

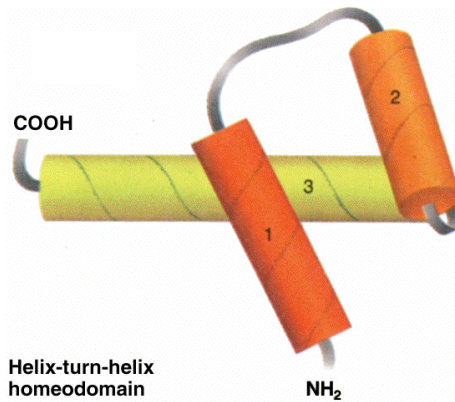
CHAPTER 18 LECTURE NOTES: CONTROL OF GENE EXPRESSION
PART B: CONTROL IN EUKARYOTES

I. Introduction

- A. No operon structures in eukaryotes
- B. Regulation of gene expression is frequently tissue specific. This tissue specific gene expression is fundamental to the function of a particular cell or tissue
- C. How does an organism express a subset of genes in one cell type and another subset in another cell type? Activation and repression.
- D. Multiple levels of regulation (transcriptional initiation, mRNA processing, mRNA stability, gene redundancy, gene amplification)

II. Transcriptional regulation

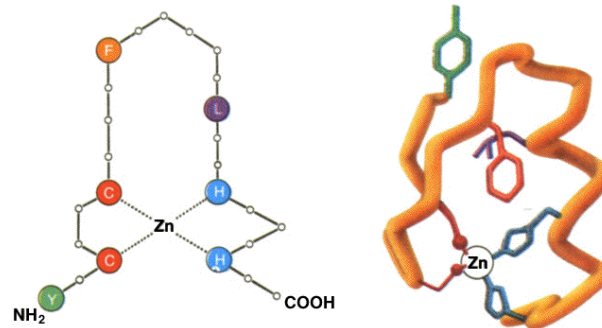
- A. As with prokaryotes, transcriptional regulation is accomplished using cis-acting DNA sequences and trans-acting factors
 - 1. Cis-acting sequences
 - a) promoters (see Chapter 13 notes)
 - b) enhancers (see Chapter 13 notes)
 - 2. Trans-acting proteins (generally have two domains – one to interact with a specific cis-acting DNA sequence and one to activate transcription)
 - a) DNA binding motifs (also called homeodomains)
 - (1) Helix-turn-helix (*Drosophila* developmental regulators; prokaryotic regulators)
Helices 1 and 2 make contact with other proteins and helix 3 contacts the DNA.



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(2) Zinc fingers (Many steroid receptors and transcription factors for mRNAs)

Histidine and cysteine bind zinc forming a finger-like structure that can bind DNA

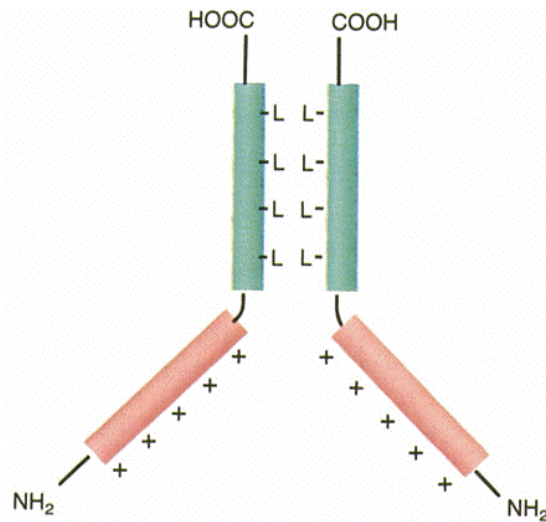


C₂H₂ zinc finger

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(3) Leucine zippers (many proto-oncogenes)

Dimer is formed between leucine rich regions of two monomers and is required for DNA binding; the DNA binding region is a positively charged region

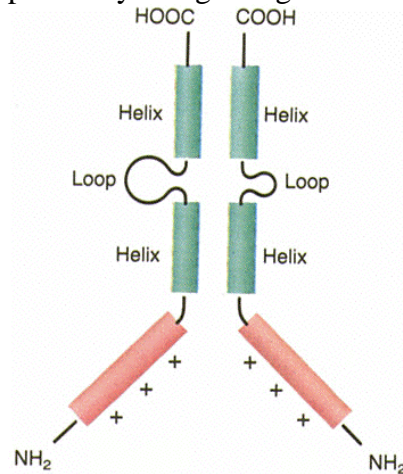


Leucine zipper

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(4) Helix-loop-helix

Dimer is formed between helix-loop-helix of two monomers and is required for DNA binding; the DNA binding region is a positively charged region



Helix-loop-helix

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b) Transcriptional activation by trans-acting factors

- (1) Stabilize RNA polymerase binding
- (2) Unwind the DNA
- (3) Attract other factors
- (4) Formation of a DNA loop that places previously distantly bound activators in proximity to each other.

B. Regulation of the transcriptional regulator's activity (Example - steroid receptors and hormones)

Eukaryotic cells are capable of activating gene expression in response to hormones secreted by other cells. Steroid hormones enter the cell by diffusing through the membrane. Once inside the cell, they bind to a specific receptor. The receptor complexed with the hormone is now capable of activating transcription of genes that contain a hormone-responsive element (HRE). In some ways the mechanism of action of steroid hormone regulation is similar to the mechanism of action of the arabinose operon regulation in prokaryotes. Specifically, a small external effector molecule (arabinose or the hormone) binds to a cytoplasmic transcription factor (AraC or steroid receptor) which then binds to a specific DNA element (araO or HRE) and activates transcription.

C. DNA methylation to regulate transcription

1. The cytosine in the sequence CG is frequently methylated in many eukaryotes.
2. There is a correlation between decreased methylation of CG sequences and increased transcription.
 - a) The inactivated X chromosome is over-methylated except for the few genes that are transcribed.
 - b) Methylation patterns are tissue specific and are heritable for all cells in the tissue
 - c) Addition of the cytosine analogue 5'-azacytidine which can not be methylated activates previously unexpressed genes

D. Histone acetylation to regulate transcription

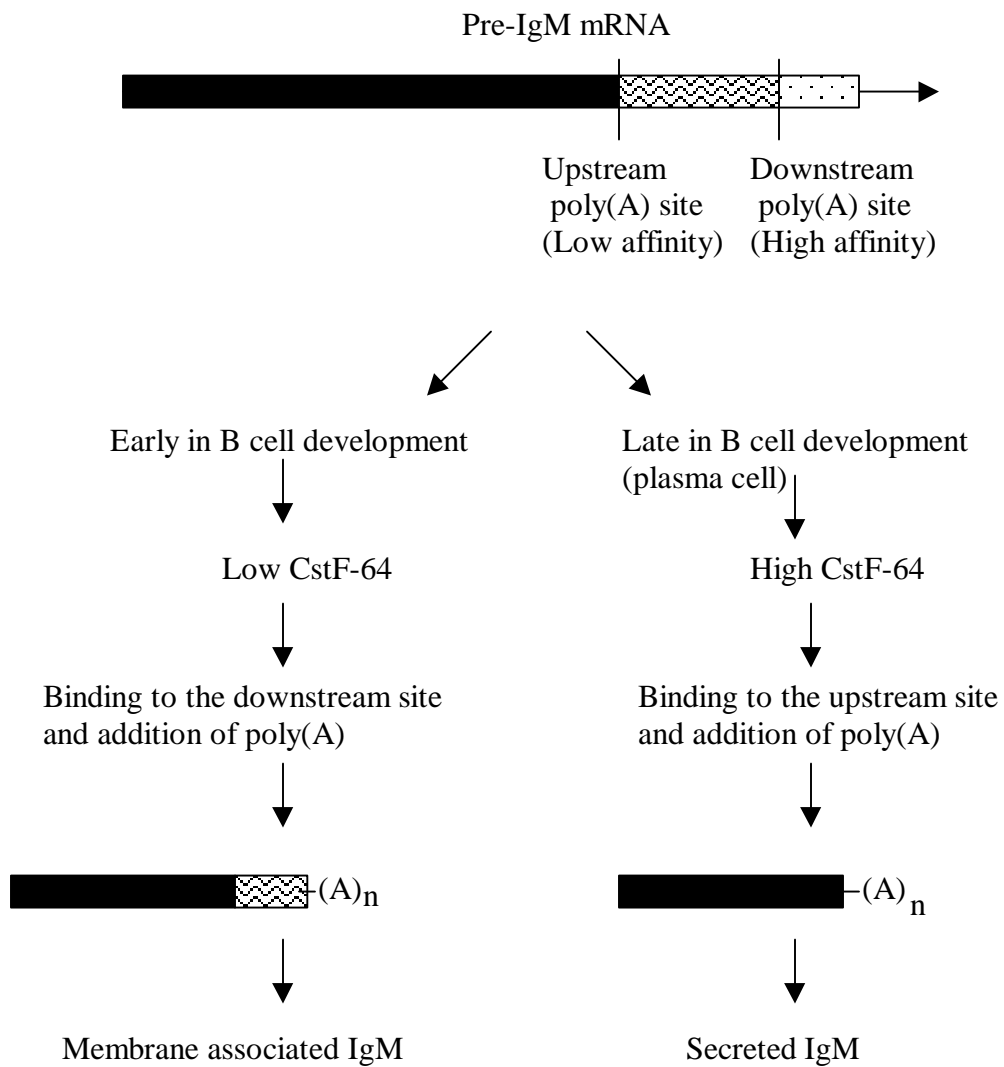
1. Histones are proteins that form a unit upon which the DNA is coiled in chromosomes.
2. Acetylation of histones may loosen the DNA around the histone to allow the transcriptional apparatus access.

III. Post transcriptional regulation

A. Differential processing

1. Poly(A) site selection (example - IgM mRNA)

B cells produce proteins called antibodies. The IgM antibody is composed of two types of protein chains called heavy and light. The pre-mRNA encoding the heavy chain has two possible sites for addition of the poly(A) tail. Early in development of the B cell, the poly(A) tail is added to the downstream site producing an IgM molecule that is associated with the cell membrane. Later when the B cell has differentiated into a plasma cell, the poly(A) tail is added to the upstream site producing a secreted IgM molecule. Recent work suggests that poly(A) site selection is regulated by the concentration of a subunit of the enzyme cleavage stimulation factor (CstF-64). In early stage B cells, CstF-64 accumulation is repressed and poly(A) selection is directed to the downstream site. Artificially overexpressing CstF-64 in early B cells results in use of the upstream splice site and production of secreted IgM. The current model is that the CstF-64 has a higher affinity for the downstream site and this is why it is preferentially used in early B cells when the concentration of CstF-64 is low.



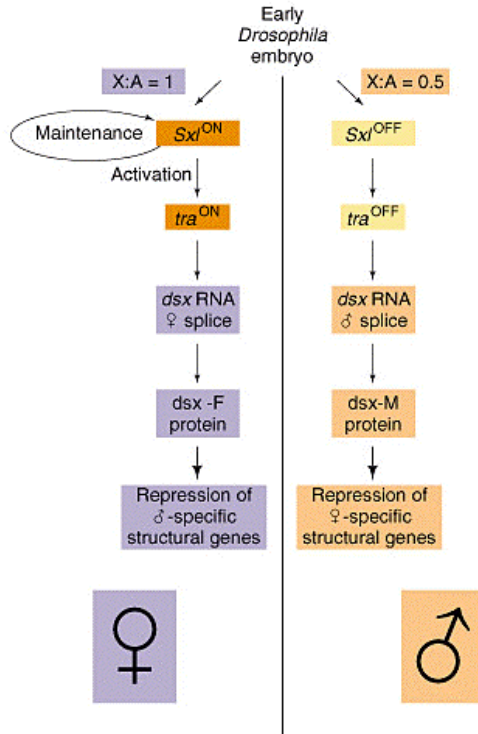
2. Splice site determination (example sex determination in *Drosophila*)

In *Drosophila*, differential splicing of one mRNA transcript (*sxl*) initiates a cascade that eventually determines the sex characteristics of the fly. A transcription factor that activates a promoter of the *sxl* gene early in development is encoded on the X chromosome of flies. This factor functions as a homodimer. Another factor, encoded on an autosome, can interact with the X encoded factor and inactivate it by formation of nonfunctional heterodimers. Early in development in XX flies, there is sufficient X factor made relative to the autosomal inhibitor so that a low level of *sxl* is transcribed. In contrast, in XY flies, since the ratio of X to autosomal chromosome is only 0.5, there is less X encoded factor relative to the autosomal inactivator and so not enough functional X factor to activate *sxl* transcription.

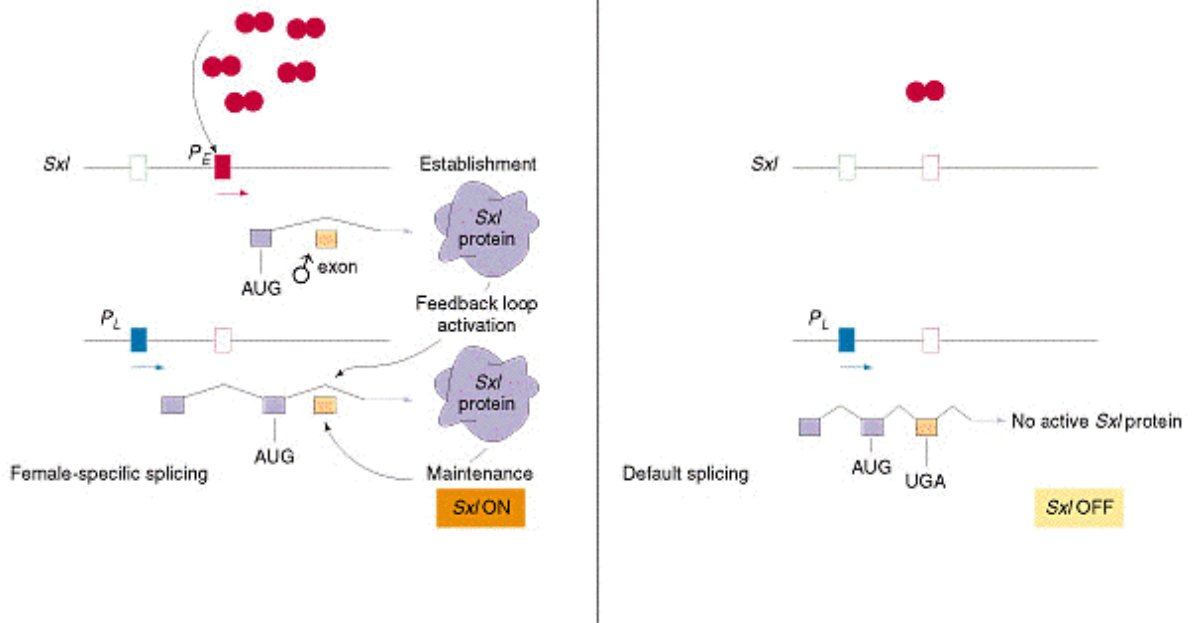
Later in development, the X encoded factor is not produced. *sxl* is now transcribed from a different promoter producing a longer pre mRNA. For this pre mRNA to form mRNA encoding a functional Sxl protein, an exon containing a stop codon must be spliced out. Sxl represses splicing at the site that would leave the stop codon in the mRNA. Since there is Sxl in the female cells, the correct splicing of the *sxl* pre mRNA transcript will occur and more Sxl will be made which will catalyze more splicing of *sxl* pre mRNA. This is a positive autoregulatory loop. In contrast in the male cells, there is no Sxl and the exon containing a stop codon is not spliced out of the *sxl* pre mRNA. Thus, no Sxl is made.

Sxl goes on to catalyze the proper splicing of another mRNA encoding Tra using a similar mechanism as for regulation of its own splicing. In the absence of Sxl (in males), splicing of the *tra* mRNA results in the incorporation of a premature stop codon and no full length Tra is produced.

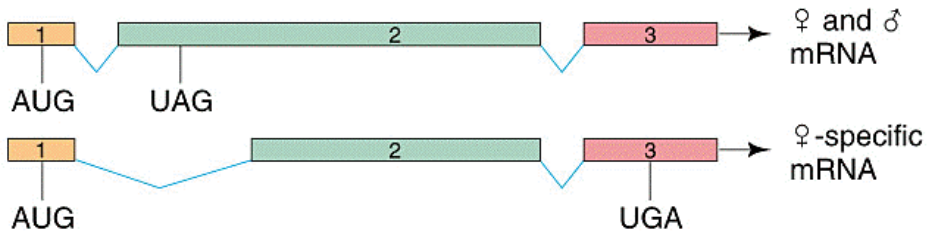
Tra goes on to catalyze the splicing of another mRNA encoding Dsx to a “female” specific conformation. The Dsx-F protein represses transcription of genes that encode male traits. In the absence of Tra (in males), splicing of the *dsx* mRNA results in the production of a mRNA encoding a “male” specific Dsx. The Dsx-M protein represses transcription of genes that encode female traits.



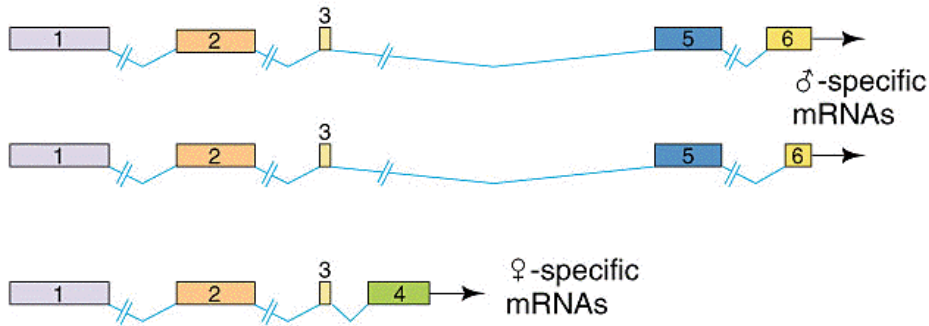
(b)



(a) *tra* alternative splicing



(b) *dsx* alternative splicing



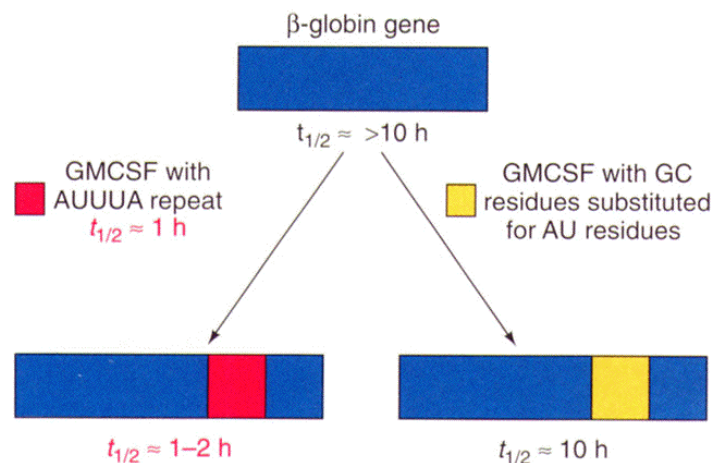
Figures 23-16, 23-17 and 23-18

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B. mRNA stability

1. Gene expression can be regulated by altering the stability of the mRNA.
2. mRNAs with short or no poly(A) tails are rapidly degraded.
3. Specific sequences in the mRNA that may affect stability. For example, a sequence of AUUUA repeats at 3' end reduces the mRNA stability. Insertion of this sequence from a gene encoding an unstable mRNA (GMCSF gene for granulocyte-monocyte stimulating factor) into a gene that encodes a stable mRNA (β -globin) decreases the stability of the β -globin mRNA as measured by mRNA half life.

(b) Activity of $A(U)_nA$ repeats



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C. Protein degradation

IV. Gene redundancy

For some genes whose products are needed in high amounts, there are multiple copies of the genes in the chromosome.

A. Hundreds of copies of rRNA genes in *Xenopus* (frogs).

1. Nucleolar organizer (region of chromosome that physically associates with the nucleolus) has 450 copies of the 18S and 28S rRNA genes.
2. 20,000 copies of the 5S rRNA genes (not associated with the NO)

B. Several hundred copies of the histone genes in sea urchin chromosomes

V. Gene amplification (The increase in the numbers of copies of a specific segment of chromosomal DNA)

A. *Xenopus* oocyte rRNA genes (from 450 copies to 10,000,000)

Occurs via repeated rounds of DNA replication of the gene followed by formation of extrachromosomal DNA circles that replicate many copies via rolling circle replication.

B. Polytene chromosome puffs

C. DHFR amplification in cultured cells

The chemotherapeutic drug methotrexate (MTX) is a potent inhibitor of the enzyme dihydrofolate reductase (DHFR) which is required for the synthesis of nucleic acids.

In laboratory cultured cells, resistance arises at a frequency 1 in 10^6 . The resistance is due to amplification of the DHFR gene. Two mechanisms have been proposed for amplification including (1) multiple rounds of unequal crossover between sister chromatids where 1 cell inherits multiple extra copies of the gene and becomes resistant to MTX (see pg. 222 for review in gene duplication section) and (2) multiple rounds of → fragmentation of some of the DNA and reinsertion of an extra copy of the DHFR gene into a chromosome that already has 1 copy.