Lecture 22

Eukaryotic gene Regulation

There a number of general ways that gene regulation in eukaryotes differs from gene regulation in prokaryotes.

• Eukaryotic genes are not organized into operons — each gene has its own promoter.

• Usually eukaryotic regulatory genes are not linked to the genes that they regulate.

• Proteins involved in gene regulation must be compartmentalized into the cell nucleus.

• Eukaryotic DNA is wrapped around nucleosomes to construct a protein DNA scaffold known as chromatin. Changes in chromatin structure can influence regulation.

Despite these differences, many of the ways that gene regulation is analyzed are the same in eukaryotes and prokaryotes. To see how eukaryotic gene regulation can be studied genetically we will look at how the yeast genes for galactose metabolism are regulated. There are a number of different unlinked genes involved in galactose metabolism that are coordinately regulated; collectively these genes are known as the **Gal** genes. On medium that does not contain galactose, the **Gal** genes are not expressed but in the presence of galactose they are induced to a high level. We will choose as a representative reporter, the **Gal1** gene which encodes galactokinase, the first enzyme in the galactose utilization pathway.

	galactokinase	
	-galactose	+galactose
Gal1$^+$	–	+
Gal1$^-$	–	–

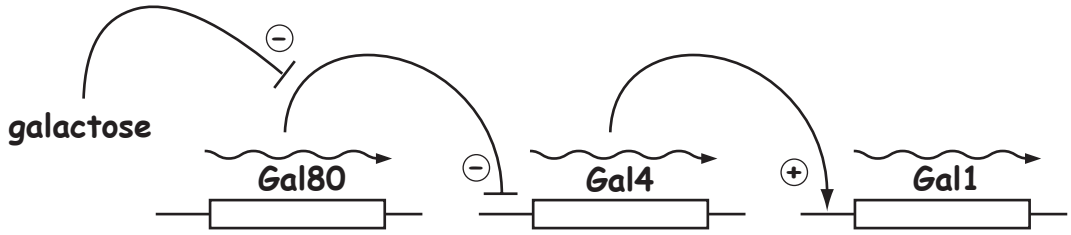
The first step in the analysis of **Gal** gene regulation was the identification of mutants that affected the coordinate expression of all of the **Gal** genes.

Gal4$^-$: The first such mutant isolated was **Gal4$^-$** which gives uninducible expression of the **Gal** genes. Although **Gal4$^-$** and **Gal1$^-$** give the same phenotype, that is no expression of galactokinase on galactose medium, it is possible to show that these mutations are unlinked by tetrad analysis. You should think about what the tetrads from this cross would look like. Finally, heterozygous diplioids constructed by mating a **Gal4$^-$** to wild type (**Gal4$^-$/ Gal4$^+$**) have normal regulation, showing that **Gal4$^-$** is recessive. Together these results suggest that **Gal4** is a positive regulator of **Gal** gene expression.

Gal80⁻: The next useful regulatory mutant that was isolated was **Gal80⁻**, which gives constitutive expression of the **Gal** genes. **Gal80** is not linked to **Gal4** or to any of the other **Gal** genes. **Gal80⁻/Gal80⁺** heterozygous diploids show normal regulation showing that **Gal80⁻** is recessive. Together these results indicate that the normal function of **Gal80** is to negatively regulate the **Gal** genes.

Assuming that **Gal4** and **Gal80** act in series, there are two different orders for the **Gal** regulatory pathways that fit the data that we have so far.

Model 1:



Model 2:

We can resolve these two possible models by performing an epistasis test to evaluate the phenotype of a **Gal4⁻ Gal80⁻** double mutant. Note that the basic requirement for an epistasis test is that the two mutants have opposite phenotypes. This requirement is met because **Gal4⁻** is uninducible while **Gal80⁻** is constitutive.

To construct the double mutant we can perform the following cross:

MAT**a** **Gal4⁻ Gal80⁺** X MATα **Gal4⁺ Gal80⁻**

And the following types of tetrads are obtained:

Type 1	Type 2	Type3
uninducible	uninducible	uninducible
uninducible	uninducible	uninducible
constitutive	constitutive	regulated (wt)
constitutive	regulated (wt)	regulated (wt)

We can see that Type 1 tetrads contain two spores of each of the parental phenotypes and are therefore parental ditypes, whereas Type 2 tetrads contain three different phenotypes and therefore must be tetratypes. Thus we can infer that Type 3 tetrads are nonparental ditypes and that the **Gal4⁻ Gal80⁻** double mutants are uninducible. Therefore Model 1 is favored over Model 2.

Now let's consider a new class of mutants known as **Gal81⁻**. Like **Gal80⁻** mutants, **Gal81⁻** mutants are constitutive, but **Gal81⁻/Gal81⁺** heterozygous diploids are also constitutive showing that **Gal81⁻** is dominant. In order to perform epistaisis tests between **Gal81⁻** and **Gal4⁻**, the following cross was performed.

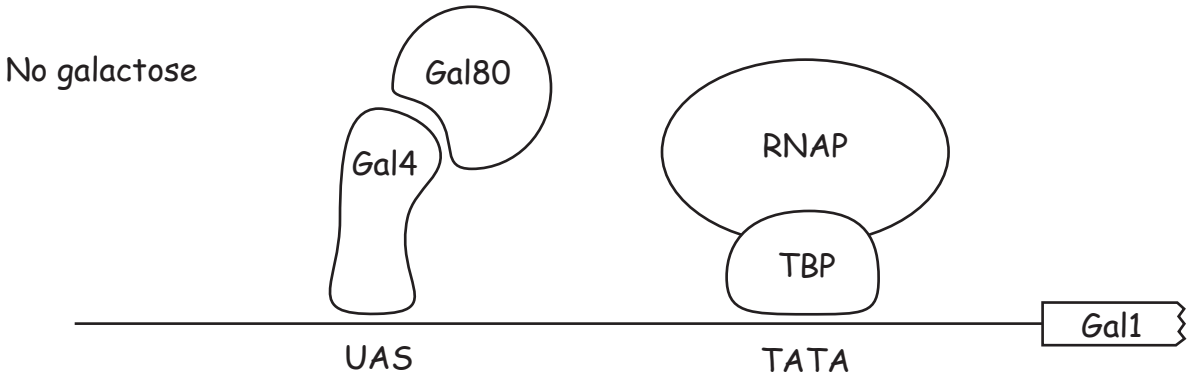
MAT**a** **Gal4⁻ Gal81⁺** X MATα **Gal4⁺ Gal81⁻**

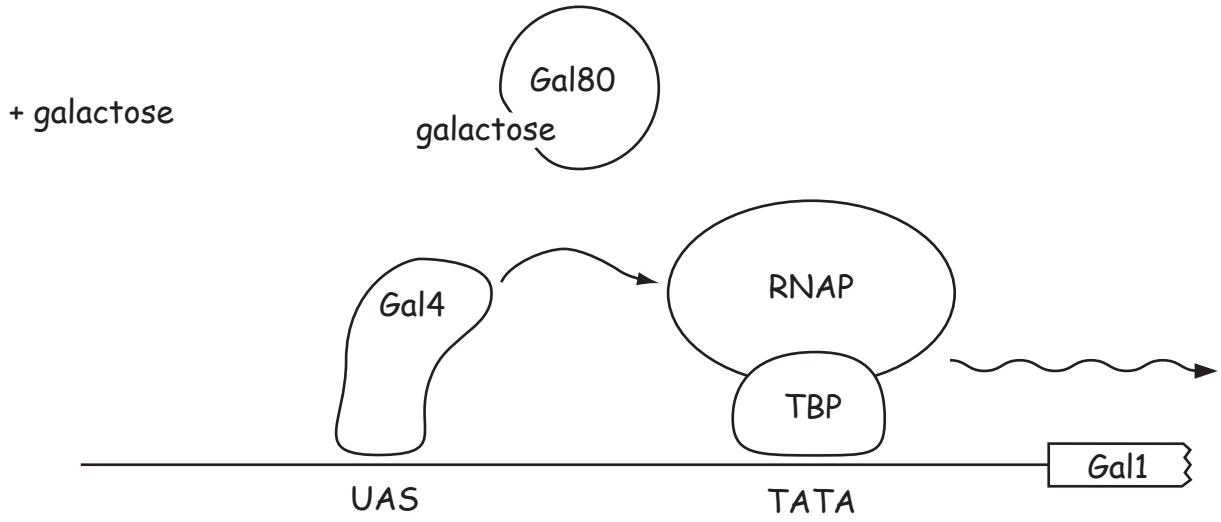
The tetrads from this cross all contain 2 uninducible spores and 2 constitutive spores. These tetrads have two spores with each of the parental phenotypes and are therefore all parental ditypes. The conclusion from this cross is that **Gal81** is actually very tightly linked to **Gal4**.

In keeping with Model 1, it was first hypothesized that **Gal80** was a transcriptional repressor of the **Gal4** gene and that **Gal81⁻** mutants were mutations in an operator sequence in the **Gal4** promoter. However, it was soon realized that this hypothesis was incorrect because **Gal4** expression is not regulated by galactose and mapping and sequencing of the **Gal81⁻** mutants showed that they lie within the coding sequence, not the promoter region, of **Gal4**. **Gal81⁻** mutants are in fact special alleles of **Gal4** and we will designate these mutants **Gal4^{81}**. Apparently, **Gal80** protein exerts its negative effect on **Gal4** by interacting directly with the **Gal4** protein (rather than interfering with **Gal4** expression). Furthermore, the **Gal4^{81}** alleles appear to be impervious to the negative effects of **Gal80** protein. It may be helpful to think of **Gal4^{81}** as being a super activator, analogous to **MalT^{c}**.

Now let's see how all of these components fit together in the context of our current understanding of how **Gal** regulation works.

Initially, **TBP** (TATA-binding protein) binds to a DNA sequence defined by the TATA consensus site. **TBP** then acts as a binding site for **RNAP** which is a large complex of proteins including RNA polymerase itself. Together **TBP** and **RNAP** constitute the so-called Basal Transcription Machinery that is necessary for transcription. However, transcription will not begin until **RNAP** is activated by a transcriptional activator. For **Gal** gene expression, the activator is **Gal4** protein. One portion of the **Gal4** protein binds to a sequence known as the UAS which anchors the protein at the promoter. The other portion of **Gal4** is an activation domain that is capable of activating RNAP but in the absence of galactose **Gal80** binds to this portion of **Gal4** and blocks activation. When galactose is present, galactose itself or a chemical derivative of galactose binds to **Gal80**, dislodging **Gal80** from **Gal4** allowing activation of **RNAP** and expression of the **Gal1** gene. (The **Gal4^{81}** alleles interfere with **Gal80** binding and give constitutive activation).


No galactose
Gal80
Gal4
RNAP
TBP
Gal1
UAS
TATA


+ galactose
Gal80
galactose
Gal4
RNAP
TBP
Gal1
UAS
TATA