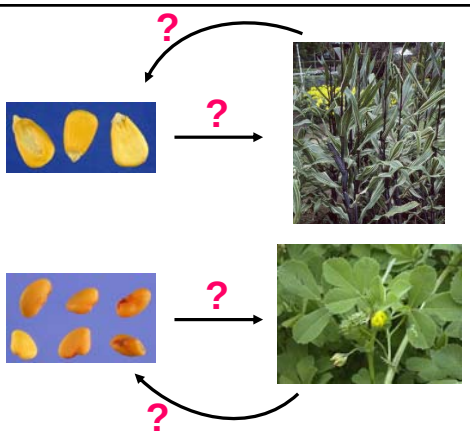
Lecture 1

General Course Introduction

Genome Size, Organization and Complexity

Prokaryotic vs. Eukaryotic gene expression



Factors affecting growth and development

External factors

Light: daylight length
spectrum
intensity

Nutrients

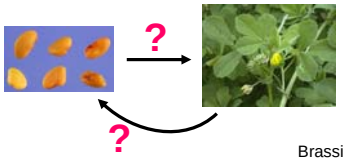
CO_2

Water

Temperature

Air pollutants

Pathogens/Insects



Internal factors

Plant hormones

Auxins

Gibberllins

Cytokinins

Abscisic acid

Ethylene

Brassinosteroids

Sugars

How do plants coordinate and regulate all these factors?

How do they adjust to varying environmental cues?

Growth, development and environmental responses are determined by the programmed expression of genes → **Regulation of gene expression**

Turning on or off genes that:

- direct synthesis of enzymes that catalyze specific biochemical reactions required for growth and differentiation.
- are involved in protein synthesis → **Structural genes**

Internal and external signals coordinate gene expression:

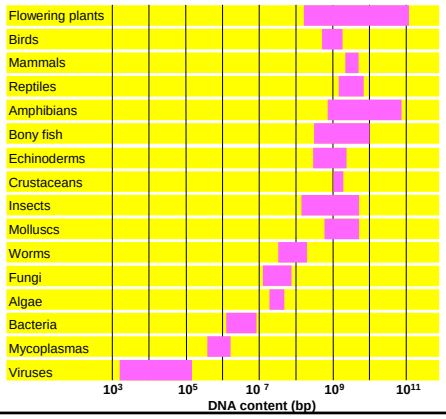
- one kind of signal or stimulus is converted into another by a sequence of biochemical reactions inside the cell to amplify the original signal and activate or repress genes
  → **Signal transduction pathways**

Genome Size

Organization

Complexity

Haploid Genome Size of Various Phyla



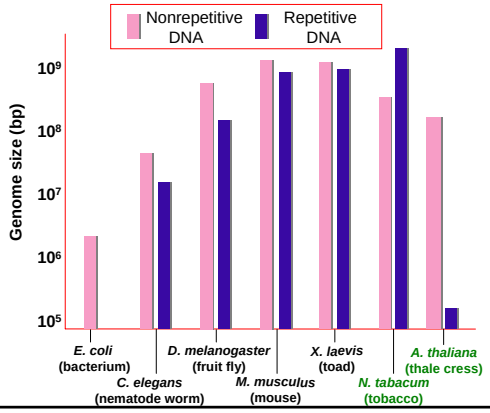
Why do plants have larger genomes?

In prokaryotes: - nearly all of the DNA consists of **unique sequences** encoding functional RNA/proteins

In eukaryotes: - unique sequences + **noncoding DNA** (for chromosome organization and structure)
- most of noncoding DNA exists as multicopy sequences of
→ **repetitive DNA**
- rest of noncoding DNA is single-copy sequences
→ **spacer DNA**

In plants with large genomes: most of the DNA is repetitive.

Nonrepetitive and Repetitive DNA in various Phyla



The Plant Researchers' pet: *Arabidopsis thaliana* (thale cress)

- A model plant
- Best-studied plant genome
- Entire genome sequenced (2000)
- Genome size: ~100,000 kb (~ 20,000 genes)
- 5 Chromosomes
- Rapid life cycle (about 6-8 weeks from germination to mature seed)
- Prolific seed production and easy cultivation
- Easy to generate transgenic plants (*A. tumefaciens*)
- Large number of mutant lines and genomic resources
- Multinational research community of academic, government and industry laboratories

Gene Expression in Prokaryotes

Constitutive vs. inducible vs. repressible
gene regulation

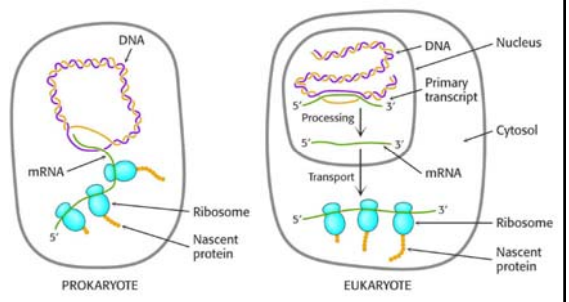
Constitutive: genes expressed at constant level all the time
(e.g. housekeeping genes regulating basic cellular processes)

Inducible: genes turned on in certain circumstances
(at low level present in non-induced conditions = basal level)

Repressible: genes turned off in certain circumstances

All involved in regulation of transcription initiation….

Gene Expression
Prokaryote vs. Eukaryote



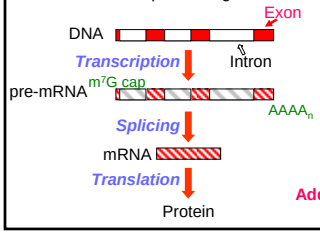
Gene Expression in Eukaryotes

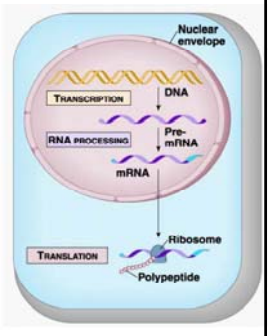
Two cellular compartments

- Transcription in nucleus
- Translation in cytoplasm

RNA processing

– 5' capping
– 3' polyadenylation
– RNA processing





Additional levels of regulation

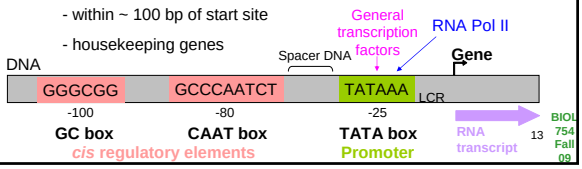
RNA polymerase II promoters and *cis*-regulatory elements

TATA box (TATAAA)

- conserved sequence in eukaryotic promoters
- Site of assembly of transcription initiation complex
- 25-35 bp upstream of start site
- highly transcribed genes

CAAT box and GC box (*cis* regulatory elements)

- binding sites of **transcription factors** (enhance rate of transcription)
- within ~ 100 bp of start site
- housekeeping genes



cis regulatory elements of transcription

- Proximal regulation elements: region of DNA directly upstream of RNA polymerase II binding site (GC box, CAAT box)
- LCR (locus control region): controls expression of genes
- Enhancers: distantly (upstream or downstream from promoter) located positive regulatory eukaryotic DNA sequences that are necessary to activate gene expression (many developmentally important genes, e.g. *AGAMOUS*)
- Response elements: *cis*-acting sequences involved in gene regulation by hormones and other signaling agents (phytochrome, plant hormones)

trans regulatory elements of transcription – Transcription factors

- Activators: proteins that, when bound to specific DNA sequences near or far away from genes, activate transcription
- Repressors: proteins that, when bound to specific DNA sequences near or far away from genes, repress transcription
- Bind to promoter proximal-elements and enhancers (or silencers)
- Composed of two parts:
  - DNA-binding domain/motifs:

    Helix-turn-helix: Anthocyanin biosynthesis

    Zinc finger: *COP1* (*CONSTITUTIVE PHOTOMORPHOGENIC*), repressor of light signaling

    Helix-loop-helix: Phytochrome-regulated genes

    Basic zipper: Abscisic acid response

    MADS: floral homeotic genes (e.g. *ag1*, *AGAMOUS*)

  - Activation or Repression domain

General Transcription Factors

- Initiation by RNA Polymerase II at almost all promoters require a set of several multi-protein transcription factors

  TFIID, TFIIA, TFIIB, TFIIF, TFIIH, TFIIE

- TFIID contains TATA box-binding protein (TBP) and TBP-associated factors (TAFs)

- TFIIH contains helicase to separate DNA strands and kinase to phosphorylate Carboxyl Terminal Domain (CTD, which is part of the large subunit of RNA Pol II; CTD is phosphorylated during initiation)

- Either assembled at start site or arrive as large complex with RNA Pol II

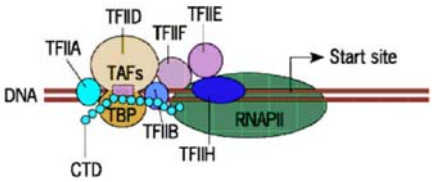
Principles of transcriptional regulation

DNA-Protein Interactions

- transcription factors (repressors or activators) are DNA-binding proteins

Protein-Protein Interactions

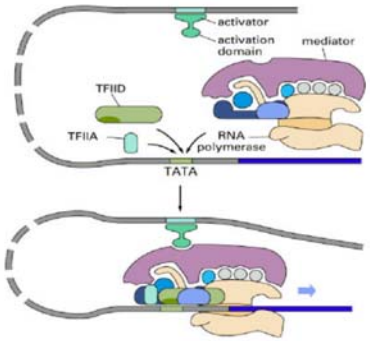
- transcription factors interact with each other or RNA polymerase



Response to environment

- light
- temperature
- hormones…

Binding of activator to enhancer recruits RNA Pol II holoenzyme complex



Cell differentiation is affected by transcription regulation

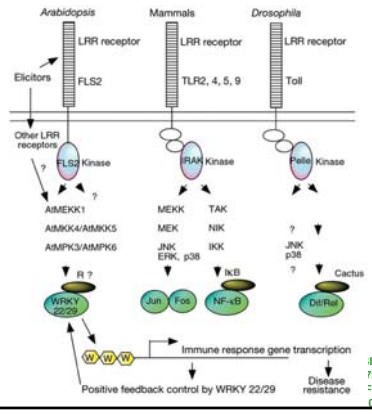
- Regulatory DNA sequence (*cis* element) consists of multiple motifs specific for transcription factors
- Multiple transcription factors can bind simultaneously to *cis* elements and act together on transcription of gene
- Properties of the cell are determined by the genes expressed
- Which genes are expressed is partially determined by the active transcription factor, which in turn are affected by external and internal factors (signal transduction)

Mechanisms of transcriptional regulation

- Changes in the amount of activity of transcription factors, i.e. by
  - hormone binding
  - phosphorylation
- Some transcription factors affect RNA polymerase complex binding or initiation
- Some transcription factors can alter chromatin structure (remove or post-translationally modify histones)
- Interaction of transcription factors

Regulation by phosphorylation – Signal transduction

- Hormone activates kinase
- Kinase phosphorylates transcription factor
- Transcription factor is activated
- Response gene is transcribed



Summary

- Growth and development are affected by external and internal factors.
- These factors influence gene expression (and signal transduction), and thus affect the properties of cells.
- Regulation of gene expression in plants (and eukaryotes in general) occurs through *cis*-acting regulatory sequences (GC and CAAT box), which modulate RNA Pol activity.
- General (and specific) transcription factors assemble into a transcription initiation complex at the promoter (TATA box) to initiate gene transcription.
- Gene transcription is also modulated by transcription factor activators or repressors that bind to distantly located *cis*-regulatory sequences.
- Protein/Enzyme concentration and turnover is regulated by the ubiquitin pathway.