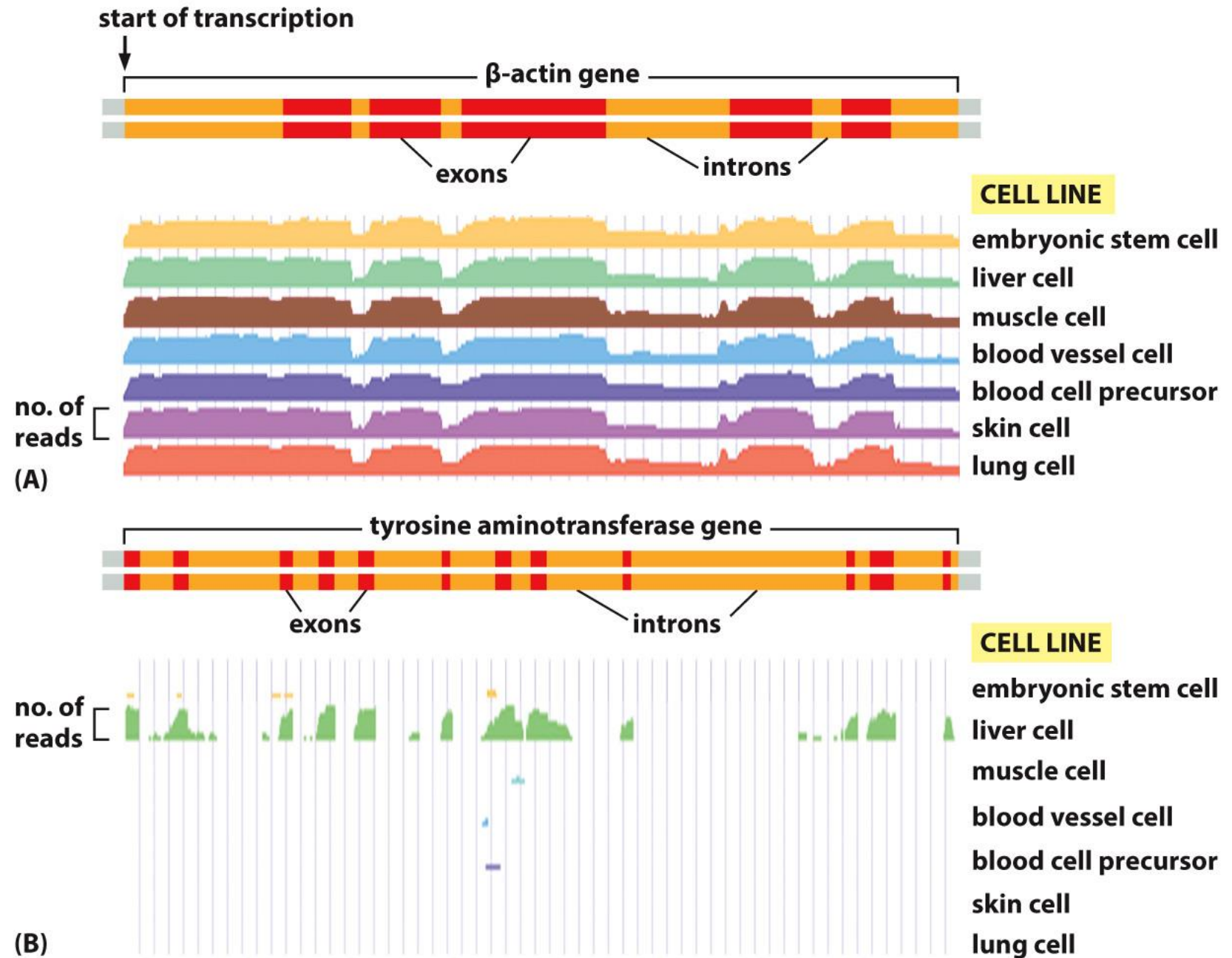


Figure 7-3 Molecular Biology of the Cell 6e (© Garland Science 2015)

# Regulation of Gene Expression

.... refers generally to the set of mechanisms used by a cell to increase or decrease the amount of a gene product it makes

- Regulation may occur at **any stage of gene expression**

i.e. – from the initiation of transcription through to post-translational modification of a protein

- The expression of one gene often regulates the expression other genes via **gene regulatory networks**

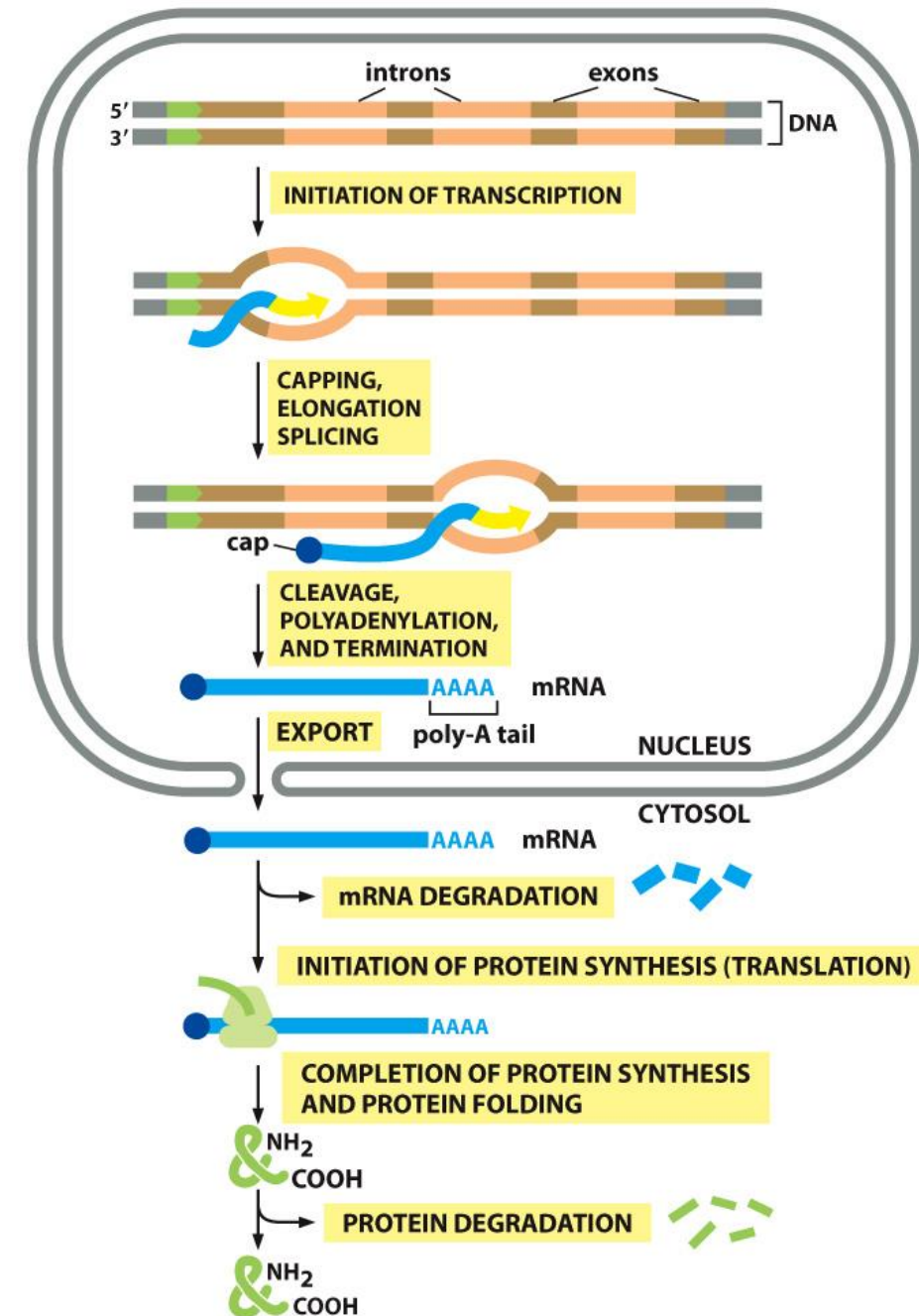
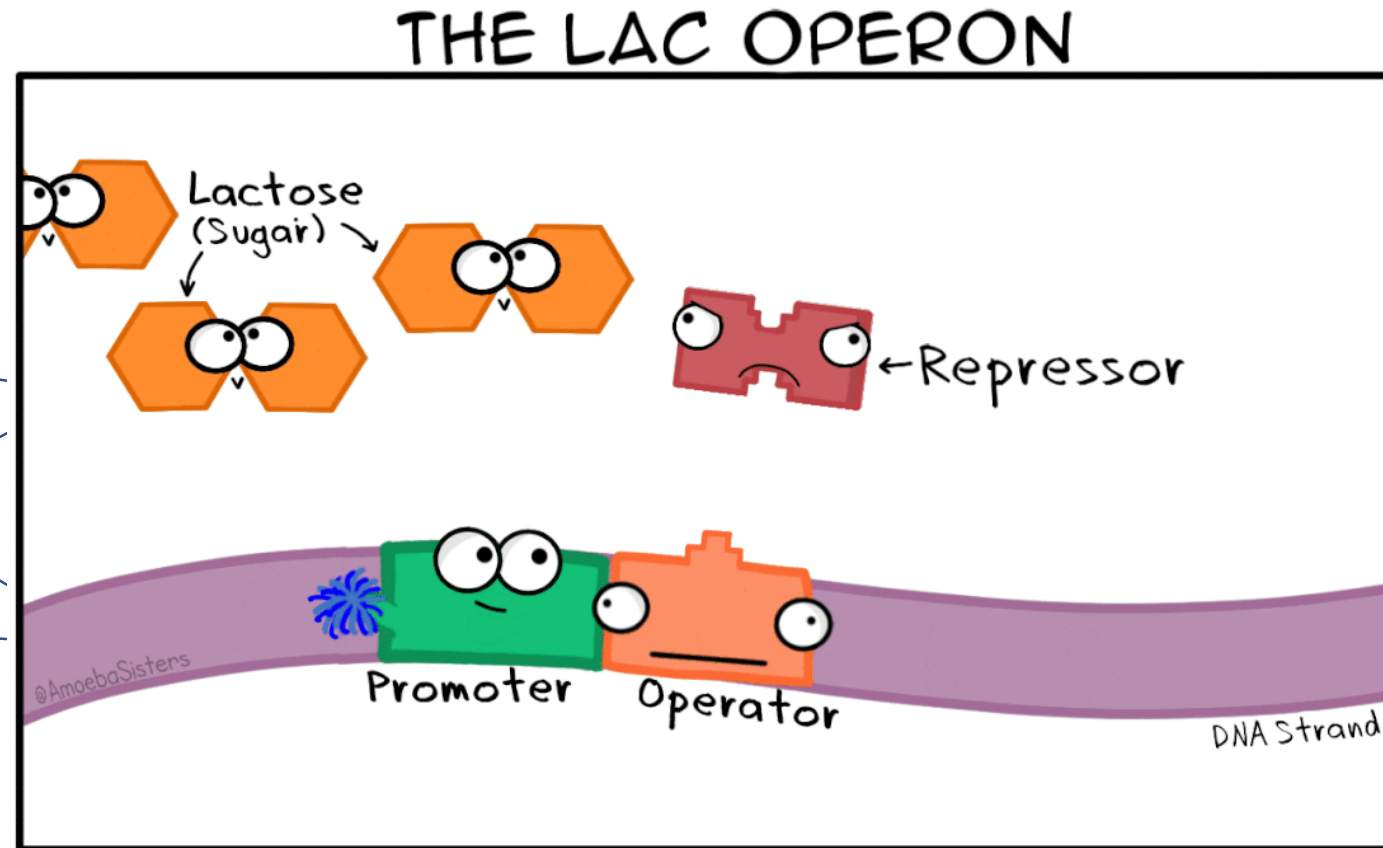


Figure 6-87 Molecular Biology of the Cell 6e (© Garland Science 2015)

# First Identified in E.Coli – the Lac Operon

The Nobel Prize in Physiology or Medicine 1965 was awarded jointly to **François Jacob**, **André Lwoff** and **Jacques Monod** "for their discoveries concerning **genetic control** of **enzyme** and **virus** synthesis."

We will discuss the Lac operon in detail next class. [Watch the amoeba sisters video as a refresher on the topic!](#)



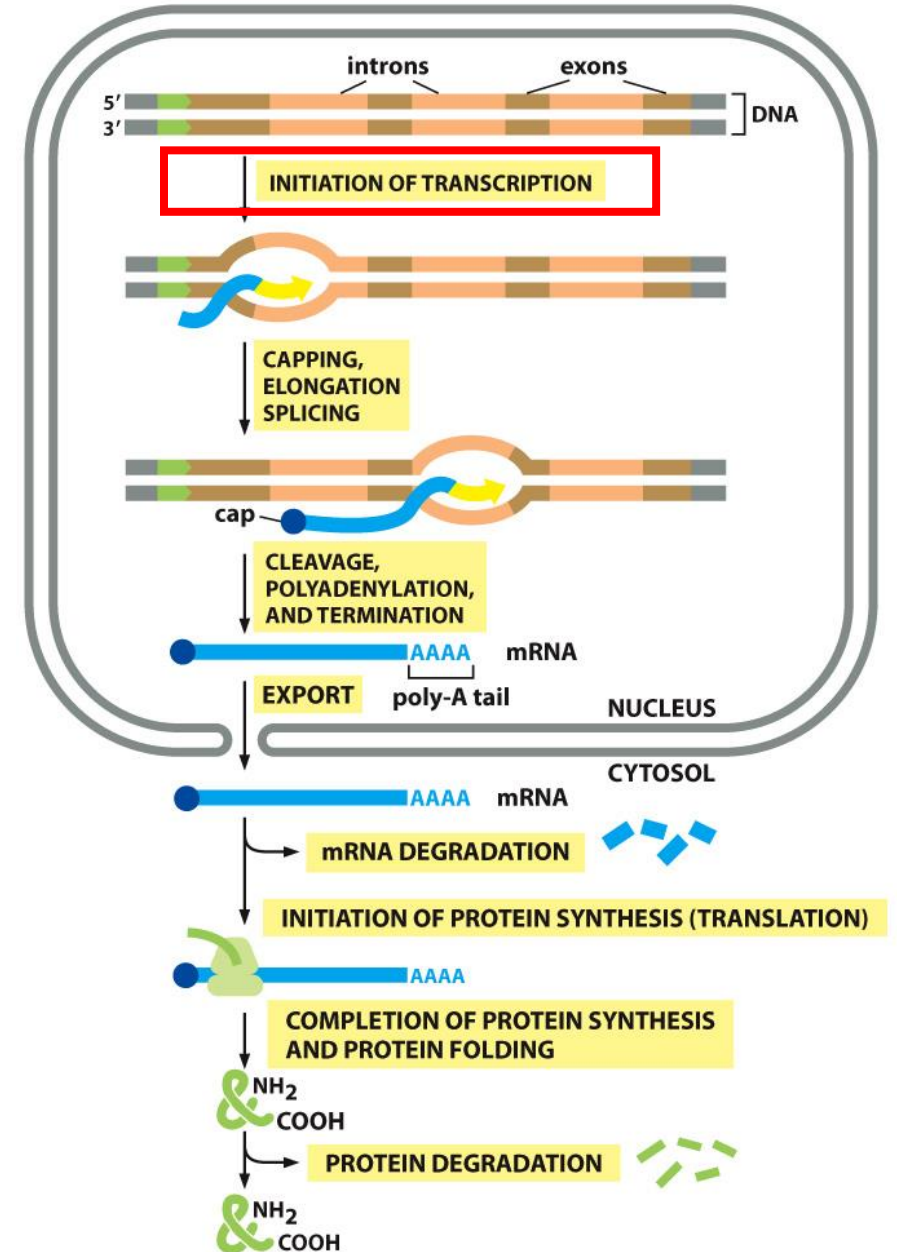
# Much of the regulation of gene expression occurs at the **INITIATION OF TRANSCRIPTION**

## Why?

- Energetically favorable
- Simplest

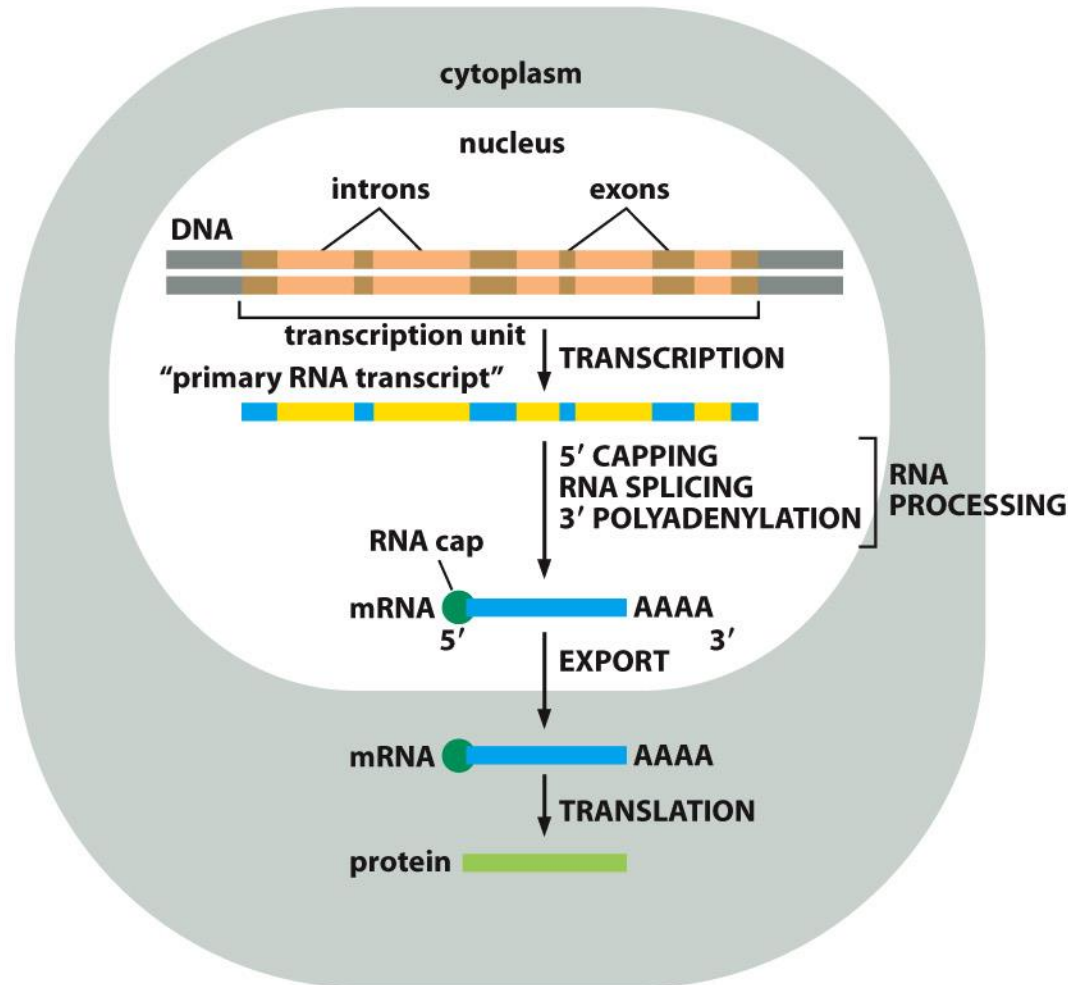
## Why not **ONLY** regulate transcription initiation?

- Allows for fine tuning
- Speed



# Regulation of Transcription Initiation

(A) EUKARYOTES



(B) PROKARYOTES

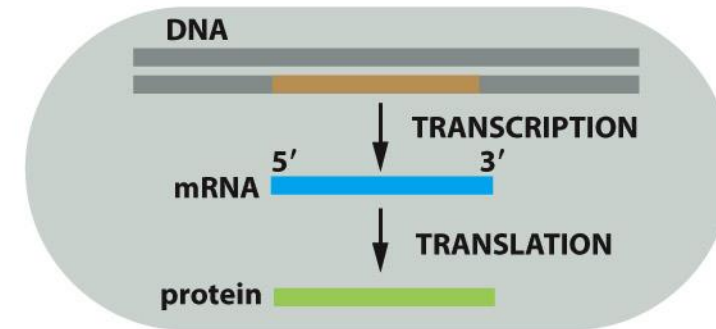
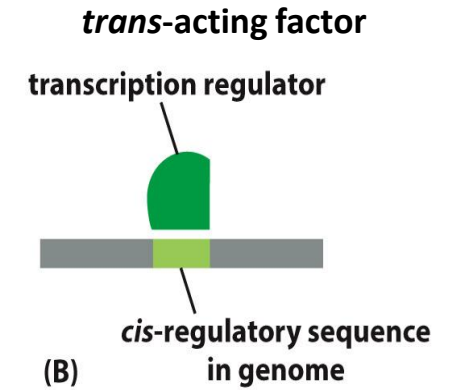
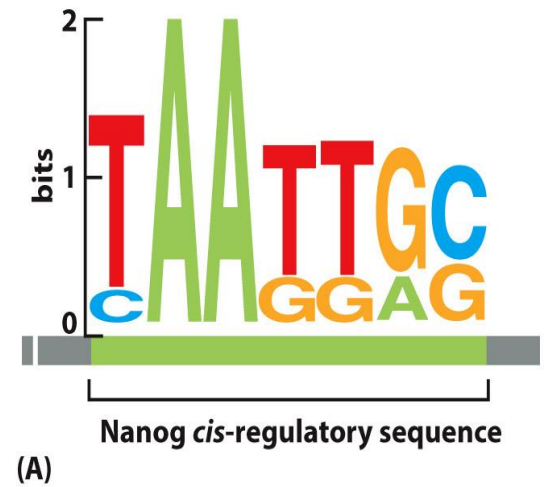


Figure 6-20 Molecular Biology of the Cell 6e (© Garland Science 2015)

# Transcriptional Regulation

## - Terminology



Transcriptional regulation occurs via a combination of:

- **Cis-regulatory elements**
  - Found on the same molecule of DNA as the gene whose transcription they regulate, they are typically non-coding DNA sequences which bind transcriptional regulators
- **Trans-regulatory elements**
  - Typically proteins which interact with the DNA of the gene(s) they regulate

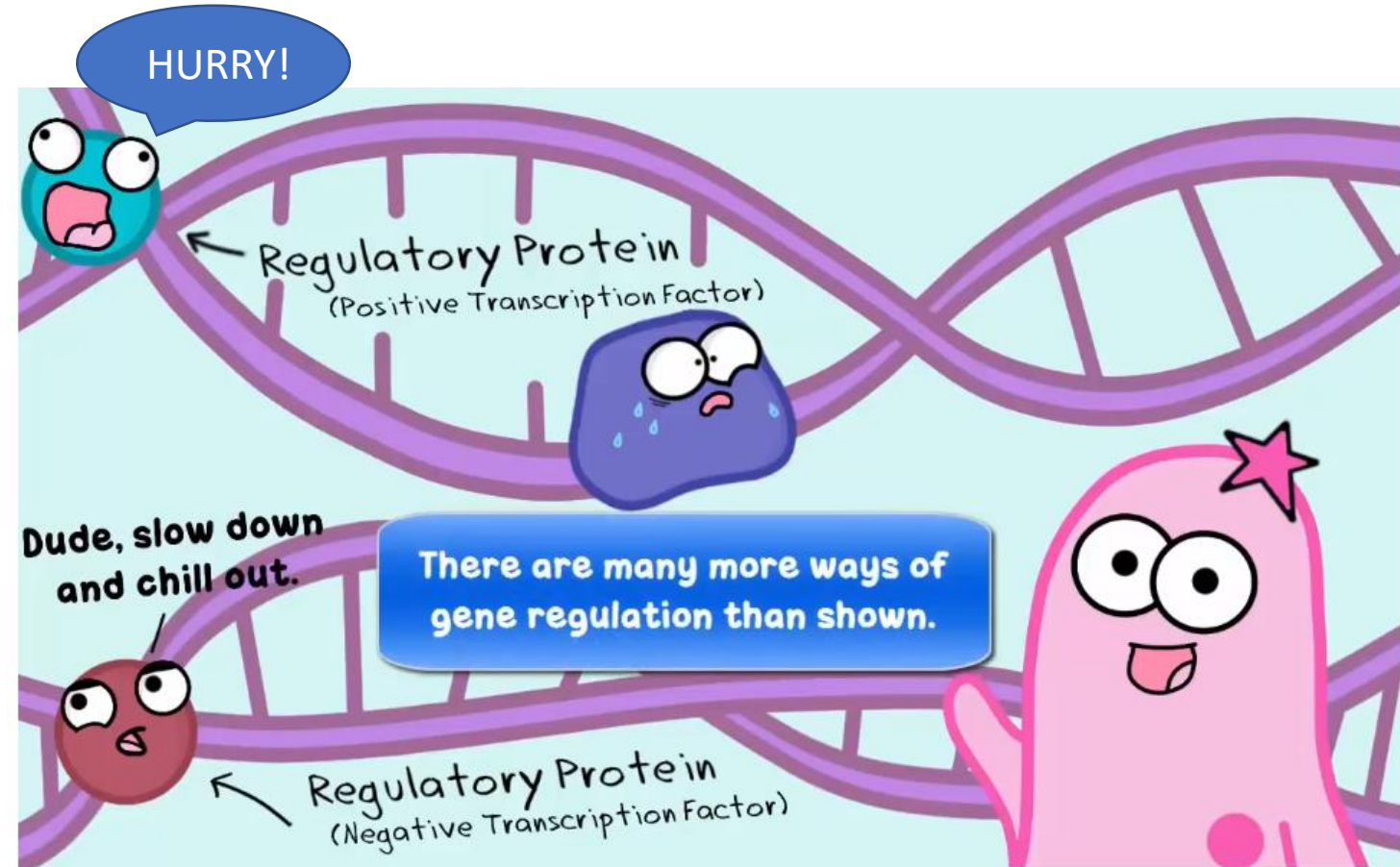
We can say that *cis*-regulatory sequences are often binding sites for *trans*-acting factors.

# Transcription Factors (TFs) Regulate Transcription

TFs are a type of Regulatory DNA Binding Protein (RDBP)

which can be an:

- **Activator** (positive regulators) which increases gene expression
- **Repressor** (negative regulator) which decrease gene expression
- RDBPs typically recognize specific sequences at or near the gene they regulate



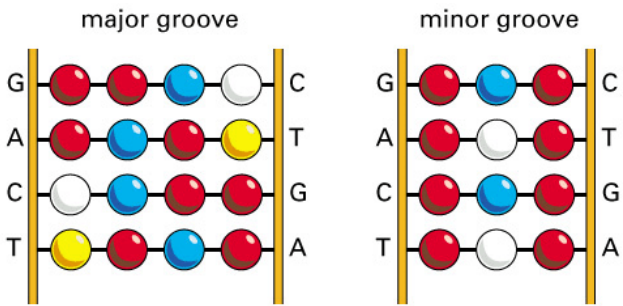
# Importance of Transcription Factors

- Approximately 10% of all protein coding genes in humans are TFs
- Deletion of a TF gene from the genome leads to profound organizational and developmental irregularities

*Table 1: Effects of Some Transcription Factor (TF) Gene Deletions in Drosophila*

TF Gene Deleted	Gene Group	Type of TF	Phenotypic Effects Observed
Buttonhead	Gap	Zinc finger	Lack of mandibular, intercalary, and antennal head segments
Hairy	Pair rule	bHLH	Ectopic expression of bristles on legs and wings
Antennapedia	Homeotic	Homeobox	Legs on the head where antennae should be

- The exposed atoms on each surface of a base pair presents a distinctive pattern which can be recognized by TFs
- Most interactions happen along the **major groove** of the DNA
  - wider (more accessible) and has more molecular features to interact with



KEY:

- = H-bond acceptor
- = H-bond donor
- = hydrogen atom
- = methyl group

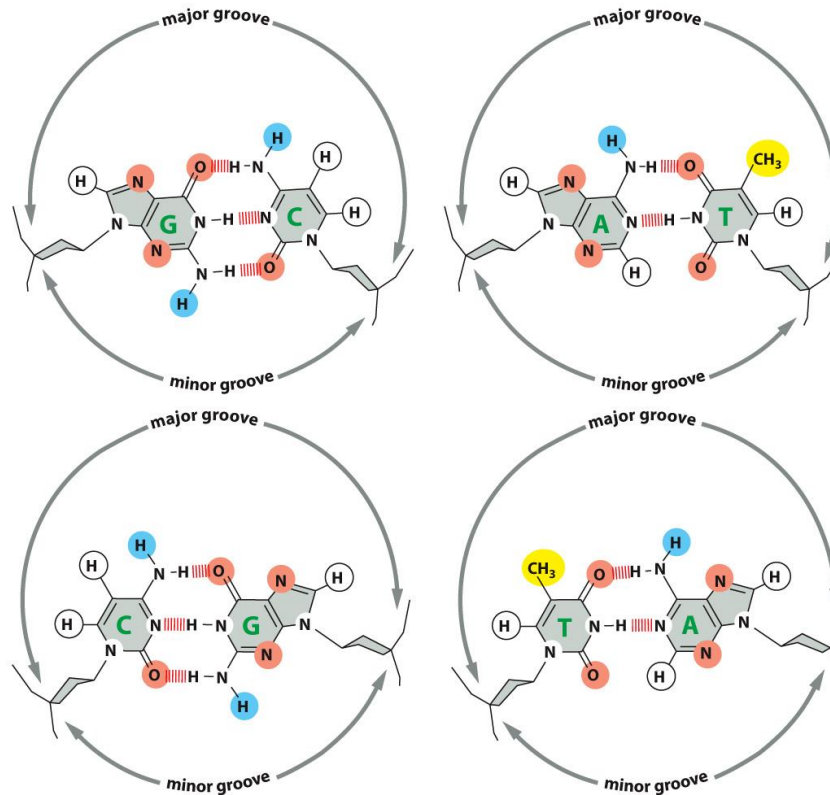


Figure 7-7 Molecular Biology of the Cell 6e (© Garland Science 2015)

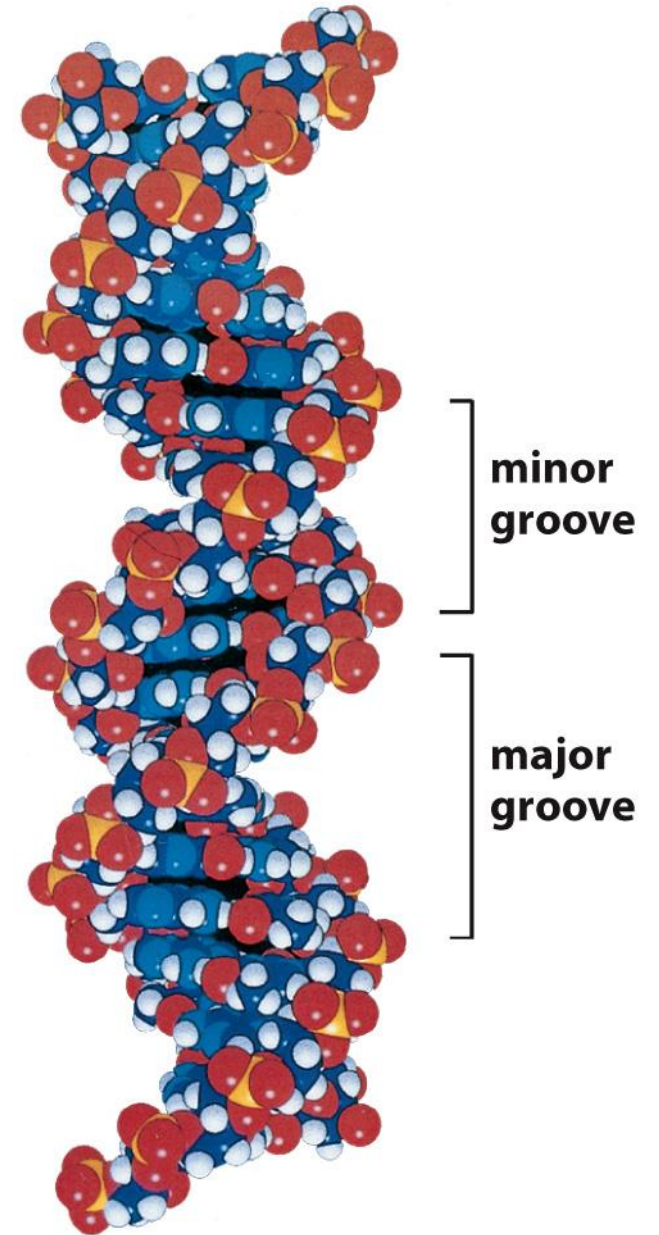


Figure 7-6 Molecular Biology of the Cell 6e (© Garland Science 2015)

Figure 7-8. Molecular Biology of the Cell, 4th Edition.

- TFs make multiple contacts via their amino acid side chains with the surface of the DNA
- Each interaction alone is relatively weak; however, the net effect of the multiple interactions creates a **highly specific** and **very strong** interaction

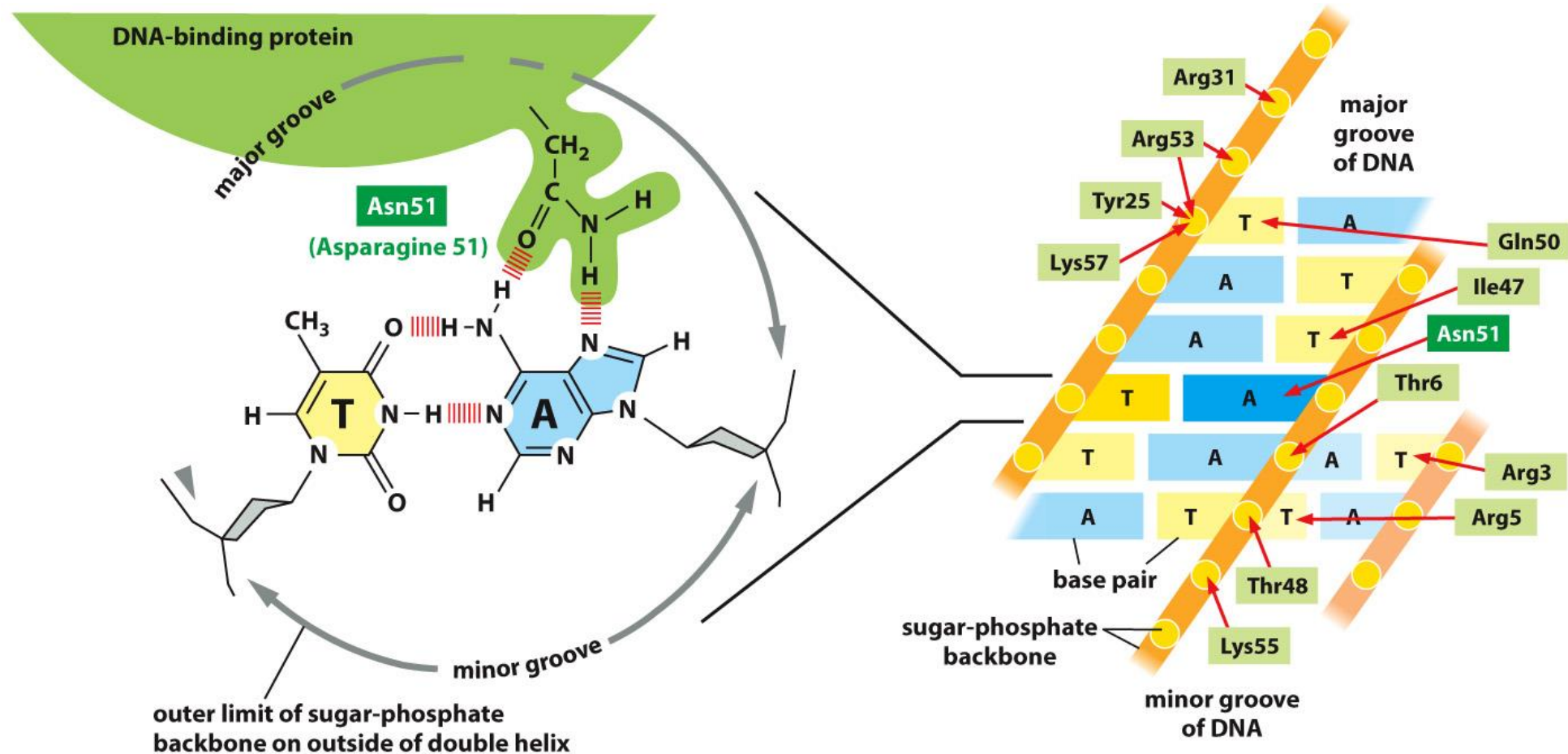


Figure 7-8 Molecular Biology of the Cell 6e (© Garland Science 2015)

# Affinity for DNA

- Most TFs bind a consensus sequence of 6-8 bp
  - How often would a 6nt sequence occur by chance?
- Many TFs dimerize, doubling the length of the DNA sequence they recognize
  - Greatly increases affinity and specificity
  - Existence of **heterodimers** further increases the number of possible combinations and possible types of control without the need for more unique regulators

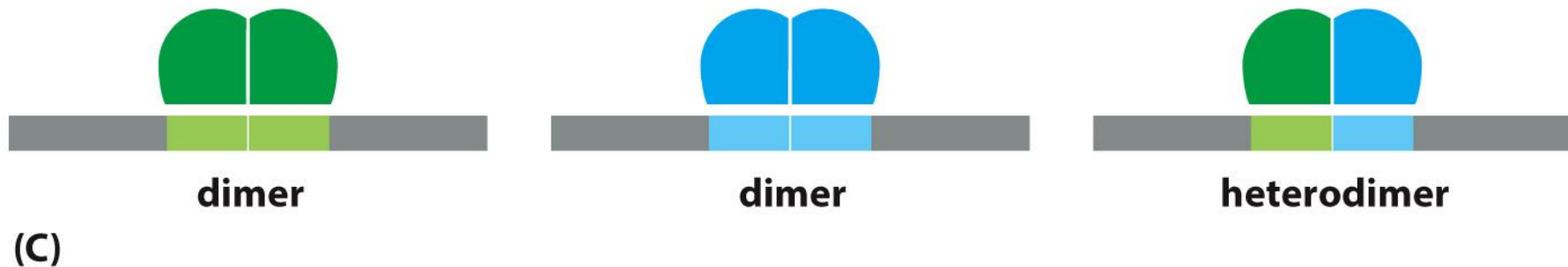
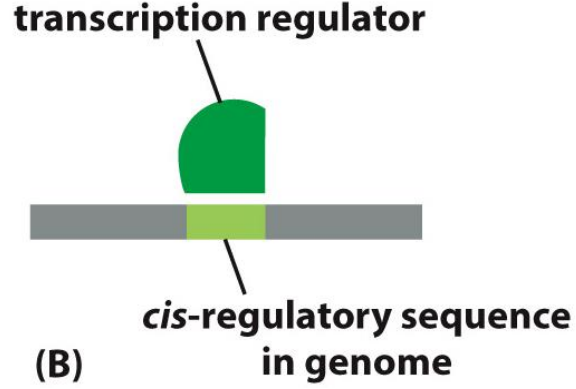
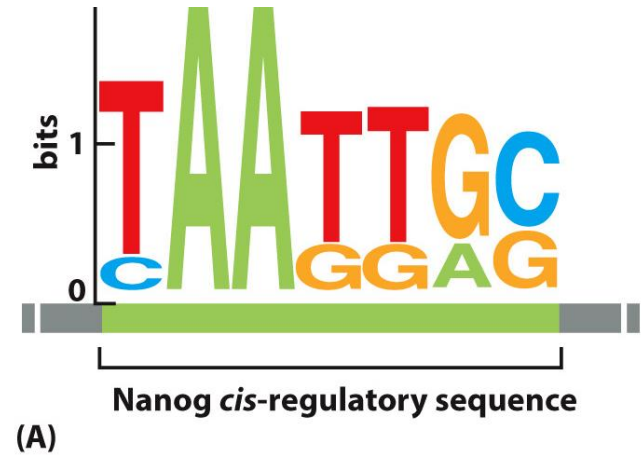


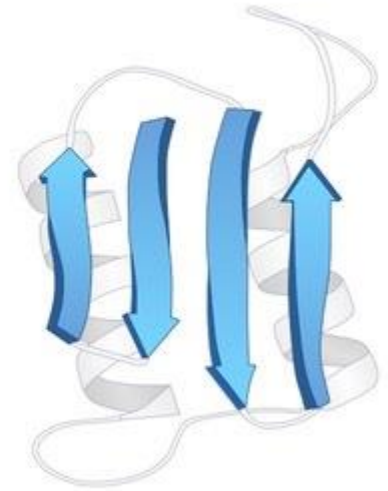
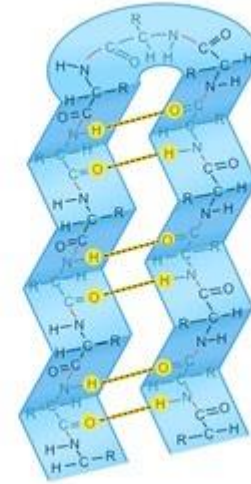
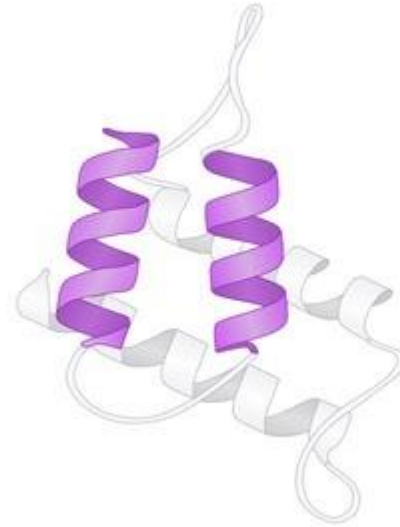
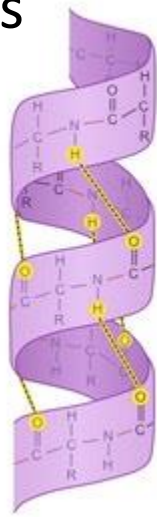
Figure 7-9 Molecular Biology of the Cell 6e (© Garland Science 2015)

# Structure

All TFs have both a DNA-binding domain and a protein-protein interaction domain.

- Most DNA binding domains interact with the major groove of the DNA through **alpha helices**

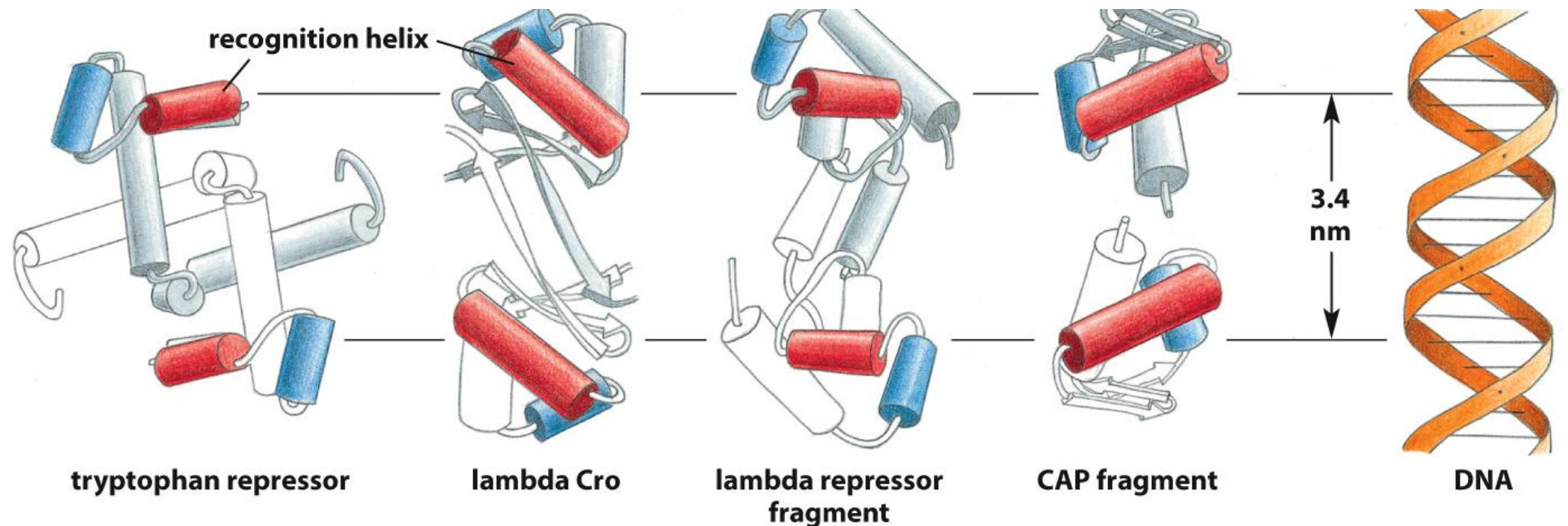
- Helix-turn-helix
  - Homeodomain proteins
- Leucine zippers
- Helix-loop-helix
- Zinc finger proteins



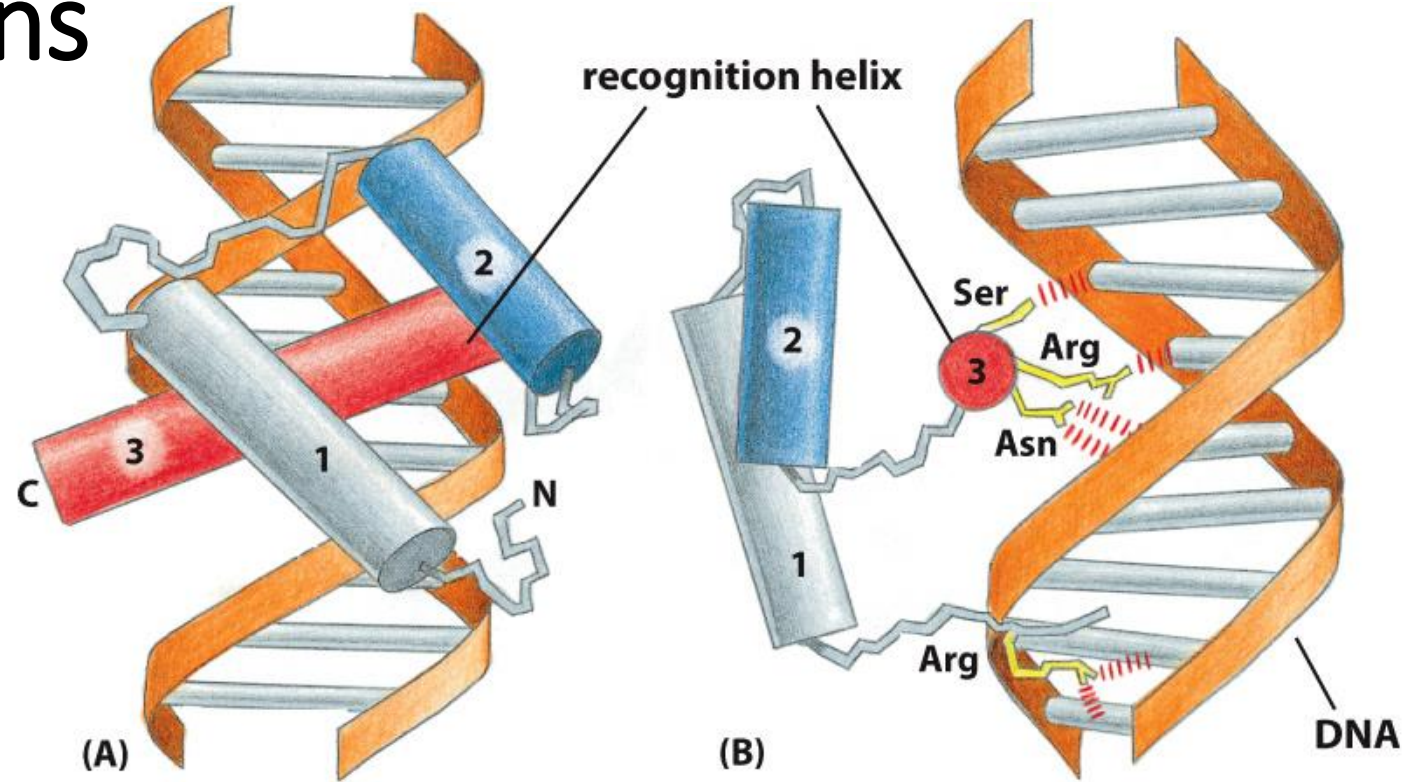
- A few will use **beta sheets**

# Helix-Turn-Helix

- Consist of 2  $\alpha$  helices connected by a short stretch of AAs which holds them at a fixed angle to one another
- The C terminal helix acts as the DNA recognition helix, fitting into the major groove
- Dimers are separated by 1 turn of the DNA



# Homeodomain Proteins

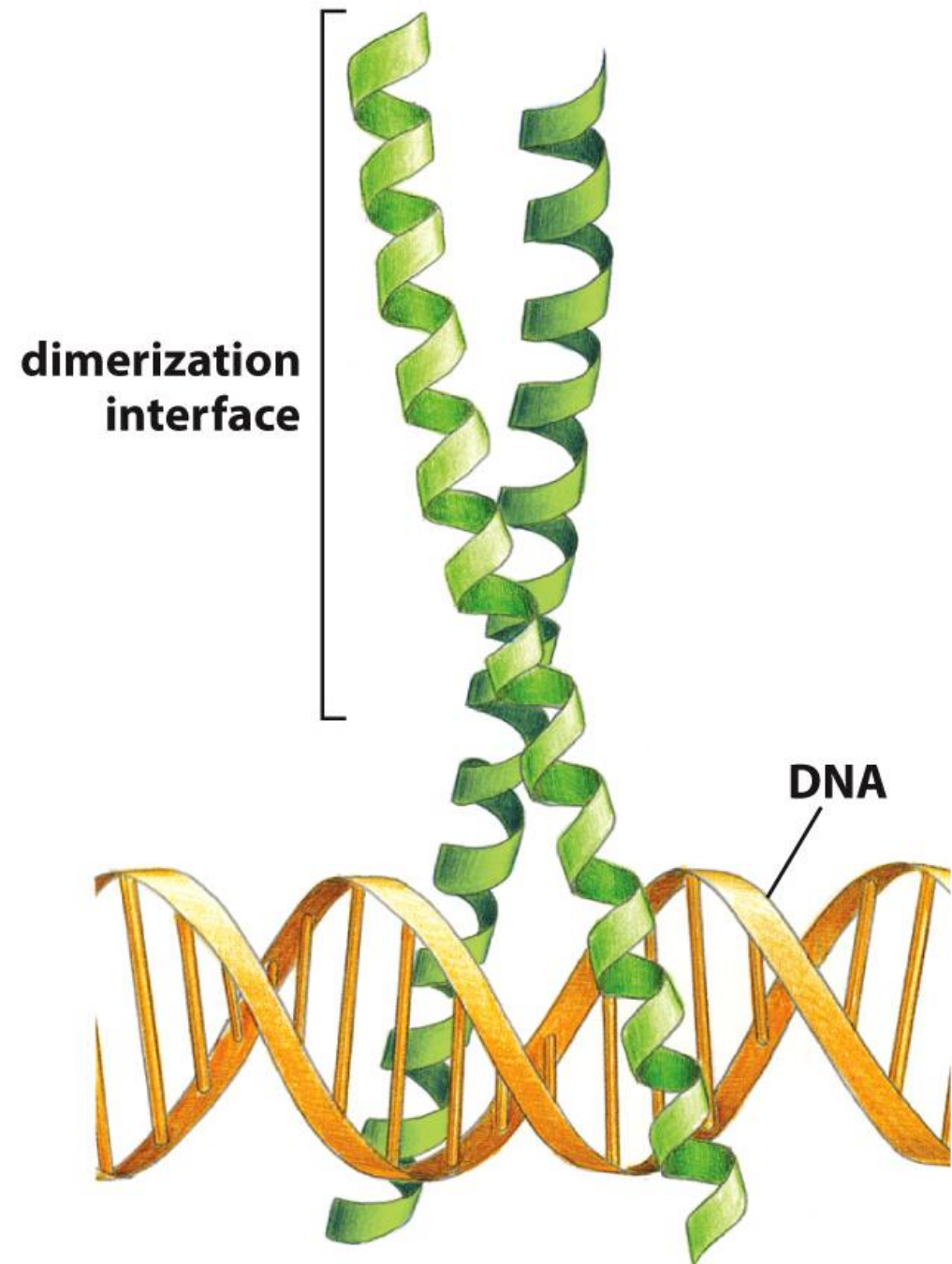


- Consist of 3  $\alpha$  helices which interact strongly with one another via hydrophobic interactions
  - Contains a helix-turn-helix motif with helix 3 acting as the DNA recognition helix, interacting with the major groove
  - A flexible arm attached to helix 1 also creates contacts with the minor groove

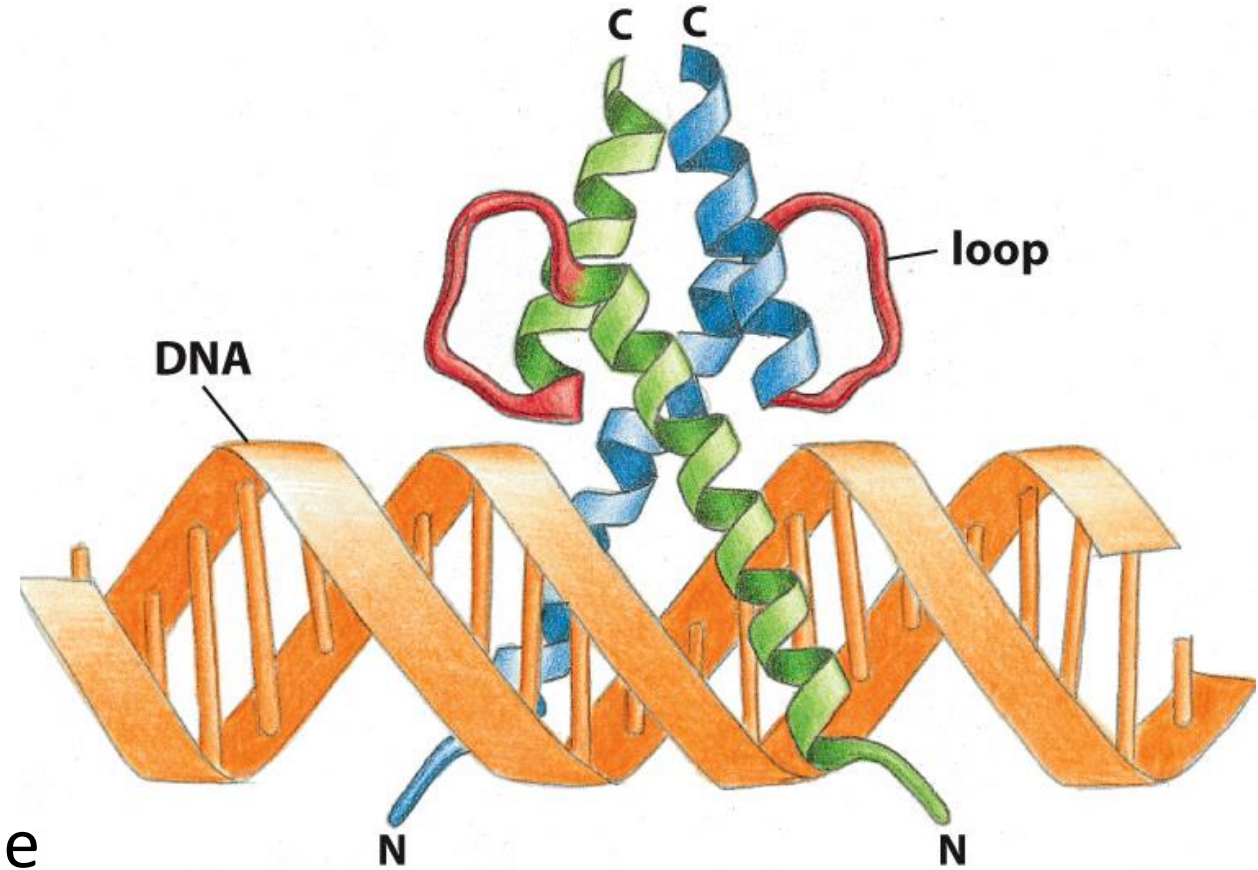
# Leucine Zipper Motif

- 2  $\alpha$  helices (one from each monomer) dimerize forming a short coiled coil via hydrophobic interaction between leucine residues
- This interaction creates a Y structure, where the branches of the Y contact the major groove of the DNA

e.x. – AP1 transcription factor



# Helix-Loop-Helix



- Same general mechanism as the leucine zipper
- The loop connecting the helices is flexible, allowing the protein to fold back and the smaller helices to dimerize

# Beta Sheet Containing TFs

Two-stranded  $\beta$  sheet family

- AA side chains which extend from the  $\beta$  sheets interact with specific bases in the major groove
- Form dimers

e.x. - TBP

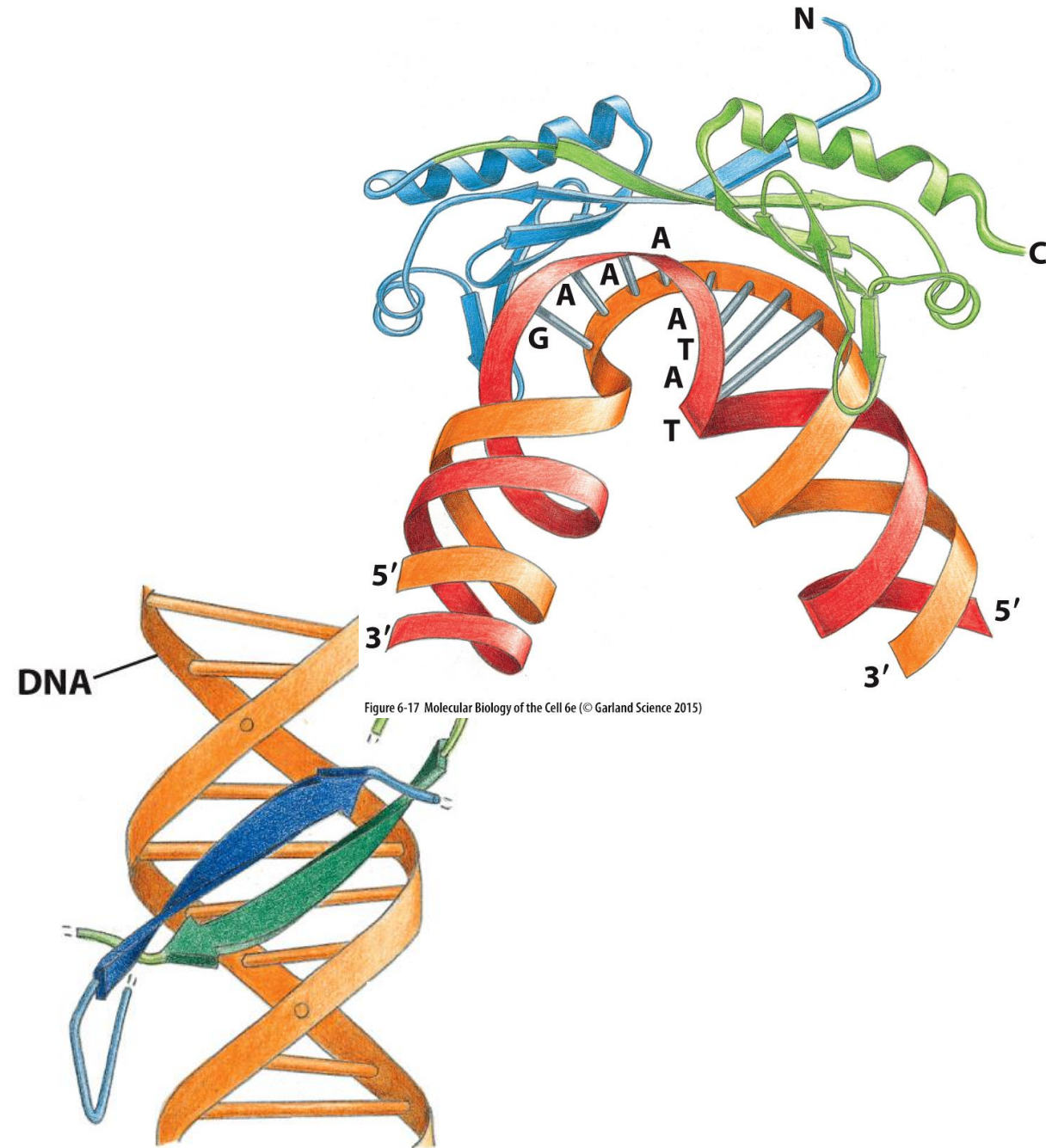
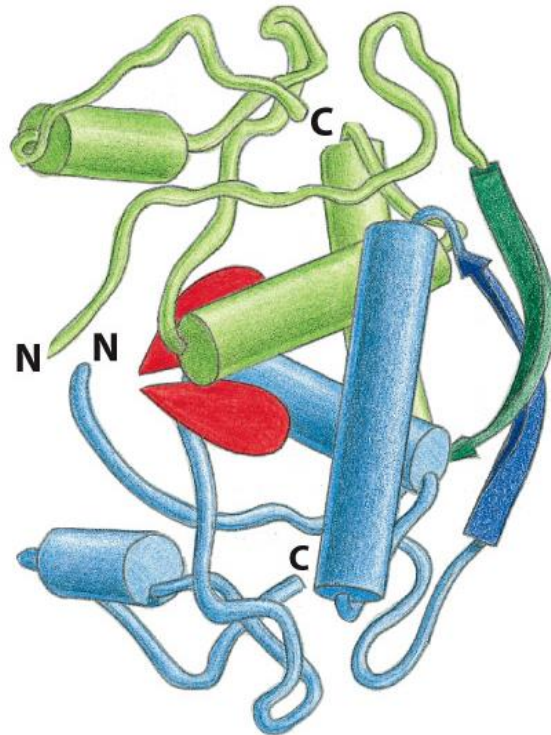
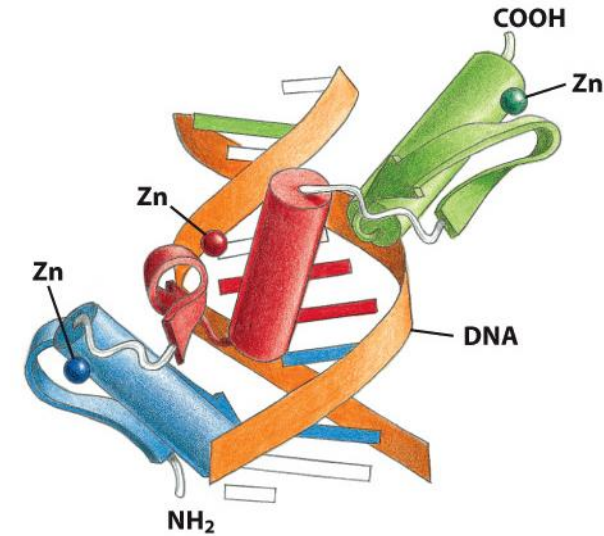
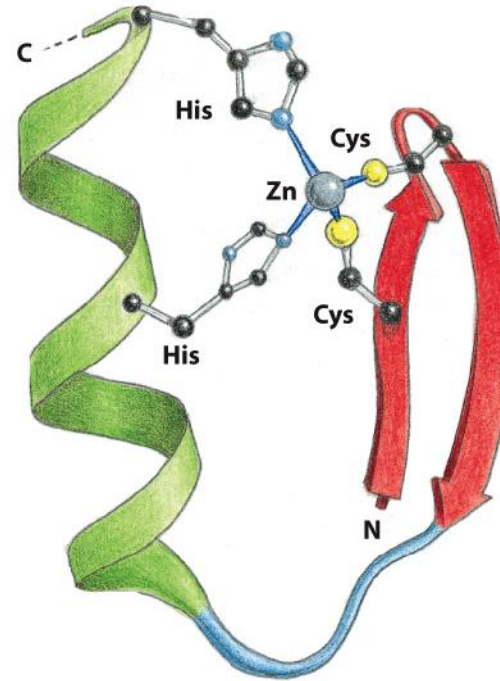
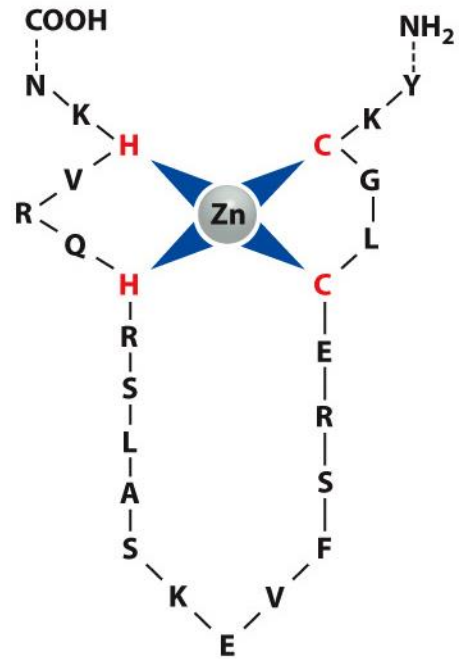


Figure 6-17 Molecular Biology of the Cell 6e (© Garland Science 2015)

# Zinc Finger Proteins

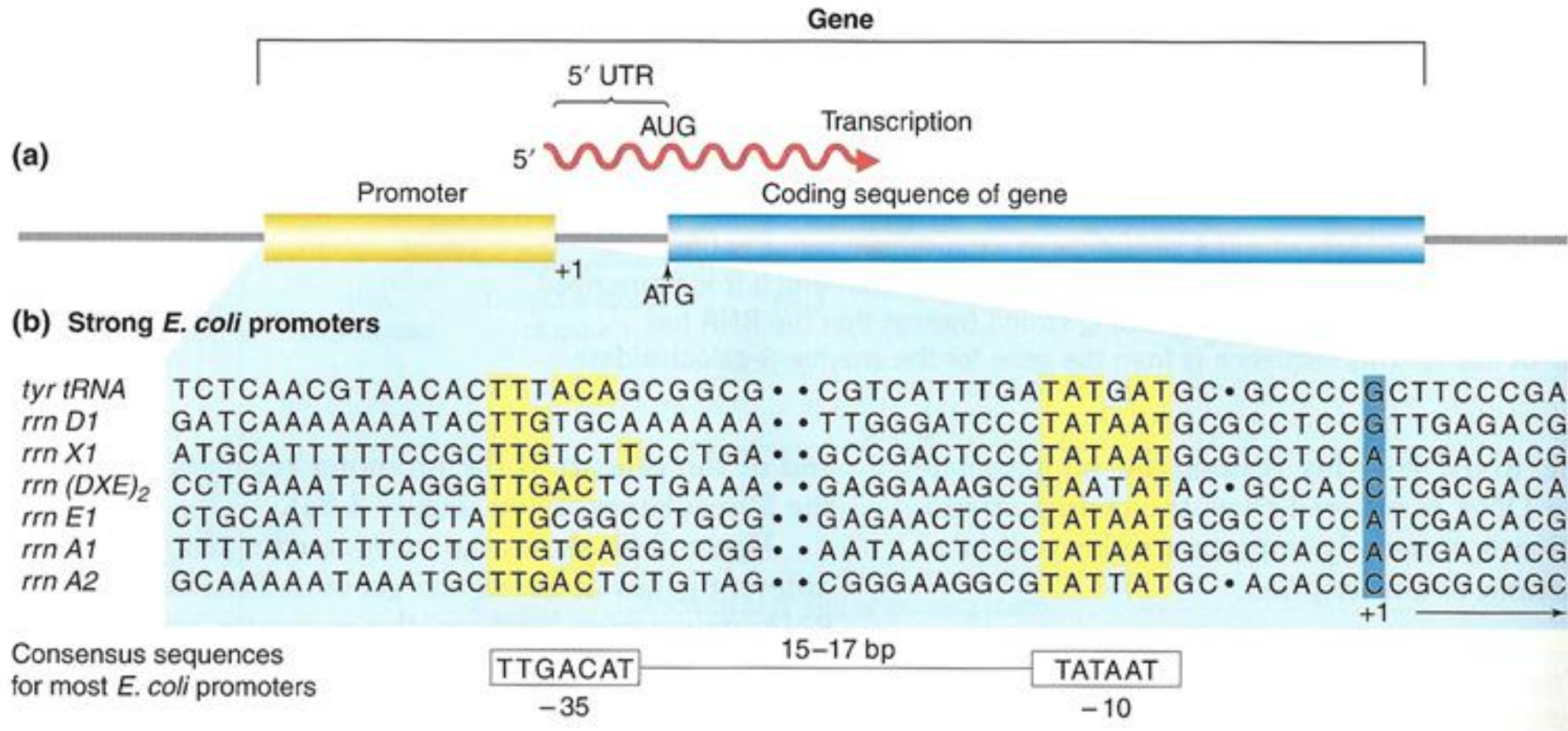


Panel 7-1 (part 5) Molecular Biology of the Cell 6e (© Garland Science 2015)

- Motif which contains 1 or more zinc ions, held in place between 2  $\beta$  sheets and an  $\alpha$  helix
- The  $\alpha$  helix interacts with the major groove of the DNA
- Often found in clusters which line up to extend the contact with the major groove

# Cis-Regulatory Sequences

general family and structure of them.



# Cis-Regulatory Sequences can be Very Distant

- DNA looping allow for physical interaction between distant regulatory elements
- Rare but does occur in prokaryotes
- Occurs for almost every eukaryotic gene (we will revisit this)

can be really far but not in prokaryotes a little bit closer.

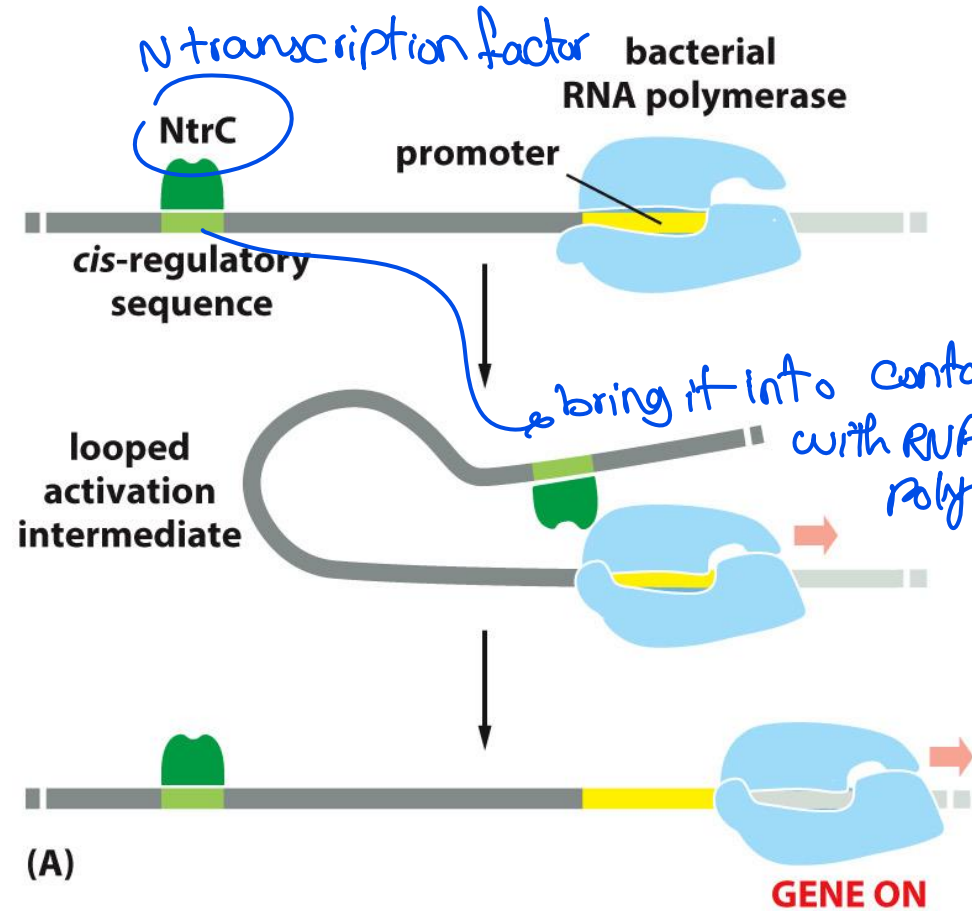
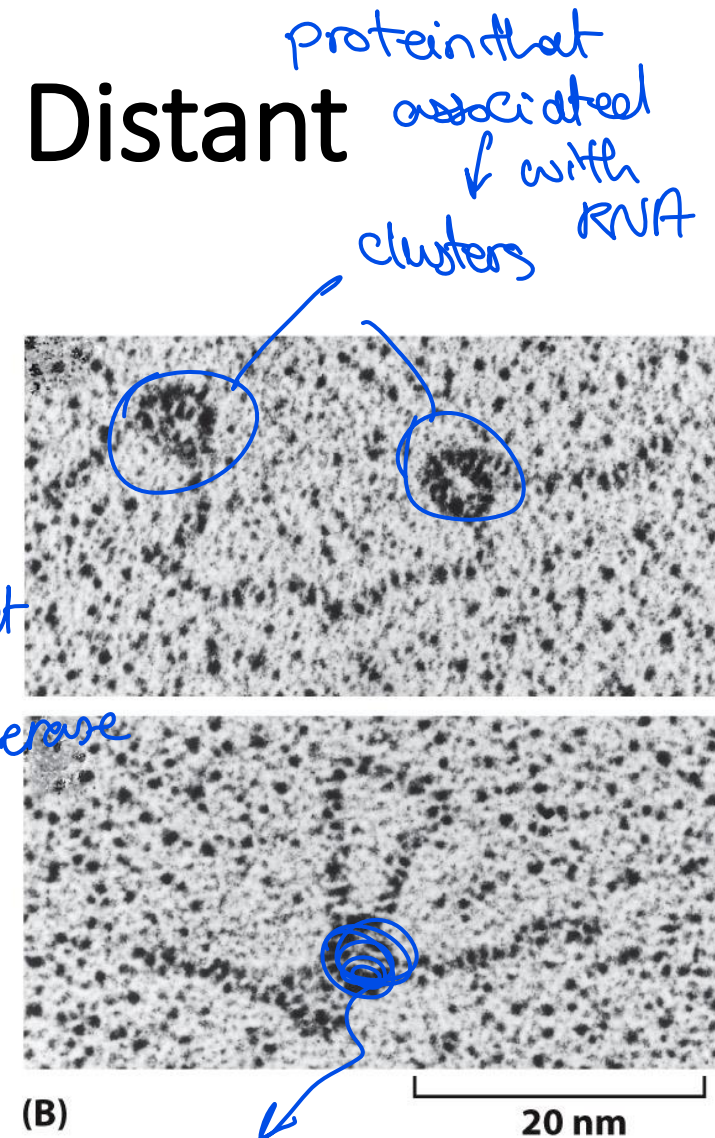


Figure 7-16 Molecular Biology of the Cell 6e (© Garland Science 2015)



# Gene Regulation - an on/off switch or a dimmer switch?

↓  
lots of  
mRNA and  
protein

↓  
not seeing  
many  
things

↳ switch level up and down from zero to 100!

# Gene Expression is Regulated Independently in Each Cell

- A striking amount of variability, sometimes referred to as **biological noise**, can be observed using single cell approaches
- Variability can be due to:
  - **Intrinsic factors** - relating to local variability within the individual cell
  - **Extrinsic factors** - relating to environmental factors local to the cell
- Various gene regulatory pathways can be differentially affected

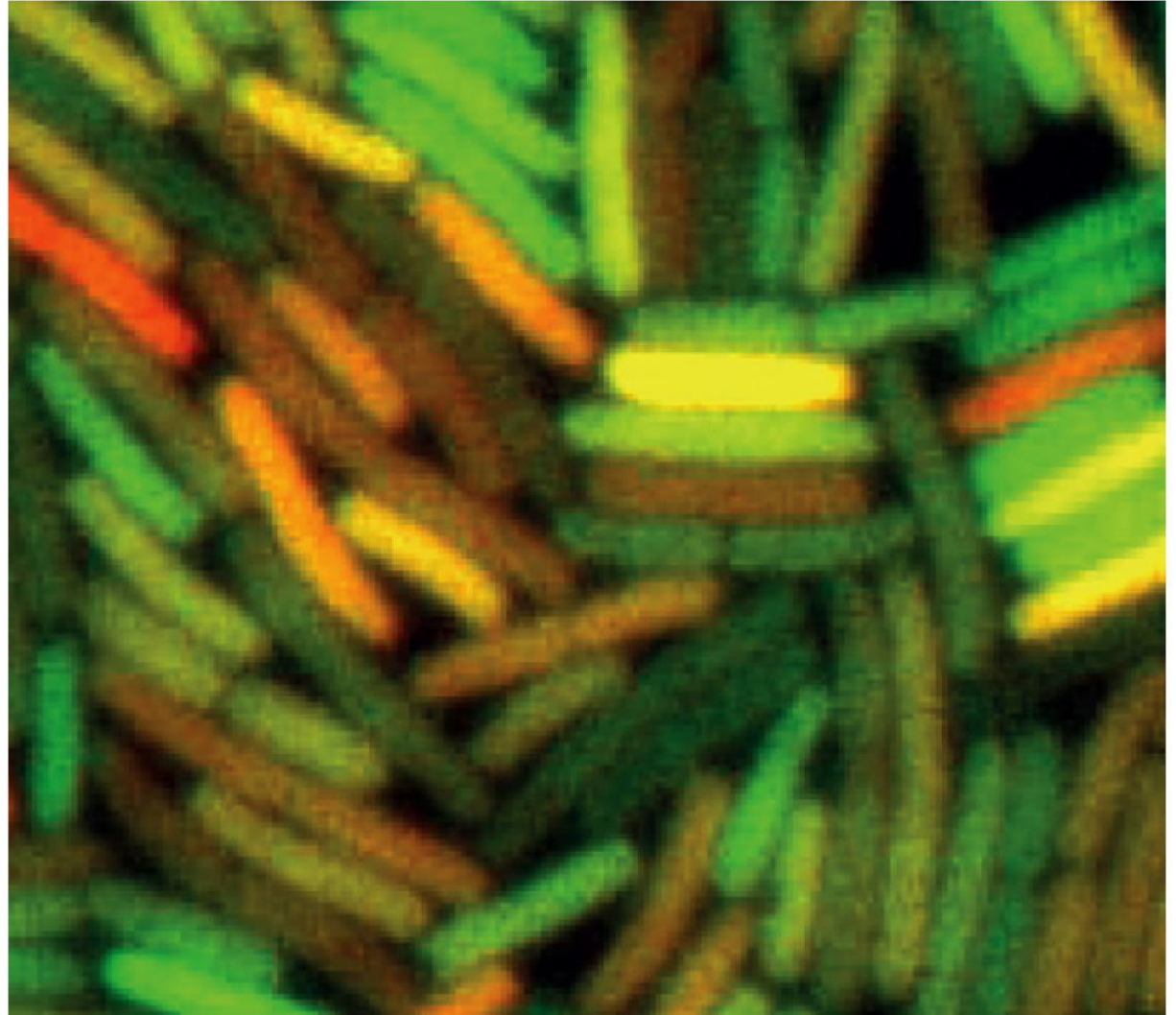


Figure 8-87 Molecular Biology of the Cell 6e (© Garland Science 2015)

# Transcriptional Regulators Form the Basis of Regulatory Circuits

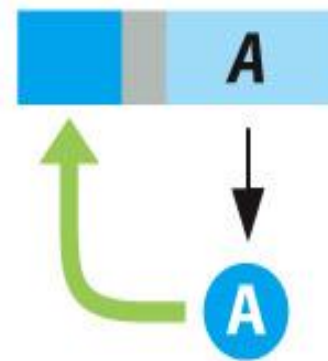
Simple systems-

**Positive feedback loop** – makes an event more likely to occur

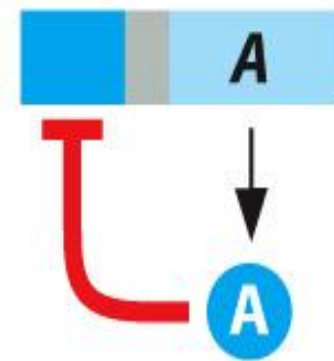
**Negative feedback loop** – makes an event less likely to occur

- Kinetics matters (more than a simple off/on)

Know the notation (arrow types)



**positive feedback loop**



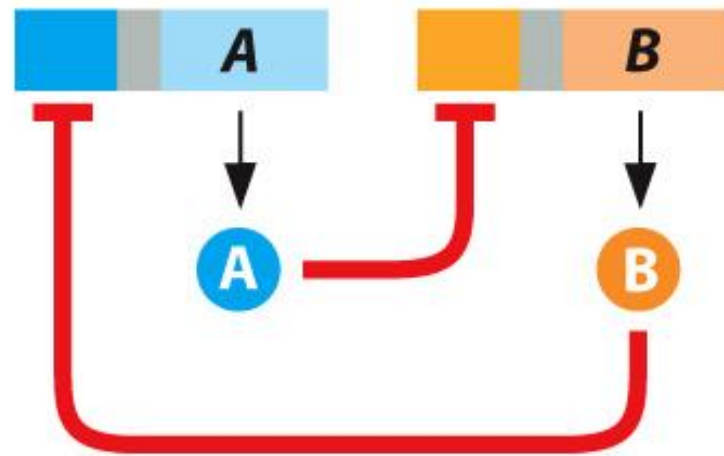
**negative feedback loop**

# Transcriptional Regulators Form the Basis of Regulatory Circuits

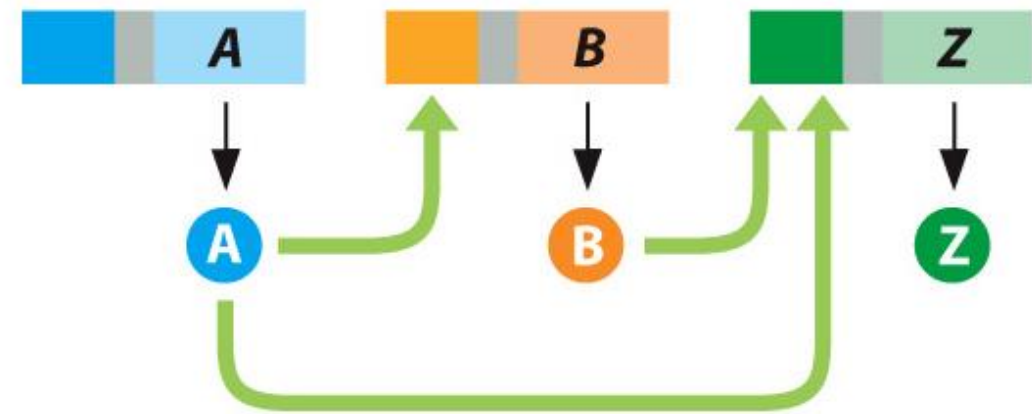
More complex

**Flip flop** – expression of 1 precludes the expression of the other

**Feed-forward** – can act as a filter (short signal vs long signal)



**flip-flop device**  
(indirect positive feedback loop)



**feed-forward loop**

# Transcriptional Regulators Form the Basis of Regulatory Circuits

More complex

**Flip flop** – expression of 1 precludes the expression of the other

**Feed-forward** – can act as a filter (short signal vs long signal)

- Kinetics matters (more than a simple off/on)

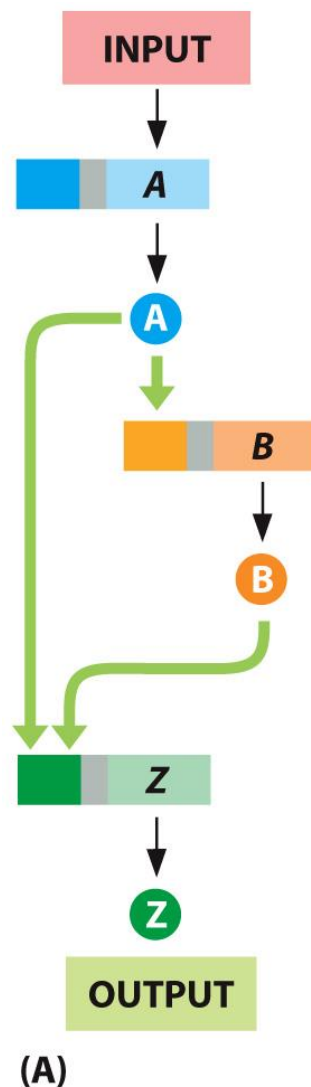
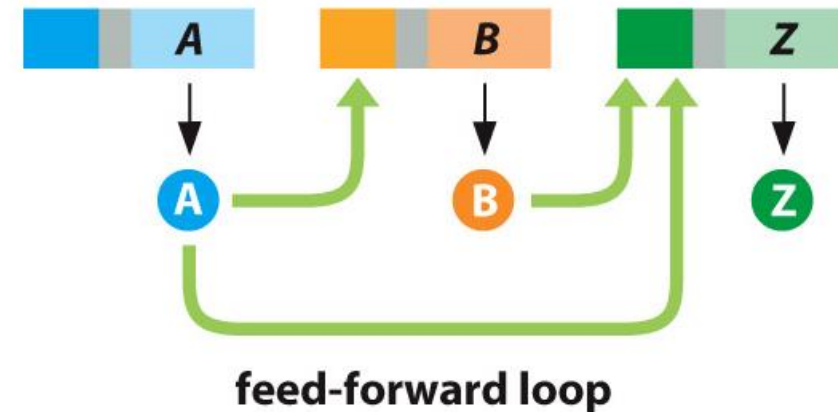
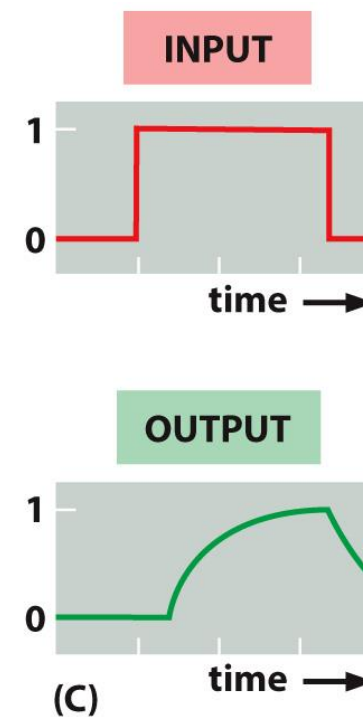
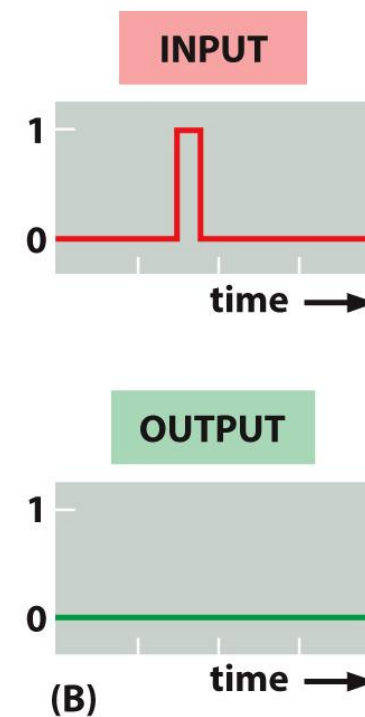


Figure 7-41 Molecular Biology of the Cell 6e (© Garland Science 2015)



# Transcriptional Regulators Form the Basis of Regulatory Circuits

These circuits perform basic logic operations but put together can create very complex systems.

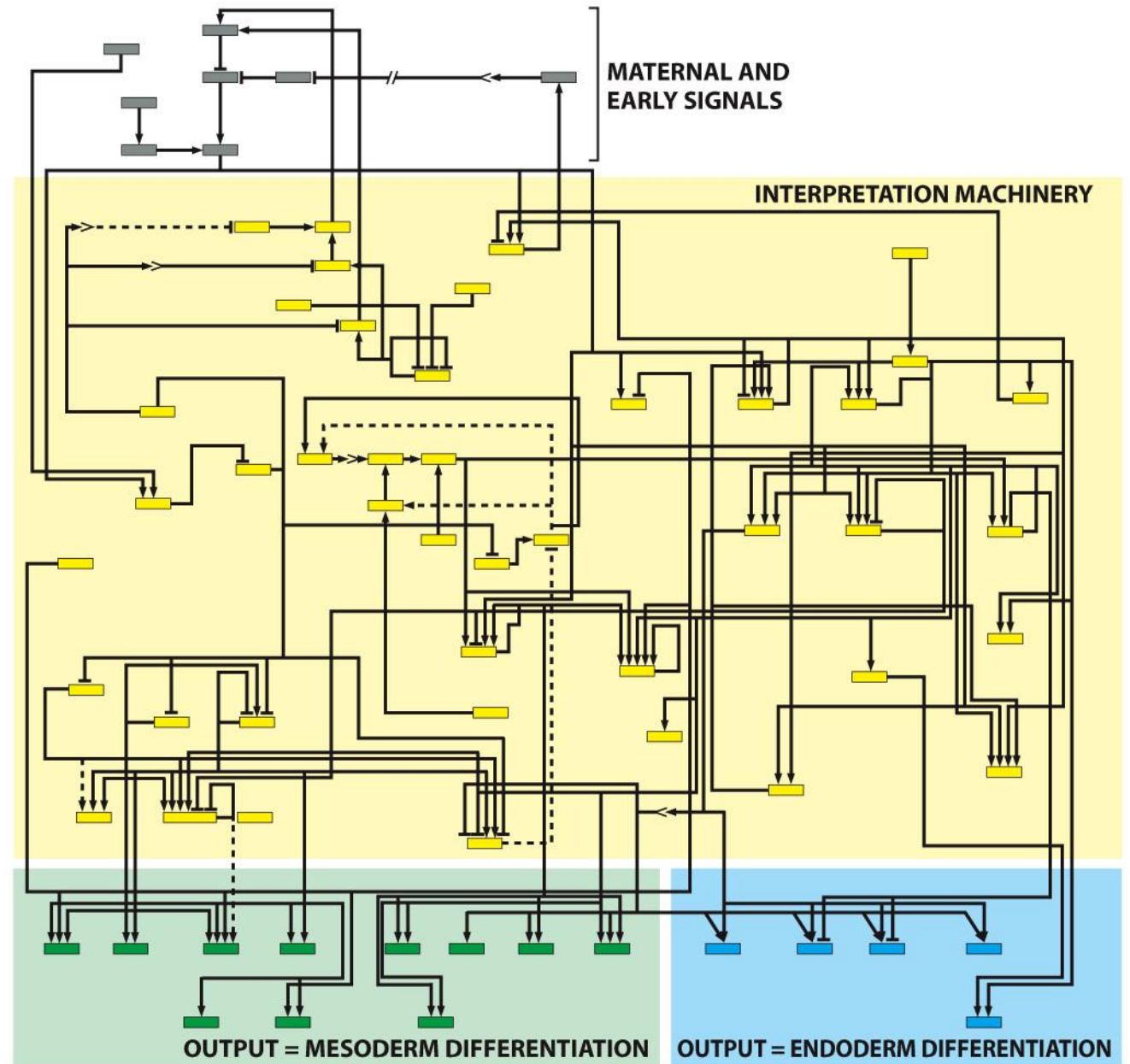


Figure 7-42 Molecular Biology of the Cell 6e (© Garland Science 2015)

# Watch Before Next Class

## Amoeba Sisters – Gene Regulation and the Order of the Operon

