MIT Department of Biology
7.014 Introductory Biology, Spring 2005

7.014 Handout

Biochemical Genetics

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Introduction and Background

We have learned that proteins can act as enzymes and catalyze all sorts of reactions in the cell. We discussed a biochemical approach to understanding cellular processes by purifying enzymes, determining their structure, and then studying the reactions they catalyze. Often though this type of study does not answer the question, "what does this protein do within the organism?" One approach to this question would be to remove the protein being studied and determine how this affects the organism. But, is this possible?

The information required to make an enzyme is encoded as a gene in the DNA of the organism. In general, one gene corresponds to one polypeptide (a gene can also correspond to an RNA, but this is not relevant to the discussion at hand). This correspondence and advances in DNA technology allow researchers to create an organism that lacks a particular protein by removing the DNA that encodes that protein. In effect, a specific mutant organism can be created and by studying this mutant we can study the effects of removing a single protein.

Even before this was a possibility, genetics played a key role in understanding many basic cellular processes. If you wanted to study olfaction but you had not identified any of the proteins involved, how could you begin? Because one gene corresponds to one protein and the genotype of an organism is often reflected in its phenotype, you could start by finding mutants that can not detect odors. The assumption would be that each mutant carries a change in the DNA that encodes a particular protein important in olfaction. The altered protein could have an altered function or be so changed that it no longer functions at all. The change in the DNA sequence is called a mutation, and the cell or organism carrying a mutation is called a mutant.

Mutants with a disruption in the olfaction process would all have the same phenotype, an inability to sense odors. This is true even if each mutant had an alteration in a different gene. For example, assume that the ability to perceive odors requires three different proteins and protein 1 is encoded by gene1, protein 2 is encoded by gene 2, etc. The phenotype of an organism with a mutation in gene 1 would be the same as the phenotype of an organism with a mutation in a gene 2; neither of these mutants would be able to perceive an odor.

Thus even without any knowledge of the genes or the proteins involved in a particular cellular process, we can begin to study it by collecting mutants that all display the same phenotype.

A Model System

For this introductory illustration of biochemical genetics we will begin with a model system. Model systems are organisms that can be easily manipulated in a laboratory environment. Widely used as model organisms are single-celled eukaryotes of the yeast family. These yeasts, like *Saccharomyces cerevisiae* or *Schizosaccharomyces pombe* are associated with the making of beer and bread. These cells are easily and quickly grown in a laboratory, can live in either a haploid or a diploid state, and can reproduce sexually or asexually. Yeast cells can be treated with chemicals or irradiated to increase the mutation rate, and they can be rather easily convinced to accept exogenous DNA. To explore more about yeast see: **http://enpc1644.eas.asu.edu/modsum/YstSUM.htm.**

The yeast life cycle.
Yeast cells reproduce asexually simply through mitosis where the mother cell divides to produce two identical daughter cells. If the yeast cell is diploid, it can instead enter a meiotic pathway and produce four haploid cells or "spores". Interestingly, each of these haploid spores can enter a mitotic cycle and indefinitely produce identical haploid daughter cells. Two haploid yeast cells can fuse or "mate" to create a new diploid cell.

Growing yeast cells.
Yeast cells can be grown easily in the laboratory. Wild-type yeast cells are prototrophs. This means that they can synthesize all the necessary cellular components *de novo* if provided with some basic carbohydrates and salts. The basic required carbohydrates and salts are supplied as *minimal medium*. Yeast cells can be grown in test tubes containing liquid minimal medium or on petri plates containing a solid form of minimal medium. A single yeast cell can only be seen with a microscope, but a single cell can divide into 10^8 identical cells in 48 hours. 10^8 cells are enough to turn a tube of liquid medium cloudy, and if the tube of medium is allowed to settle, a coating of cells is visible at the bottom of the tube. When a single cell divides on solid medium, all the daughter cells stay grouped together. 10^8 cells grouped together form a *colony* about as big as the following dot.

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Yeast cells can also be grown in or on *rich* medium. Rich medium contains carbohydrates and salts and all the amino acids and other nutrients that yeast cells could need. When presented with amino acid supplements (or other nutrients), yeast cells do not invest cellular energy to make them *de novo*, but instead scavenge them from the medium.

When a mutation occurs in the genome of a yeast cell that eliminates a protein required for biosynthesis, the mutant yeast cell can no longer grow on mininal medium. Such mutants are called *auxotrophs*. If the mutation damaged a gene encoding an enzyme needed for arginine synthesis, then the mutant cell would not grow on minimal medium but would grow on minimal media that was supplemented with arginine. Alternatively, if the mutation damaged a gene encoding an enzyme needed for nicotinic acid synthesis, then the mutant cell would not grow on minimal medium but would grow on minimal medium that was supplemented with nicotinic acid. Both the arginine auxotroph and the nicotonic acid auxotroph could grow on *rich* medium.

Making and Identifying Mutants

Mutations like those discussed above are naturally occurring due errors in mitosis or meiosis. However, to study a biochemical pathway using genetics, many mutants are needed. To this end, wild-type yeast cells can be *mutangenized* by irradiation or with chemicals that damage DNA. In a mutagenesis, the starting cells are usually haploid, and are treated with levels of mutagen such that most cells carry a single mutation. Why would you want to begin with a haploid strain?

You are hoping to identify cells that are missing a gene encoding a protein important in the pathway that you are studying. In a haploid cell, a mutation in a gene that results in a non-functioning protein is likely to have an effect. If you began with diploid cells, both copies of a gene would need to be mutated to prevent protein function. In a diploid, a single mutation could be masked by the normal copy of the gene because the phenotype of the diploid would be wild-type.

Depending upon the process being studied, creativity and insight is needed to accurately predict what phenotype your mutants should have and identify interesting mutants in a large population. Take the olfaction example for instance. You might begin such a study using fruit

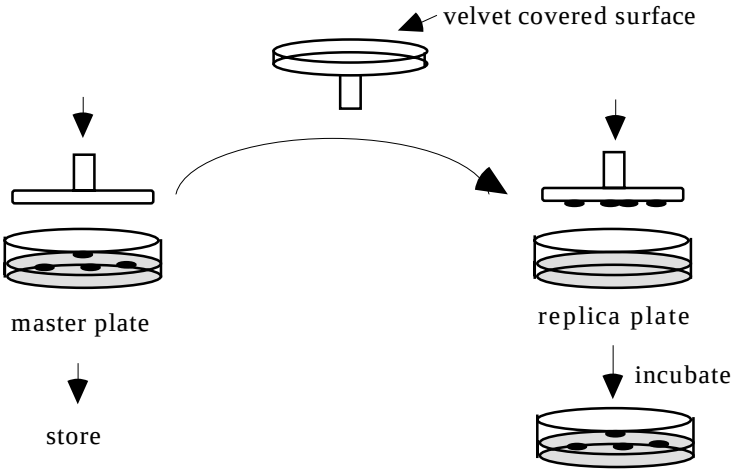
flies; and then search for mutants that are unable to negotiate a maze to find the target fruit. You may well collect flies that can not sense odors, but among them are flies that can sense odor perfectly but are poor fliers.

Let's outline a "mutant hunt" with a straight-forward example. Say we want to study arginine biosynthesis in a yeast model system. We begin with a haploid, wild-type strain. This strain can grow on minimal medium. We mutangenize many of these cells in liquid and then transfer (or *plate*) the treated cells onto solid agar medium† in petri plates. What type of media would you use for these solid agar plates?

For reasons that will become clear, we plate our mutagenized cells on rich medium and many colonies are seen two days later. How do we know which of the many colonies contain cells that are defective in arginine biosynthesis? Remember that the mutants of interest to us carry defects in arginine biosynthesis and can not grow unless the medium contains arginine. So we simply need to determine which of the colonies growing on rich medium have cells that can not grow in the absence or arginine. Hum…?

Replica Plating, Screening and Selecting

In a real experiment, you may have 10,000 or more colonies to test, so the concept may be simple, but the execution would be tedious and time consuming. A technique called *replica plating* developed by Esther and Joshua Lederberg, makes it possible to quickly *screen* many colonies for mutants of interest. The basic principle is the same as that of the rubber stamp. Regardless of the deficiency, most cells form colonies on the rich medium plates, these plates are *called non-selective*. We just need to copy those colonies onto plates that can distinguish cells with mutations in the arginine synthesis pathway from others. The non-selective, rich medium plate serves as a *master* plate. A sterile transfer device*, such as a piece of velvet is gently pressed onto the master plate. The loops and threads of the material pick up a small number of cells from each colony on the master plate. All the colonies and their spatial relationship to each other are now represented on the velvet. The velvet is then applied to consecutive *replica* plates. Some of these replica plates are *selective* and mutants of interest fail to grow. In our case, selective plates lack arginine.



*The prototype of the transfer device was the application pad that came in Esther Lederberg's powder compact.

In such a screen, we can identify mutants that have lost the ability to grow with out arginine. These cells will not form a colony on the replica plate but can be retrieved from the master plate by comparing the position of the colonies on the two plates.

We could follow the same experimental outline if our mutant hunt was designed to identify mutants that had acquired a new ability, say resistance to anti-fungal drugs. In this case the non-selective medium would not contain drug, but the selective medium would. On the drug containing replica plate we could *select* only for cells that had gained the ability to grow in the presence of drug.

These procedures would be repeated until a large collection of mutants had been identified. Remember that all our mutants will have the same phenotype, in our example they require arginine, even if each mutant has an alteration in a different gene. Each mutant was individually isolated, and we will randomly call them the arg1 mutant, the arg2 mutant, the arg3 mutant, etc. Until we know more, we will assume that the arg1 mutant has a mutation in the *arg1* gene and is defective in the ARG1 protein, and that the arg2 mutant has a mutation in the *arg2* gene and is defective in the ARG2 protein

Analyzing the Collection of Mutants

You now have a collection of haploid mutants that each carry only one mutation. You might begin by asking the following questions:

1. Is the phenotype of each mutant dominant or recessive?
2. Which of the mutants have mutations in the same gene?
3. Which of the mutants have mutations in different genes?
4. How many different genes are mutated in this population of mutants?
5. Do these genes encode proteins that function in a particular order, and if so, in what order do the proteins function?

Each of these questions can be answered using one of three tests, a test of recessivity, a test of complementation, or a test of epistasis.

Test of Recessivity

The first step is to determine if the phenotype of each mutant is recessive. This information is vital in interpreting the other tests. Recall that a recessive phenotype is a phenotype that is not seen in a heterozygote. In our case, a heterozygote would be a diploid yeast cell made from a haploid mutant and a haploid wild-type (wt or +) cell. This diploid cell would carry a single mutation. If the phenotype of the mutant is recessive, then this diploid cell will appear wild-type. If the phenotype of the mutant is dominant, then this diploid cell would be the same as the mutant. In general, recessive phenotypes correspond to loss-of-function mutations.

For example, we could take our haploid arg1 mutant, mate it with a wild-type haploid cell, and prevent the resulting diploid cell from undergoing a meiotic division. The *arg1/+* diploid cell will then multiply by mitotic cell divisions. If the phenotype of the arg1 mutant is recessive, the *arg1/+* diploid cells will grow on minimal medium, without arginine supplementation. At a molecular level, the haploid arg1 mutant had defective ARG1 protein and was thus missing an enzyme needed to synthesize arginine. Arginine is an amino acid and without it the cell can not produce the proteins need for cellular functions. The *arg1/+* diploid has one good *arg1* gene, can make sufficient amounts of normal ARG1 protein, and can thus synthesize the arginine needed to make cellular protein.

Complementation Test

A single test called a complementation test can answer the next three questions.

- Which of the mutants have mutations in the same gene?
- Which of the mutants have mutations in different genes?
- How many different genes are mutated in this population of mutants?

A complementation test is much the same as the test for recessivity, but a complementation test involves making a diploid using different combinations of the haploid mutants.

If the diploid looks like the wild-type organism, the two mutations are said to complement. The underlying basis is the same as described above for our example. The haploid arg1 mutant has a mutation in the *arg1* gene, makes defective ARG1 protein, and thus is missing an enzyme needed to synthesize arginine. Likewise, the arg2 mutant has a mutation in the *arg2* gene and this mutation prevents the resulting ARG2 protein from functioning. Without ARG2 protein, the arg2 mutant, like the arg1 mutant can not grow on minimal medium.

A diploid cell made from the arg1 mutant and the arg2 mutant would have one good copy of the *arg1* gene (donated by the arg2 mutant), and one good copy of the *arg2* gene (donated by the arg1 mutant). This diploid would be able to produce functional ARG1 and ARG2 proteins, and because this diploid cell can make all the proteins required for arginine synthesis, it can grow on minimal medium.

Remember that, without information, we randomly assigned names to these mutants. What if by chance, both the arg1 mutant and the arg2 mutant had mutations in the same gene? The resulting diploid cell would not have a functional ARG1 protein, and would not grow on minimal medium. In this case, the arg1 mutant and the arg2 mutant fail to complement.

Data from a complementation test might look like this where the intersection of a column and a row shows the phenotype of the resulting diploid.

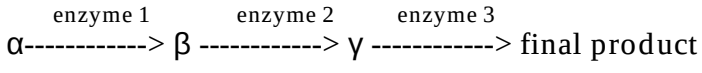
Mutant	arg1	arg2	arg3	arg4	arg5	wildtype
arg1	fails	fails	complements	fails	complements	complements
arg2		fails	complements	fails	complements	complements
arg3			fails	complements	fails	complements
arg4				fails	complements	complements
arg5					fails	complements
wildtype						complements

These data suggest that the five mutants have identified two genes. The arg1, arg2, and arg4 mutants each have a mutation in the same gene. The arg3 and arg5 mutants each have a mutation in the same gene, but a different gene than the one damaged in the arg1, arg2, and arg4 mutants.

Epistasis Test

Once you know how many different genes are represented by your mutants, you can perform an epistasis test. An epistasis test can determine if these genes encode proteins that function in a particular pathway, and determine the order in which these proteins act.

Given:



You could assume that an organism lacking only enzyme 1 would not form β and could not therefore make the final product. However, if this organism had an exogenous supply of β, enzyme 2 could convert β into γ and enzyme 3 could convert γ into the final product. Alternatively, an organism lacking both enzyme 1 and enzyme 2 would not form β, and even if this organism had an exogenous supply of β it could not make γ. Indeed, the organism lacking both enzyme 1 and enzyme 2 would need an exogenous supply of γ to complete the pathway and make the final product.

Given the pathway above, you might also find that an organism lacking enzyme 1 would accumulate α, where an organism lacking enzyme 2 would accumulate β. What would you predict for an organism lacking both enzyme 1 and enzyme 2? You would expect α to accumulate.

In effect, epistasis tests use just this sort of logic and a collection of mutants to define and order biochemical pathways. Let's look at our example of arginine synthesis in a yeast model system. Assume that the mutants unable to synthesize arginine were and categorized into four complementation groups. We assume that each of these groups represents a different gene, and therefore at least four different enzymes are required for the arginine synthesis pathway.

To continue, we need to begin to distinguish between the different mutants. Often intermediates in a biosynthetic pathway are known and mutants can be differentiated by examining their growth on media supplemented with various intermediate compounds. Each mutant will grow on a defined set of media that is different from the media that supports growth of a different mutant. In our example, N-acetylornithine, ornithine, citrulline, and arginosuccinate are intermediates in the arginine pathway.

Our mutants were each tested for the ability to grow and on the various media shown below.

		Compounds added as supplement				
Mutant	Gene affected	N-acetylornithine	ornithine	citrulline	arginosuccinate	arginine
Mutant 1	arg1	No growth	grows	grows	grows	grows
Mutant 2	arg2	No growth	No growth	grows	grows	grows
Mutant 3	arg3	No growth	No growth	No growth	grows	grows
Mutant 4	arg4	No growth	No growth	No growth	grows	grows
wildtype	none	grows	grows	grows	grows	grows

The growth characteristics of Mutant 1 predict that the *arg1* gene encodes an enzyme, ARG1, necessary to convert N-acetylornithine into one of the other intermediates and this enzyme functions early in the pathway. The growth characteristics of Mutant 2 allow us to say that the *arg2* gene encodes an enzyme necessary to convert ornithine into one of the other intermediates and this enzyme functions next in the pathway. Working through the data in the chart allows us to propose the following pathway for arginine synthesis.

We would further test this proposed pathway by creating haploid organisms that carry two deficiencies (double mutants) and determining the phenotype of these double mutants. If we produced a haploid mutant lacking both the *arg1* and the *arg2* genes, we would predict that this double mutant would have the same growth pattern as seen above with Mutant 2.

The double mutant should only grow on minimal media supplemented with citrulline, arginosuccinate, or arginine. These data would place the function the protein encoded by *arg1* before the function of the protein encoded by *arg2*.

Alternatively, we could study the pathway by determining what intermediate accumulates in the mutant cells. Mutant 1, which lacks only *arg1* would accumulate N-acetylornithine. The double mutant lacking both the *arg1* and the *arg2* genes would also accumulate N-acetylornithine. This data would also place the function the protein encoded by *arg1* before the function of the protein encoded by *arg2*.

Mutations in genes encoding essential proteins

So far we have discussed mutants that could grow, given the right circumstances. What, however, would happen if the gene that was mutated encoded DNA polymerase? Without DNA polymerase, cells could not replicate their DNA, they could therefore not undergo mitosis and form new daughter cells. This mutation interrupts an *essential* function, and regardless of the type of medium, these mutants could not grow. How then can we use genetics to study an essential process?

The mutations discussed so far have been mutations in a gene that resulted in a lack of the encoded protein or a non-functioning protein. There is a different class of mutations where the change in the DNA still allows production of a functioning protein, but only under certain conditions. Mutants with this type of mutation are called *conditional* mutants, and one kind of conditional mutant is a *temperature-sensitive* (ts) mutant. Temperature-sensitive mutants carry a mutation in a gene that produces a temperature-sensitive protein. At the permissive temperature (low temperature) when the mutant protein is translated, the mutation results in only a minor change in the protein so it folds into the appropriate three-dimensional shape, and is stable and functional. The mutant appears normal at permissive temperature.

At the non-permissive temperature (high temperature), the increased thermal energy reveals the change in the protein caused by the mutation. The mutant protein unfolds, becomes unstable and no longer functions normally. If the protein affected is essential, at non-permissive temperatures the mutant fails to grow. Thus, although the mutation is always present, the mutants appear wild-type at the permissive temperature and mutant at the non-permissive temperature. The existence of this type of mutant has allowed genetic analysis of essential cellular processes such as protein secretion and the regulation of cell division.

Conclusion

We have outlined a genetic approach in a yeast model system to studying many diverse cellular processes, even without prior knowledge of the genes or the proteins involved. As researchers, we might study yeast cells for their applications in the brewing and baking industries, or we might study yeast because some species are important human and crop pathogens. More likely, we study yeast to understanding cellular processes that are important in all cells, from yeast to man. A good example of the relevance of a yeast genetic study is how identification key molecules in the control of cell division in yeast have lead to important discoveries in the field of human cancer biology. So the next time you read an editorial demanding that taxpayer money is not spent on research of newt taste buds (just as an example), wait before you take up the cause. Take a broader view of the possible benefits and ramifications that such a study may have.