

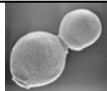




7.03, 2006, Lecture 23 Eukaryotic Genes and Genomes IV

In the last three lectures we have thought a lot about analyzing a regulatory system in *S. cerevisiae*, namely **Gal** regulation that involved a hand full of genes. These studies monitored the increased transcription of **Gal** genes in the presence of galactose (and the absence of glucose); we saw that this regulation is achieved by particular proteins, or multiprotein complexes that bind to specific sequences in the promoter region upstream from their target genes.

What if I told you that it is now possible to do the following in *S. cerevisiae*:

- Monitor mRNA expression level for every gene in *S. cerevisiae*, in one single experiment.
- Monitor all the binding sites in the *S. cerevisiae* genome for each transcription factor in a single experiment.
- Determine all possible pair-wise interactions for every *S. cerevisiae* protein.

Obviously I wouldn't mention these possibilities if they weren't already happening. What I want to do today is to introduce you to the idea of carrying out genetic analyses on a global, genome-wide scale, and hopefully give you some examples that are relevant to what we have already learned along the way. So, this will be

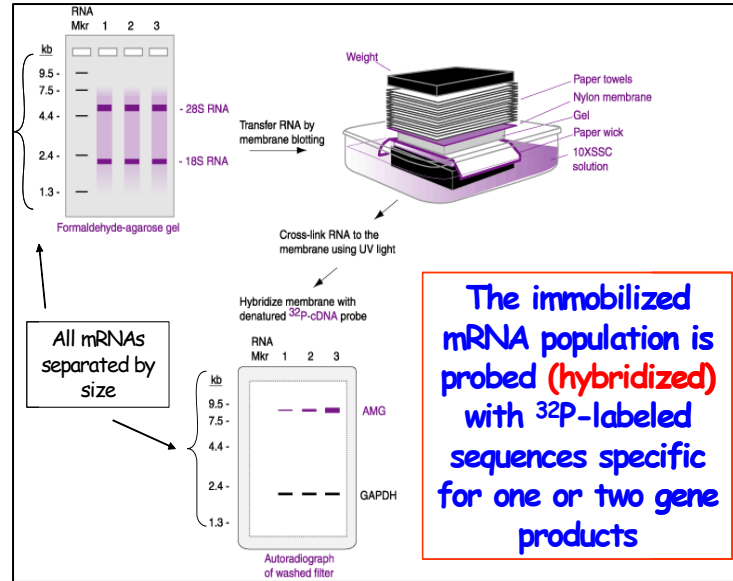
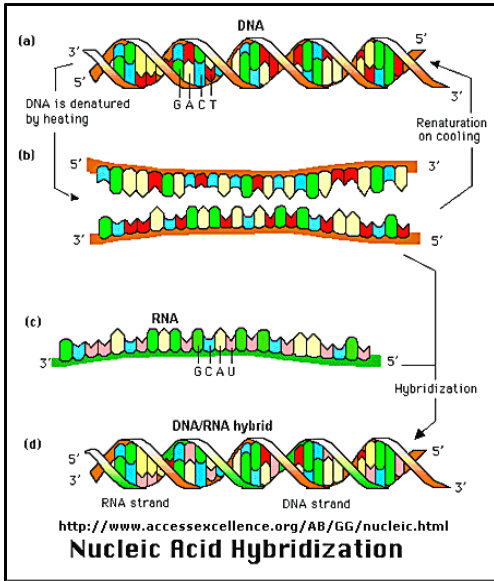
Numbers of genes per haploid genome	
 5,800	
 14,000	 22,500
 19,000	 22,500

a technology oriented lecture, but with some application to what we have already learned about gene regulation in eukaryotes. It should also be mentioned that what will be described for *S. cerevisiae*, is theoretically possible for any organism whose genome has been completely sequenced and the location of all the genes in that genome have been established. So, what we will learn today is being, or will be, applied to higher eukaryotes and mammals.

- **Monitor mRNA expression level for every gene in *S. cerevisiae*, in one single experiment: Global transcriptional profiling.**

Before we consider how it is possible to measure the levels of thousands of mRNA species, we will have to step back to consider how the levels of one or two mRNA species can be measured by Northern Blot analysis....and I know you must have learned this in 7.01 if not in high school. Northern blot analysis is based upon the fact that DNA and RNA molecules that possess complementary base sequences that will hybridize together to form a double stranded molecule. If the complementarity is perfect the duplex molecule is stable, if it is imperfect (with

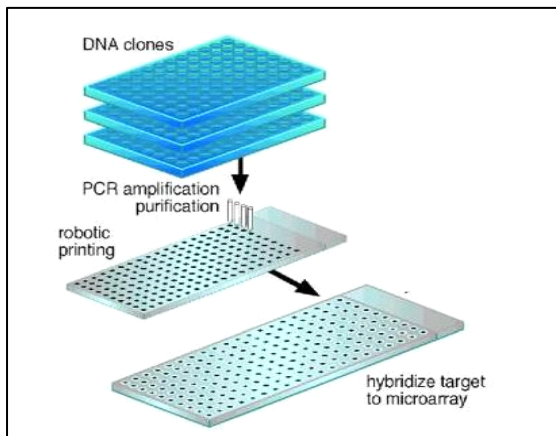
base pair mismatches) it is relatively less stable. This provides the specificity needed to identify perfectly matched DNA:RNA duplexes (on Northern Blots) and DNA:DNA duplexes (on Southern Blots). This specificity is needed to be sure we are measuring the level of one particular transcript and that this is not contaminated with signal from closely related transcripts. RNA is isolated from



cells, size fractionated on a gel; the thousands of mRNAs species form a smear on the gel which is punctuated by the strong ribosomal RNA bands (28S and 18S) that do not interfere with the analysis.

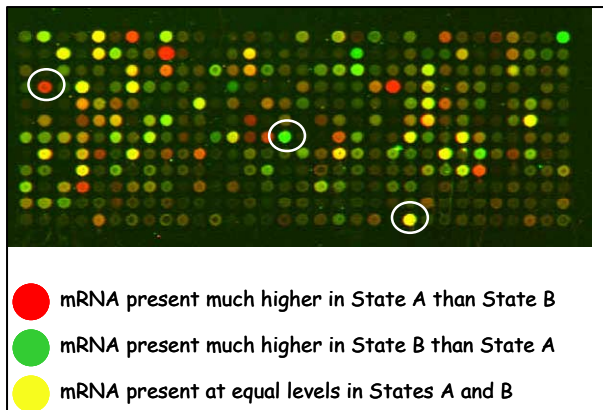
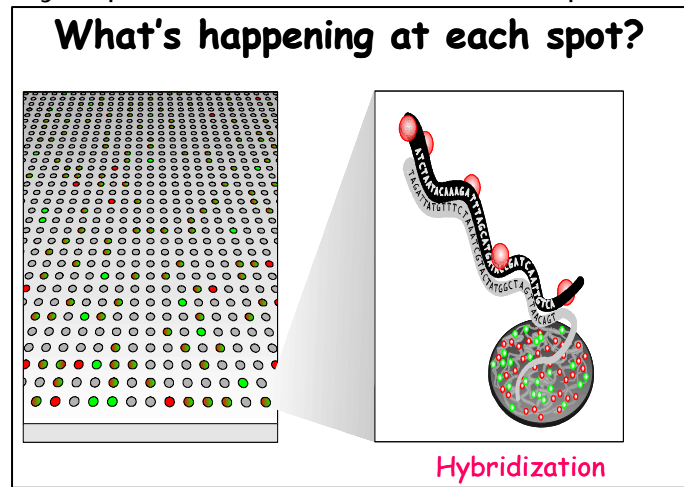
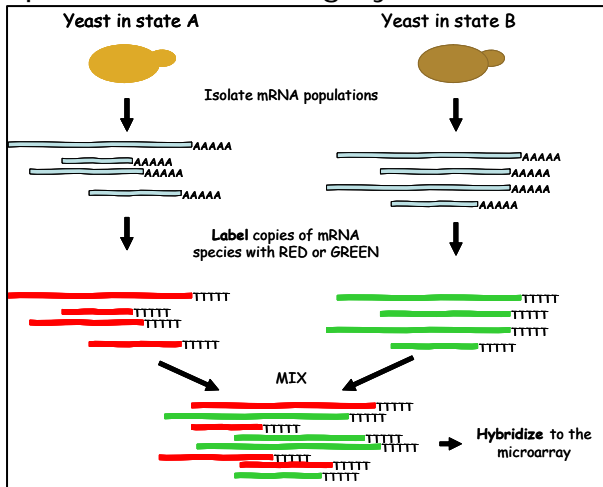
Northern Blots
 Immobilized mRNA population hybridized with labeled DNA probe representing one or two genes

DNA Microarrays
 Immobilized DNA probes representing all possible genes hybridized with labeled mRNA population



The breakthrough in developing microarrays for analyzing mRNA levels was to reverse the logic – instead of immobilizing the mRNAs for hybridization with one or two labeled complementary DNA (cDNA) probes, all possible cDNA probes are immobilized on a solid surface (usually glass slides). The spotting of probes is achieved robotically; the DNA probes are designed to specifically hybridize to only one nucleic acid sequence that represents a single mRNA species. The thousands of DNA probes are dispensed from 96-well, or 384-well plates to an addressable site on the solid surface. The mRNA population from each cell type is purified and then copied such that the copy is fluorescently labeled. This fluorescent population is hybridized to the immobilized probes, and the intensity of the fluorescence at each probe spot is proportional to the number of copies of that specific mRNA species in the original mRNA population.

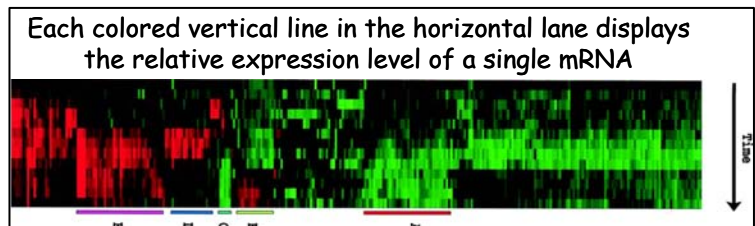
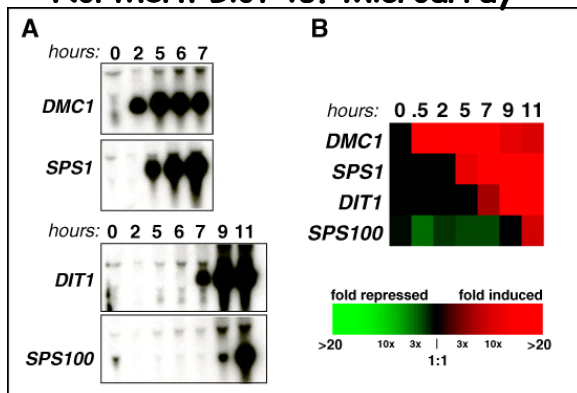
So let's look at how this would actually work in a real experiment. **mRNA** is isolated from yeast cells in **state A** (e.g., minus galactose) and from yeast cells in **state B** (e.g., plus galactose), and copies of each population is made such that one fluoresces red and the other fluoresces green. After mixing, these fluorescent molecules are hybridized to the slides containing ~5,800 DNA probes, each one specific for detecting hybridization of many copies of an individual mRNA species.



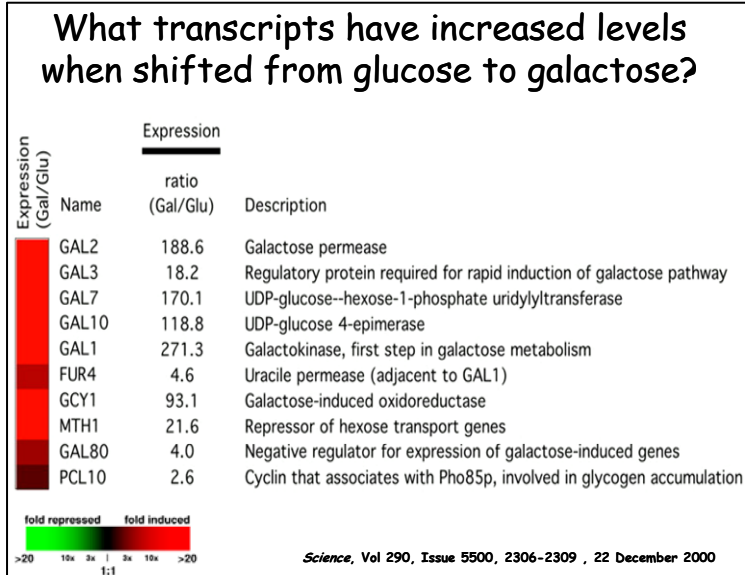
The location and identity of each probe on the microarray slide is known, and each probe is specific for a single mRNA. The color and intensity of the fluorescence is measured by scanning the slide with lasers, and the relative abundance of each mRNA in the cells of **State A** vs **State B** can be calculated from the emitted fluorescence, i.e., the relative level of 5,800 mRNAs can be compared between two populations of yeast cells.

Presenting data for thousands of mRNA transcripts is clearly a challenge. You could present endless tables of data, but our brains are much more adept at recognizing shapes, patterns and colors. Colored representations of up and down regulation of transcripts levels is the preferred way to present data.

Northern Blot vs. Microarray



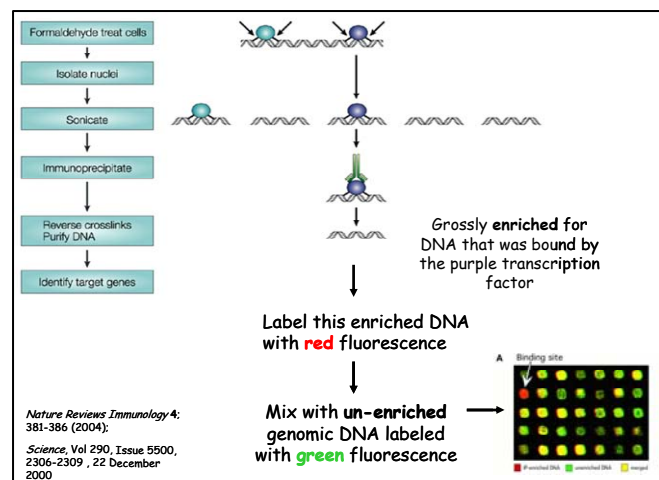
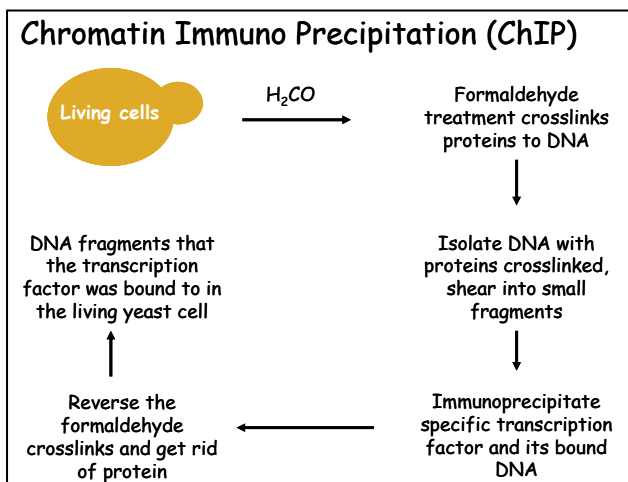
For our purposes here, let's look at what genes are up-regulated when a glucose grown culture of *S. cerevisiae* is shifted into galactose; what genes are up-regulated under these conditions? Obviously transcripts for **Gal1**, **Gal7** and **Gal10** genes will be up-regulated, as we have discussed in the last couple of lectures. But upon looking globally, it is now clear that some other genes are also up-regulated.



But upon looking globally, it is now clear that some other genes are also up-regulated. In addition, **Gal2** (galactose permease) and **Gal80** (the negative regulator of the **Gal4** Transcriptional activator) are also induced; this was previously known, although we didn't discuss it directly in the previous lectures. but upon looking globally, it has become clear that some other genes are also up-regulated. (This figure shows just a small snapshot of the response.) These additional genes are **Fur4**, **Gcy1**, **Mth1**, and **Pcl10**, and their co-regulation along with the **Gal** genes was previously unrealized. We will be coming back to this later in the lecture.

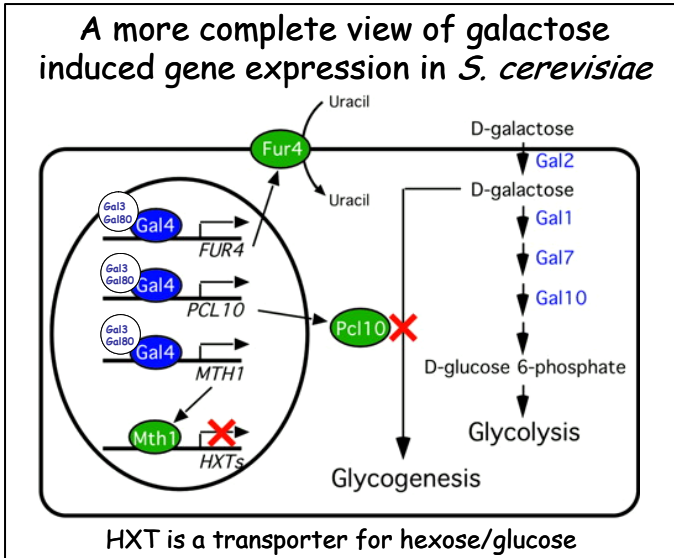
- Monitor all the binding sites in the *S. cerevisiae* genome for each transcription factor in a single experiment.

In the last lecture we talked about deletion analysis of cis-acting regulatory sequences identifying the location of **UAS** and **URS** sequences upstream of the **Gal1** gene. That the **Gal4** transcriptional activator protein binds to the DNA sequence present at the **URS_{GAL1}** can be shown to happen in the test tube, but showing that it is actually bound in a living cell is another matter. A method was recently developed for doing just that, and this method has been further



developed to determine transcription regulator binding across the whole genome. This method takes advantage of the fact that formaldehyde crosslinks proteins to DNA in a way that can later be reversed.

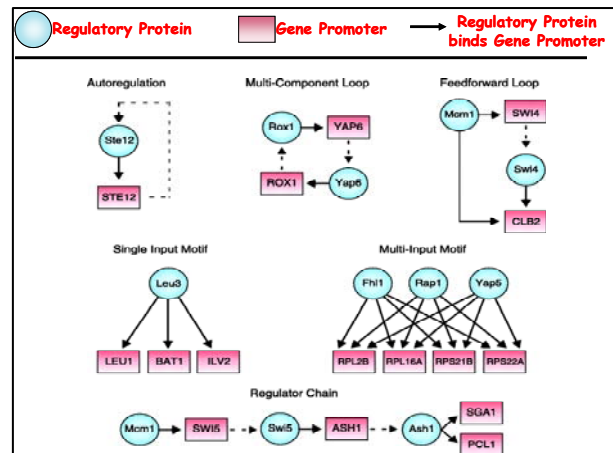
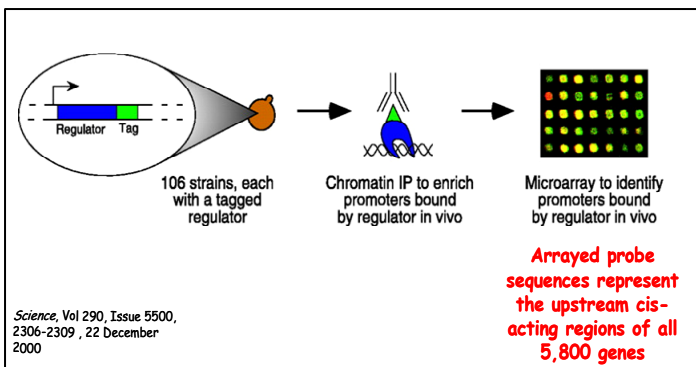
For galactose grown yeast cells chromatin immunoprecipitation (ChIP) with an antibody that pulls down the **Gal4**



protein revealed some surprises. In addition to confirming that **Gal4** binds to the promoter regions upstream of the expected **Gal** genes, the **Gal4** protein also binds to the promoter regions of 4 other genes, namely **Fur4**, **Pcl10**, **Mth1** (shown in the adjacent figure) and **Gcy1** (not shown). Note that these genes were shown to be induced by galactose in the previous section. Just how the up-regulation of **Fur4**, **Pcl10** and **Mth1** might contribute to optimizing the metabolism of galactose is shown in this figure, but

the role **Gcy1** plays is unclear. Clearly, taking a global look at what genes are up-regulated in the presence of galactose, and taking a global look at what promoters are bound by the **Gal4** regulator, has clearly enriched our view of how *S. cerevisiae* adapts to the presence of this sugar.

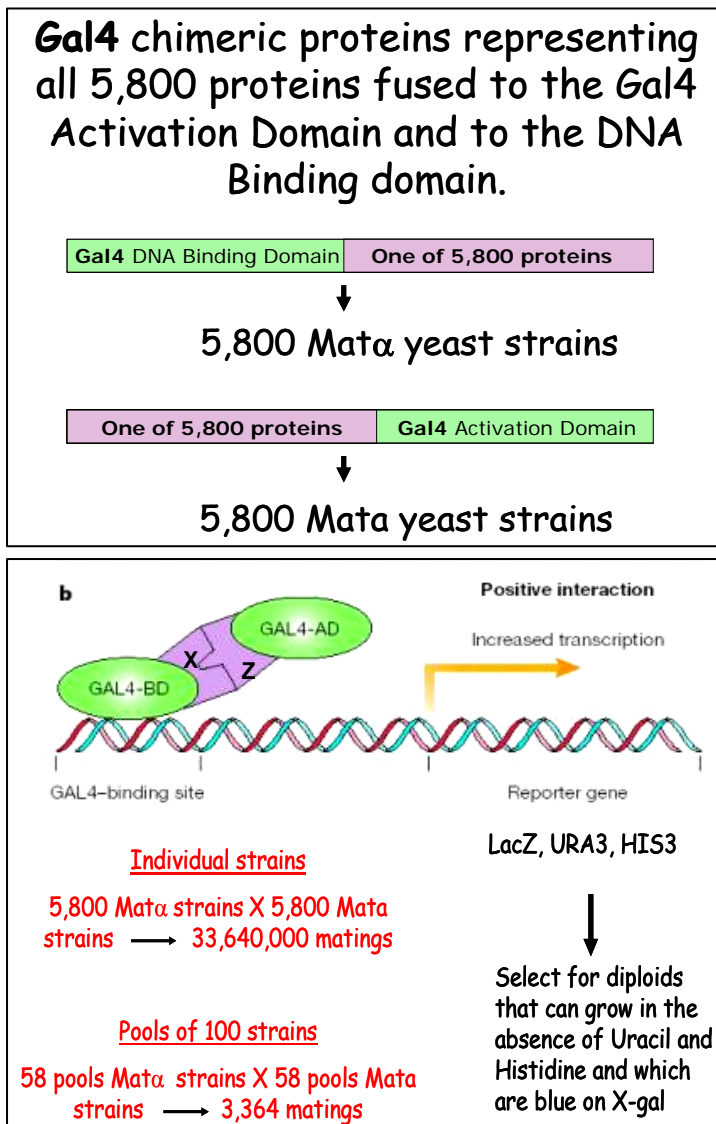
The ChIP approach, followed by hybridization to DNA microarrays, was originally limited to monitoring binding of transcriptional regulators for which there were good precipitating antibodies. However, this limitation was recently eliminated by fusing an epitope TAG to each regulator gene. This epitope TAG is recognized by a strong antibody, and so a single antibody can “pull down” (immunoprecipitate) >100 different regulatory proteins, each of which is expressed in its own yeast strain.



This has enabled a massive study to identify all of the target genes for each of 106 transcriptional regulators in *S. cerevisiae* growing in a defined medium. A compilation of all the data has revealed a number of fundamentally different regulatory motifs; these are shown in the adjacent figure. For the most part the **Gal4** regulatory network (now shown) represents a simple Single Input Motif.

This approach has already been extended to human cells and it will not be long until detailed regulatory mechanisms are defined for humans, in the way it is now happening in yeast. It is now possible to go on to monitor which genes the transcriptional regulators bind to under different environmental conditions, and from there to build more dynamic models for how these genetic regulatory mechanisms operate and ultimately how they co-operate with each other.

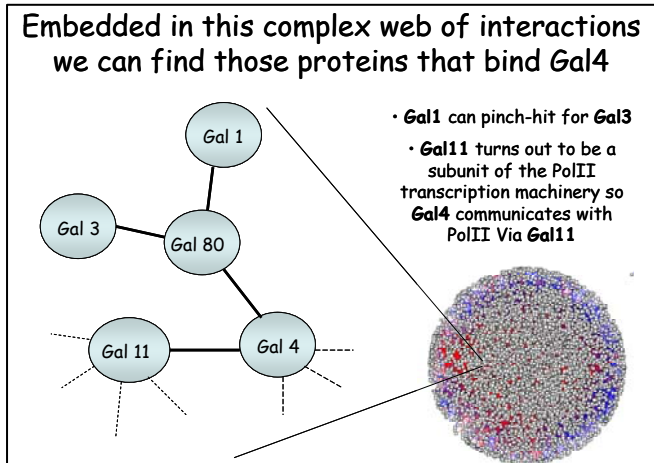
- **Determine all possible pair-wise interactions for every *S. cerevisiae* protein.**



The third global scale analysis we will consider is the systematic determination of protein-protein interactions in *S. cerevisiae*. This essentially involves a systematic test of all pair-wise combinations between all 5,800 yeast proteins. Individual matings to test >33 million combinations isn't feasible, so mating pools of 100 strains in all combinations has become the preferred approach. Only the diploid strains where the **Gal4 DB**-fusion and the **Gal4 AD**-fusion proteins interact will be able to grow on galactose medium without uracil and histidine, as well as turning blue when grown on galactose and X-gal. The plasmids present in such diploids are then sequenced to determine which proteins are fused to the **Gal4 AD** and **DB** domains.

This systematic approach to cataloguing all possible protein-protein interactions for yeast proteins yielded many more interactions than originally thought. Admittedly the yeast two hybrid is

quite noisy, giving many false positive interactions, but even so alternative methods (that we do not have time to consider in detail) have confirmed many of these interactions. When all of the known protein-protein interaction data is assembled, we see the surprising fact that > 5,000 proteins can be connected



together by > 14,000 protein interactions in a continuous web. Indeed, the interaction data for **Gal4** embedded within this web makes sense and adds some new information. Such "Interactomes" are being developed for all the usual organisms, and the *C. elegans* interactome is particularly well developed. One of the major revelations has been that proteins from pathways that were previously thought to be totally unconnected, turn out to have interacting proteins.