MMG 301, Lec. 24 Regulation of Gene Expression

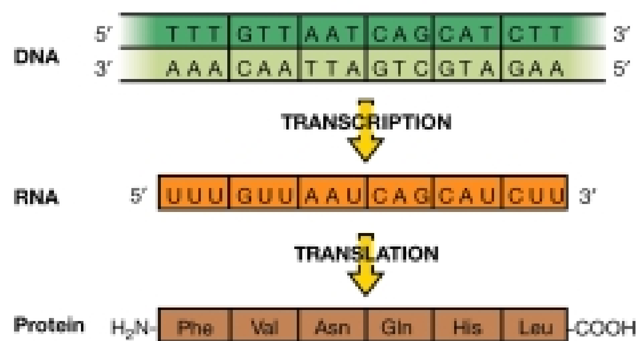
Questions for Today:

What are key features of general transcription?

1. What are *alternate sigma factors* all about?
2. How are *repressors* used for regulation (2 ways)?
3. How are *activators* used for regulation (2 ways)?
4. What are 2-component regulators?
5. How can one monitor regulation of gene expression?

General transcription

Recall: Replication
Transcription
Translation



<u>Key terms for general transcription:</u>

Core RNA polymerase: $\alpha_2\beta\beta'$

Sigma factor: σ^{70} needed for initiation of "house keeping genes"

Promoter sequence: σ recognition site

-10 and –35 base sequences

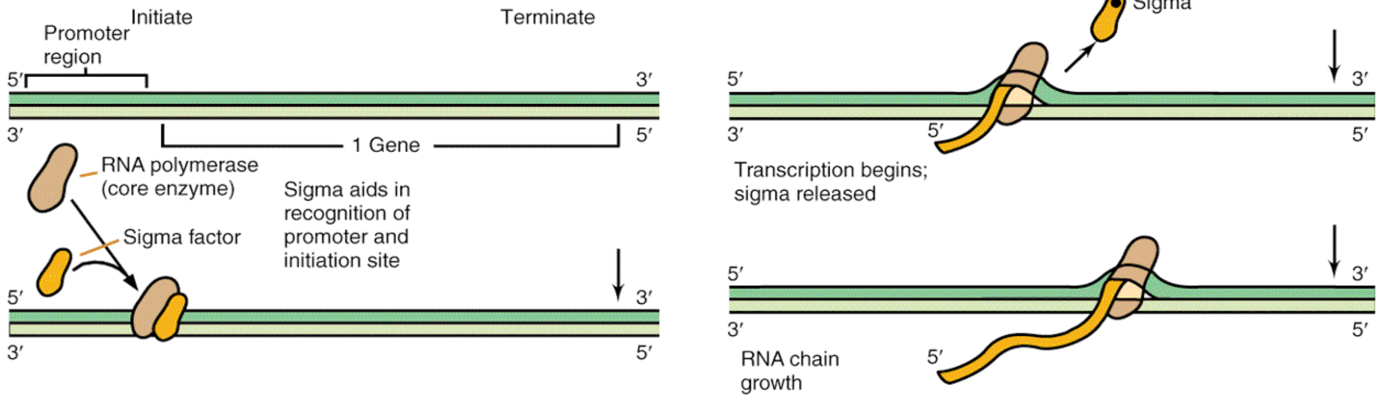
Initiation: $\sigma\alpha_2\beta\beta'$ binds, DNA melts, RNA made

RNA chain growth: 5'-to-3' direction

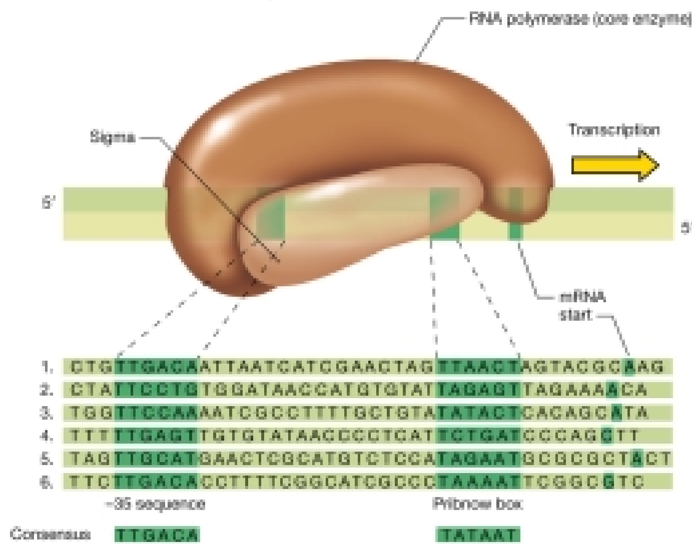
Termination via stem-loop structure:

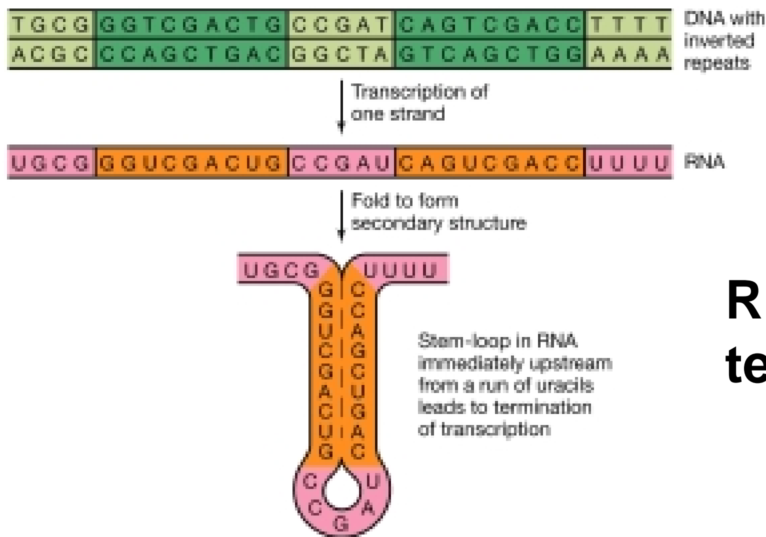
Termination via Rho-dependent termination:

Major steps in Sigma-70 transcription



Promoter region



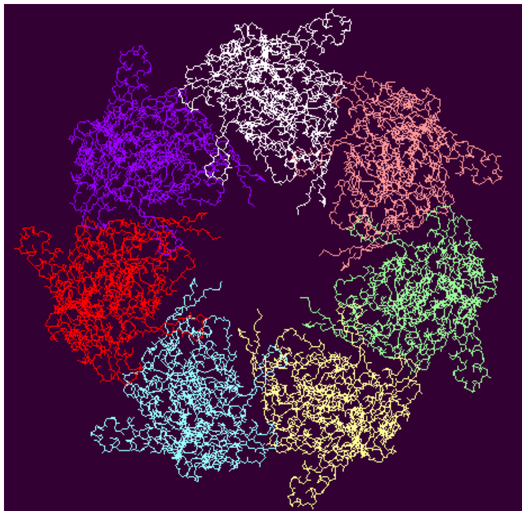


Rho-independent termination

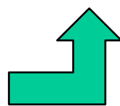
This general transcription machinery works well for routine needs, but the transcription of many genes is tightly regulated by various means as described below. *Why is it important to regulate transcription*?

Alternative Sigma factors

σ^{32} is synthesized when cells face a sudden shift in growth temp or "heat shock" (e.g., from 30°C to 42°C). This sigma factor recognizes, binds to, and leads to transcription of ~36 genes that protect the cell (e.g., by refolding denatured proteins).

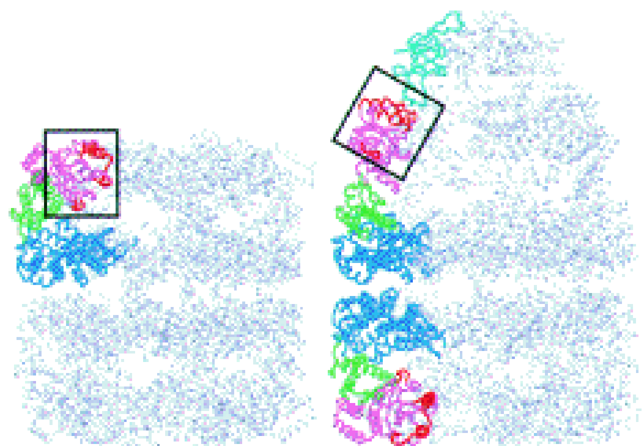


Two proteins turned on by sigma-32: the GroEL/GroES chaperonin system where 7 GroEL subunits form a box with GroES forming the lid. Unfolded proteins are refolded in the box



Top view (7GroEL)

Side view (GroES & GroES)



σ^{54} is made when cells are grown without added ammonia or “nitrogen starvation”. This sigma factor turns on transcription of ~12 genes that allow growth on other N sources.

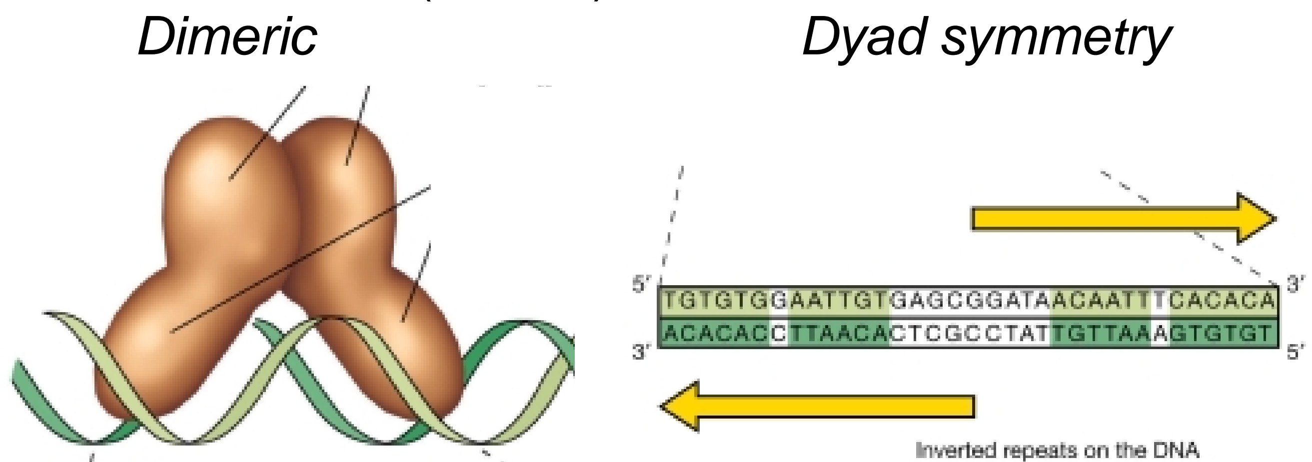
Different sigma factors function in regulation of flagella synthesis (σ^{28}), sporulation (several used), survival in stationary phase (σ^{38}), and other roles.

Use of an alternative sigma factor allows for simultaneous control of multiple genes/operons: *regulon*. Each of these factors recognize a *distinct promoter sequence*. The number refers to the size (in kDa)

Repressors

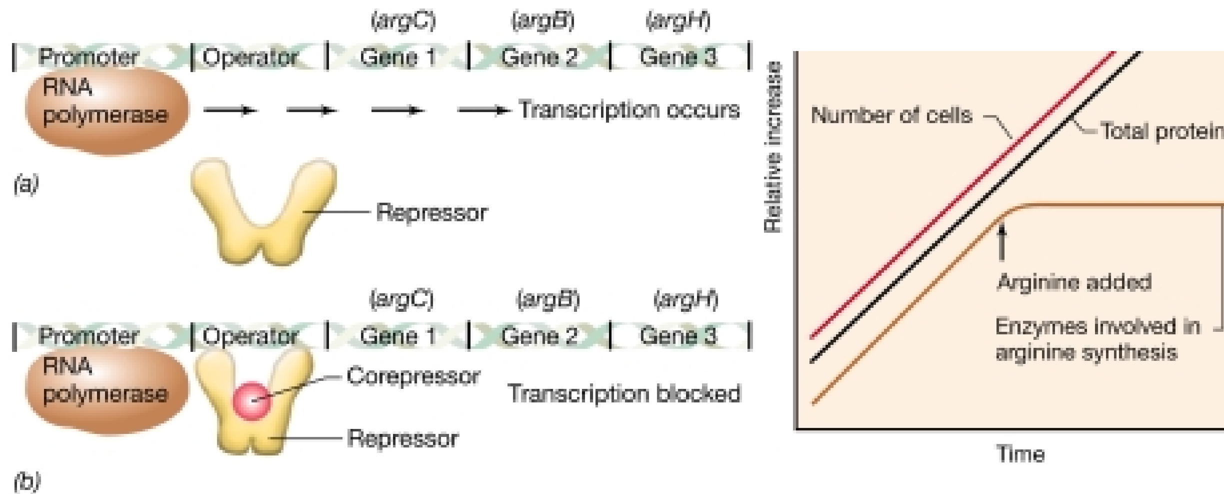
Repressors are proteins that bind to specific sites on DNA (operator sites) and hinder transcription (HOW?).

Dimeric *Dyad symmetry*

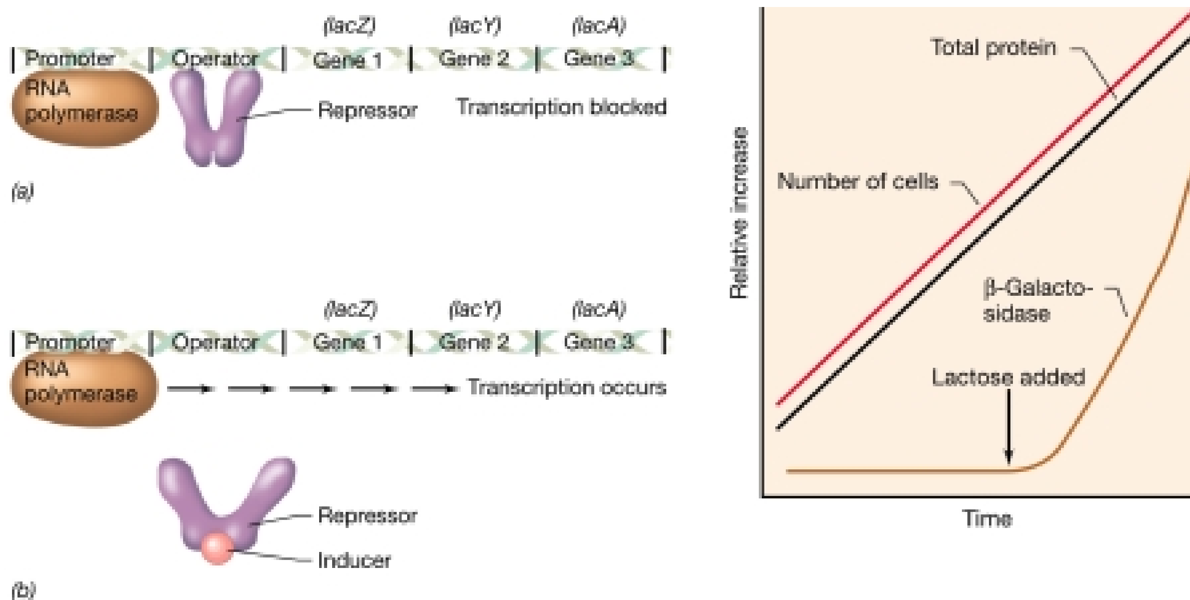


How are repressors used for regulation? – <u>Two mechanisms</u>:

<u>Corepressor:repressor complex</u> binds to DNA
example: *argCBH*. The cell turns *off* synthesis of the genes in the presence of Arg.

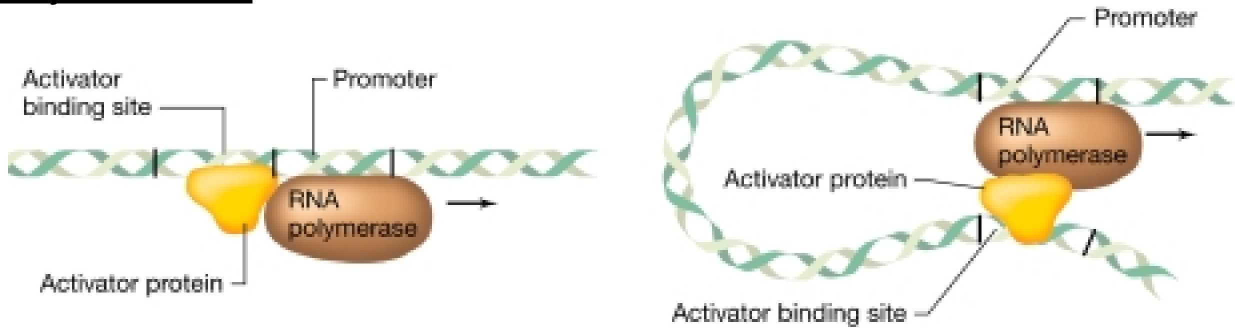


<u>Inducer:repressor complex</u> is released from DNA
example: *lacZYA*. The cell turns *on* the synthesis of the lactose utilization genes in the presence of lactose.

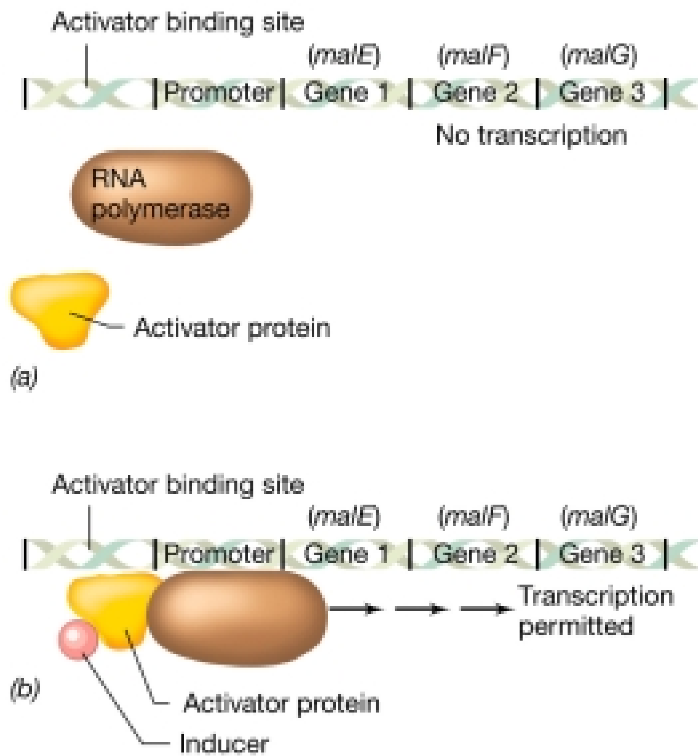


Activators

Activators are proteins that bind DNA at specific sites (activator binding sites) that may be distant from the promoter and stimulate binding of RNA polymerase



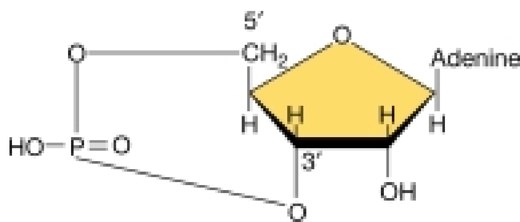
Activators may act alone (above) or with an *inducer* (e.g., maltose stimulation of *malEFG*).

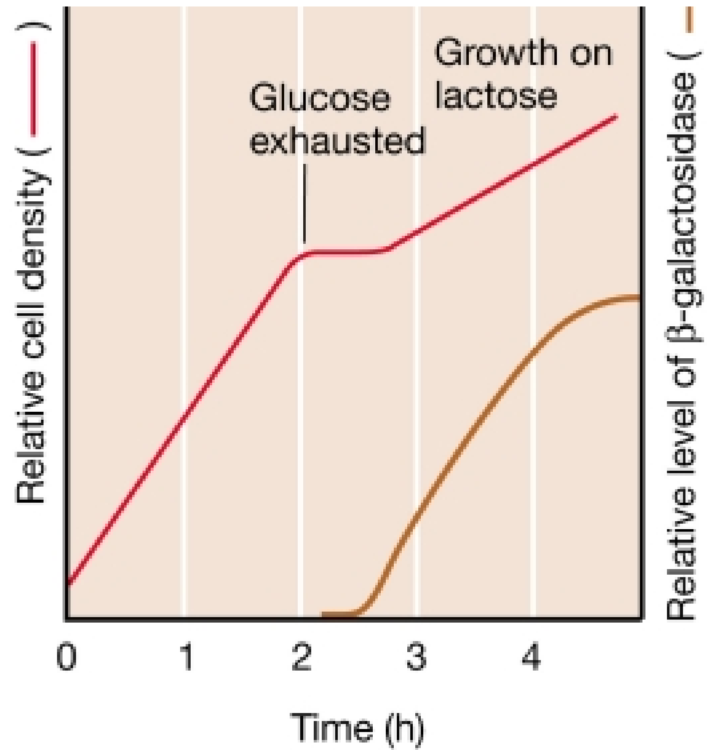


Another important type of regulation using an activator protein is "*catabolite repression*" (yes, this is a confusing name!).

This process occurs during growth in the presence of two substrates (e.g, glucose + lactose). The cells exhibit *diauxic growth*:

Glucose is the preferred carbon source. This nutrient is used up, then the lactose utilization genes are turned on.





This process involves the *catabolite activator protein* (CAP) and the *inducer* cyclic AMP.

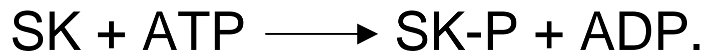
<u>High Glucose</u>	<u>Low Glucose</u>
Low cAMP (pumped out)	high cAMP
CAP does not bind DNA	cAMP:CAP binds DNA
lac genes are not transcribed	*lac* transcription

Two-component regulatory systems

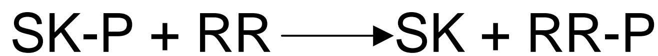
Sensor kinase (SK) recognizes an environmental signal (e.g., CheA in flagellar control).

Response regulator (RR) is a DNA binding protein (recall CheB).

SK is autophosphorylated

SK + ATP  SK-P + ADP.

Phosphate is transferred to RR

SK-P + RR  SK + RR-P

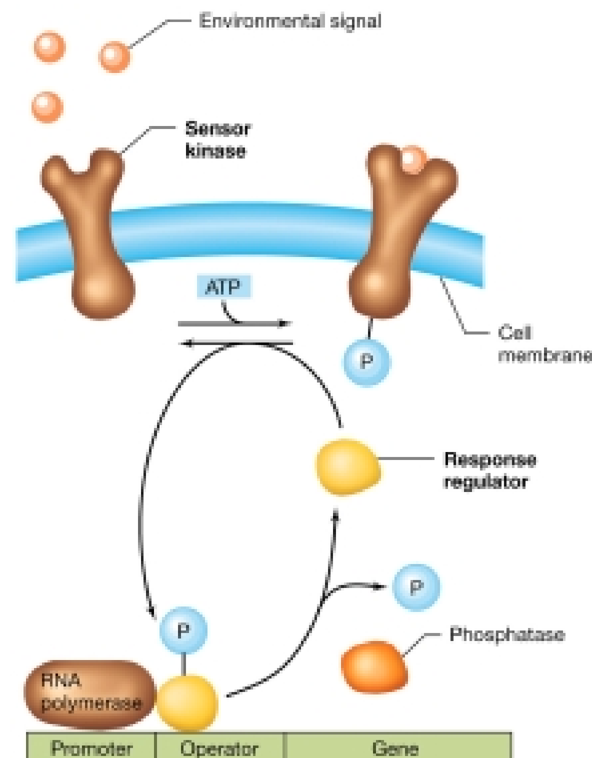
Phosphorylation of RR generally increases DNA binding affinity.

RR-P may be an activator or a repressor (depending on the system).

Phosphatase converts RR-P to RR + phosphate.

These are very common in bacteria.

E. coli has ~50 2-component systems!



A few extra regulatory complications

Any one gene of interest may be regulated by several different processes. For example, it may exhibit “baseline” levels of transcription due to Sigma-70 type activity, while also being “upregulated” or “downregulated” by one or more other regulatory system.

Some proteins may act as either activators or repressors, depending on the environment of the binding site, the presence of co-repressors or inducers, or extent of phosphorylation.

There are several “*global regulatory systems*”

ArcA repressor/activator: ~50 genes switch on/off in response to oxygen.

LexA repressor: ~20 genes turned on when cells senses DNA damage (SOS response).

FNR activator: ~70 genes affected by switch to anaerobic conditions.

OxyR activator: ~30 genes turned on by oxidative stress (e.g.., catalase, peroxidase).

CAP regulates ~300 genes!

Alternative sigma factors.

and more!

Methods to monitor regulation

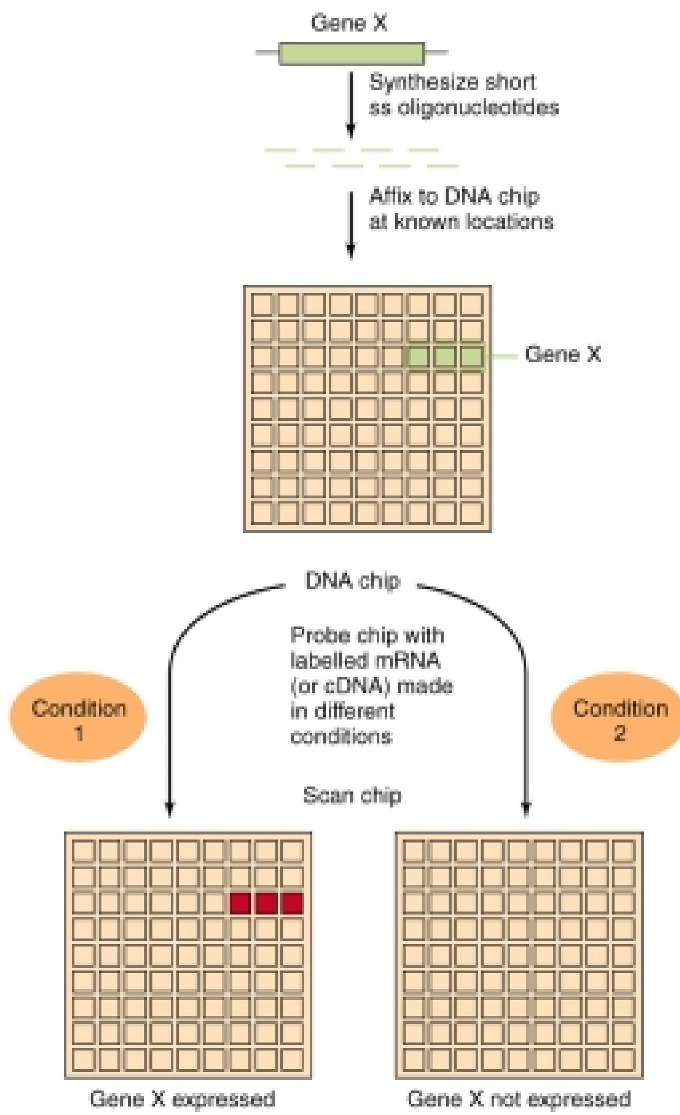
Examine changes in RNA levels

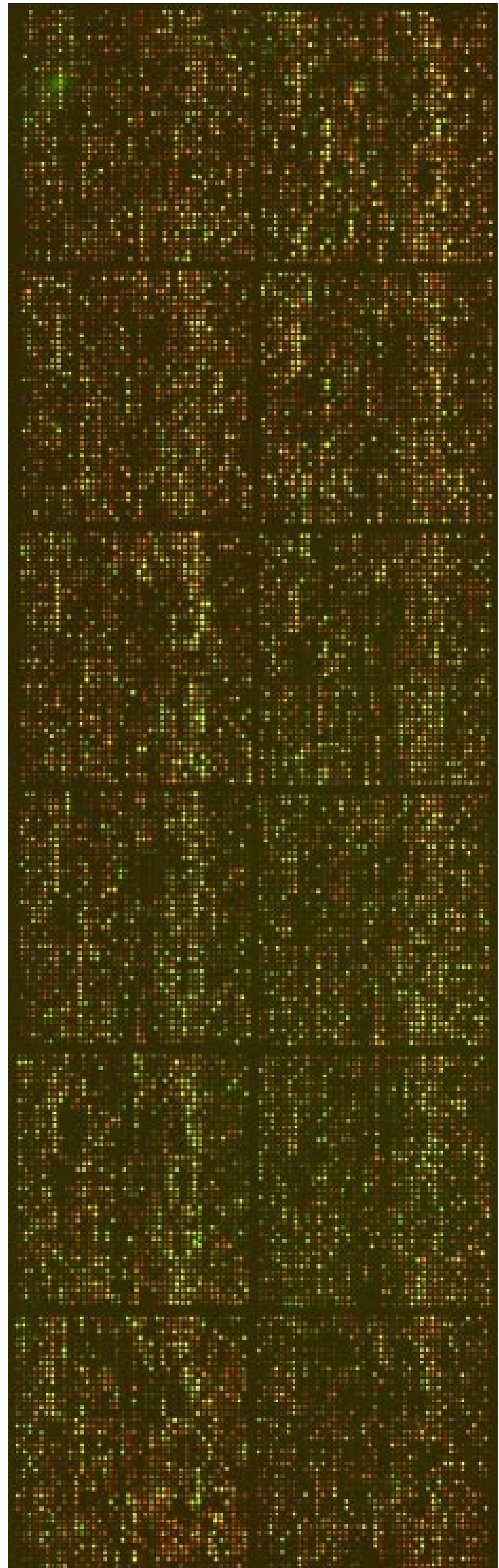
The expression of any one gene of interest can be monitored by developing a probe to that RNA:
-- synthesize a matching DNA oligonucleotide
-- label with ^{32}P or a fluorescent dye
-- isolate RNA from cells under controlled conditions (inhibit RNA-degrading enzymes)
-- hybridize DNA to RNA (often after performing gel electrophoresis to separate RNAs by size)
-- wash away excess probe
-- quantitate radioisotope or fluorescence intensity
-- compare to controls

What about global regulation? *Want to look at all RNA molecules simultaneously* (~4300 in *E. coli*):
-- Generate a DNA chip or DNA microarray
-- Each spot on a slide or plate (with 1000's of spots) contains a bound oligonucleotide matching one of the genes.
-- Hybridize with total RNA from cells grown with various conditions, then wash away excess.
-- Quantitate the extent of hybridization *for each spot* and compare among samples.
-- This type of analysis is an important component of Bioinformatics.

Microarrays

Dealing with this much data is challenging!





Examine changes in protein levels

-- The level of any one particular protein of interest can be studied by using antibodies to that protein, or may be visualized by protein gel electrophoresis.

-- If dealing with an enzyme, one can monitor the level of enzyme activity under varied conditions.

The bigger challenge is *global monitoring* of the >1000 proteins in a cell

-- 2-Dimensional gels separate proteins by charge (isoelectric focusing) and size.

-- Stain proteins and monitor intensity of spots

-- Identify spots of interest by N-terminal microsequencing or mass spectrometry.