

From mutation to gene - 1. cloning by complementation and marker rescue

Paper to read for this section :

Kranz J.E. and Holm C. (1990) Cloning by function: an alternative approach for identifying yeast homologs of genes from other organisms. *Proc. Natl. Acad. Sci. USA.* **87**:6629-33.

The first part of the course has focused on how to isolate strains carrying mutations in genes that encode products involved in some biological process. From complementation studies, we can determine whether two mutations affect the same gene or different genes, and from the statistics of how mutations are recovered from a mutant hunt, we can estimate the number of genes involved.

The next section of the course continues our description of the classical approach to molecular genetics; the next step is to see how having a mutation allows us to isolate the DNA that comprises the gene; i.e. cloning the affected gene. We'll explore two classes of approaches to cloning. In this section, we'll go over methods that require the ability to reintroduce foreign DNA into the experimental organism. In the next chapter, we'll describe methods that work in organisms where transformation is not practical on a large scale.

Getting DNA into cells

Methods for putting cloned DNA molecules into organisms are available in a wide variety of systems. The most straightforward methods involve mixing purified DNA with cells that have been treated in some way to encourage them to take up the DNA. This process is called **transformation** in microorganisms and in plants; in animal systems, especially in mammalian systems, the same process is called **transfection**, to distinguish it from oncogenic transformation, the changes in a cell that make it have tumor-like properties.

Cells that can take up DNA are called competent cells. Many bacterial species, including *Streptococci*, *Bacilli* and *Haemophilus* are naturally competent during part of their life cycles. In other species, such as *E. coli*, washing the cells in different salt solutions (e.g. CaCl₂) makes the cells competent. As mentioned in Szybalski's article, conditions that precipitate DNA onto cultured

mammalian cells are used for transfection. Transformation of many plants can be performed by injecting DNA into their ovaries so that developing embryos are exposed to the DNA. Injection is also used to introduce DNA into *Drosophila* embryos and nematodes. Injection methods have a throughput problem in that they have to be done one at a time. An alternative that works in some situations is to use the DNA to coat particles that can be shot into a tissue with a pneumatic gun. Since many of these biolistic particles can be loaded into a kind of molecular biology shotgun shell, this allows DNA to be blasted into many cells in parallel. Another common and violent method to introduce DNA into cells is by electroporation. Electroporation works by causing holes to form transiently in membranes when cells are given a very short (~5 msec) high voltage electric shock. DNA that is in the external solution equilibrates between the inside and the outside of cells while the holes are open; proteins can be introduced in the same way, and it can be shown that cellular contents also leak out during electroporation. In fact, if a plasmid-containing strain of *E. coli* is mixed in an electroporation experiment with a different strain that lacks the plasmid, the electric shock allows the plasmid to leak out of the first strain and into the second.

Genes can also be transferred into cells into different kinds of cells by taking advantage of some of the ways that nature moves genetic material from cell to cell. Viruses have evolved to efficiently move their own genomes into susceptible hosts. Cloning vectors based on phages such as M13, P1 and allow efficient transfer of cloned genes between *E. coli* strains. DNA ligated into cosmid or phage vectors based on can be packaged into phage particles *in vitro*; in the early days of DNA cloning, the efficiency with which packaged DNA could be introduced into *E. coli* was many orders of magnitude higher than could be achieved by transformation. Cloning vectors based on adenoviruses or retroviruses can be used to introduce cloned DNA into animal cells. These kinds of vectors are being used in trial experiments in gene therapy.

Plasmids have also evolved mechanisms to move from cell to cell. DNA cloned into vectors based on broad host range plasmids can be used to move cloned genes among a wide variety of bacterial species. Some bacterial plasmids have evolved to transfer DNA from bacterial hosts to eukaryotes. The TI plasmid of *Agrobacterium tumorfaciens* can mobilize a segment called the T-DNA for transfer

into plants. In nature, the T-DNA encodes genes that cause tumors called crown galls to form in infected plants. Plasmid vectors based on the TI plasmid are widely used in plant molecular biology. Transfer of a cloned DNA into *Arabidopsis* can be done by inverting a potted plant into a suspension of *Agrobacterium* containing a plasmid with the desired DNA. [XX Talk to arabadopsis students about what happens after conjugation]. It turns out that *Agrobacterium* is also able to transfer DNA into a variety of nonplant cells as well.

How can you tell that DNA has been taken up by a cell? Observing transformation or transfection depends on being able to assay for genetic markers on the input DNA. How this is done depends on whether or not you want to be able to propagate the transformed or transfected cells. In studies using mammalian tissue culture cells, people often just look for expression of a transfected gene somewhere in a culture dish. These kinds of **transient transfection** assays are often used to determine whether or not a particular DNA clone carries promoter elements needed to drive expression of a reporter gene. As long as some subset of the transfected cells express the reporter, the experimenter will observe it.

Isolating a cell line or strain carrying a genetic marker introduced by transformation or transfection can be thought of as being analogous to isolating a mutation. In a sense, transformation is a form of mutagenesis; the genotype of the transformed cells is altered. However, unlike most other forms of mutagenesis, transformation can introduce new genes to the cell.

As in a mutant hunt, finding a transformed cell often involves looking for a relatively rare event. Thus, the availability of selectable markers makes transformants easier to find. In the first observation of transformation, described by Griffith in 1928, pneumococci (probably some species of pathogenic *Streptococcus*) of one serotype were converted to another serotype by treating the former with killed cells of the latter. The rare transformants could be observed because the large majority of untransformed cells could be killed with antisera against the parental serotype. These experiments led to the demonstration by Avery, McCarty and McCleod in 1944 that the “transforming substance” was DNA. As we discussed in Chapter 4, transformants produced by mixing *E. coli* with plasmid DNA are usually isolated by selection for a plasmid-encoded antibiotic resistance.

DNA introduced by transformation can have several fates. Stable maintenance requires that the

transforming DNA be able to replicate; if it can't, then it will be diluted out when the transformed cell replicates its own DNA and divides. In a transfection experiment, where DNA cloned in a bacterial plasmid is precipitated onto mammalian tissue culture cells, most of the input DNA is lost by dilution or degradation.

If the transforming DNA contains an origin of replication that can function in the recipient cell type, it can be propagated through many cell generations. Since most DNA cloning involves passing DNA clones through *E. coli*, cloning vectors usually contain origins of replication derived from bacterial plasmids or phages. A plasmid that contains an origin that functions in yeast and an origin that functions in *E. coli* is called a yeast **shuttle vector**. It can replicate in either type of cell, using one origin or another.

Depending on the kind of origin used, plasmids in yeast and in *E. coli* can be maintained at different levels, or **copy number**. At one extreme, typical pUC plasmids that are widely used in *E. coli* have copy numbers in the order of 100. The origins used in bacterial artificial chromosomes (BACs) typically have copy numbers around 1 to 4. Similarly, yeast plasmids based on the origin of replication of an endogenous yeast plasmid called the 2 micron circle (2 μ) are high copy plasmids in yeast. Low copy plasmids in yeast contain origins derived from chromosomal DNA. Such an origin is called an ARS, for Autonomously Replicating Sequence. Note that a shuttle vector can be a high copy plasmid in *E. coli* and a low copy plasmid in yeast, or vice versa.

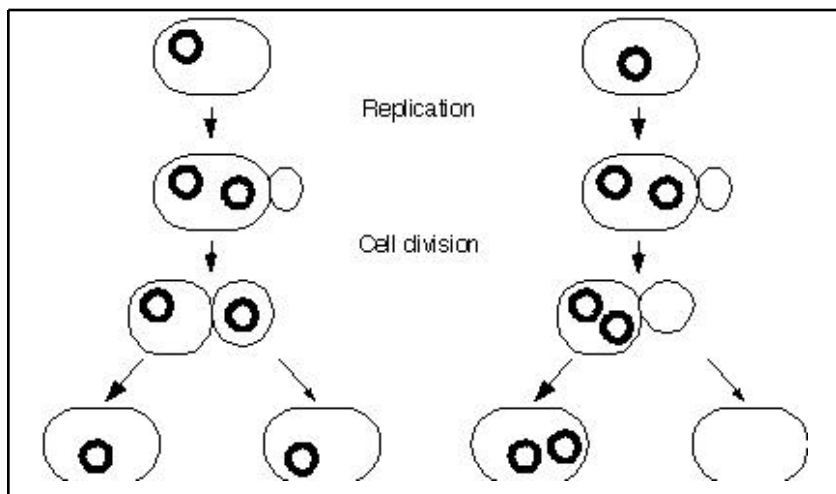


Figure 5-1. Plasmid loss due to random segregation.

Low copy plasmids have to deal with the problem of partitioning correctly during cell division. In the absence of sequences needed for active partitioning, low copy plasmids are easily lost (Figure 5-1). If the plasmids are distributed randomly in the cell when it divides, sometimes all of the copies will be on one side or the other of the division plane. The probability of all of the plasmids being on the same side of the cell decreases as the copy number increases; high copy plasmids are maintained without any active partitioning mechanism.

ARS plasmids are low copy plasmids. CEN plasmids, such as the ones used by Kranz and Holm in the reading assignment, contain sequences for yeast centromeres in addition to an ARS. This causes the plasmids to segregate in mitosis through the same active mechanisms used to separate the normal chromosomes; each of the daughter plasmids is pulled to the opposite pole of a dividing cell by microtubules in the mitotic spindle.

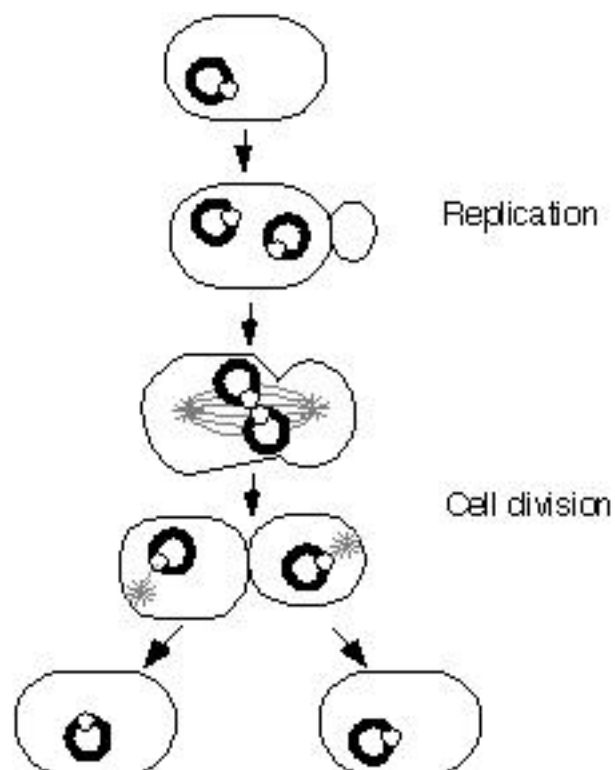


Figure 5-2 Segregation of CEN plasmids

Cloning by complementation

In a complementation test, complementation occurs when the genome provided by one parent supplies the gene product missing in the other. Note that complementation only requires an extra copy of the mutated gene. The rest of the genome is basically irrelevant. Thus, complementation can also occur when a wild-type copy of a mutated gene is supplied by a cloned DNA fragment. In a system where many transformants can be examined, such as *E.coli* or yeast, complementation can be used to find the specific plasmid that carries DNA corresponding to a particular gene among millions of cloned DNA fragments in a library.

For example, we could isolate the *URA3* gene by transforming a *ura3* strain with a yeast library and plating the resultant transformants on minimal media lacking uracil. The majority of plasmids, which contain other yeast chromosomal fragments will not be able to complement the deficiency in orotidylate decarboxylase. A clone containing the *URA3* gene would supply the missing enzyme and would allow the transformant to grow in the absence of uracil.

Cloning related genes from different species

Cloning by complementation is so simple and routine in *E. coli* and yeast that it was not worth assigning a paper just to illustrate cloning a yeast gene by complementation of a yeast mutant. The paper by Kranz and Holm illustrates an important extension of cloning by complementation - the ability to use complementation to isolate genes that encode products with the same functions from different organisms.

The use of DNA from different species to complement mutations was first shown by Struhl, Cameron and Davis in 1976. They used a library of yeast DNA cloned into a phage vector to lysogenize an *E. coli* strain that was a histidine auxotroph due to a mutation in the *hisB* gene, which encodes imidazole glycerol phosphate dehydratase. A clone carrying the *HIS3* gene, which encodes the yeast version of the same enzyme allowed *hisB* lysogens to grow on media lacking histidine.

Cloning *HIS3* relied on the conservation of metabolic pathways across distantly related species. The *E. coli hisB* gene and the yeast *HIS3* gene are **homologous**, which means that they are related

by descent from a common ancestral gene. Homologs that have the same physiological function, such as *hisB* and *HIS3*, are called **orthologs**. Note that not all proteins with the same function are orthologs; in some cases the same function evolved more than once. In such cases of **convergent** evolution, the resultant proteins are not related to one another by descent from a common ancestral gene. Note also, that the descendants of the same ancestral gene can acquire different biochemical and physiological functions; genes that have this kind of relationship are called **paralogs**.

As a model eukaryote, yeast is often useful for isolating orthologous genes from other organisms. Since the complete sequence of the yeast genome was released in 1996, sequence comparisons have estimated that more than 30% of all yeast open reading frames have clear homologs among mammalian proteins (Botstein, Chervitz and Cherry (1997) *Science* **277**:1259). Orthologous gene products will often complement mutations in yeast. For example, the mammalian homologs of various cell cycle components have been cloned in yeast by complementation of ts mutations in *CDC* genes, a class of genes involved in the cell cycle in yeast.

Complementation shows that two gene products can substitute for one another, and suggests closely related biochemical functions. However, the physiological functions of complementing genes may be quite different. For example, some subunits of the mammalian CCAAT factor were cloned by complementation of mutations in the yeast genes. Although the yeast and mammalian CCAAT transcription factors both bind the same DNA sequence element, the CCAAT factor regulates very different target genes in yeast and in mammals.

Conditional growth

The paper by Kranz and Holm illustrates one general strategy for cloning by complementation in yeast and illustrates many of the methods used in yeast molecular biology. The paper may be somewhat confusing because they are actually trying to demonstrate the feasibility of a strategy that is more subtle than what we are trying to cover here. I will first describe how their methods could have been used to clone the genes encoding topoisomerase II from yeast or *Drosophila*. In fact, these clones had already been isolated by other methods. At the end of the section I will try to explain the general strategy they were trying to illustrate in the paper.

An important aspect of cloning by complementation is that you have to be able to transform the library into a cell that is alive. In other words, the mutant cells that you use have to be able to live under some condition where the gene product is nonessential, but there has to be another condition where the mutation prevents growth of the cells so you can select for complementation. In many cases this can be accomplished by using different media or growth temperatures. Temperature-sensitive mutations are especially useful for the study of genes whose products are essential for growth in all kinds of media, such as components of replication complexes, general transcription factors or cell cycle regulators.

Sometimes temperature-sensitive mutations are not available in a particular function, or the orthologous gene product you are trying to find is from an organism that doesn't grow at the nonpermissive temperature for the ts mutations you have. In such cases, conditional growth can be achieved by two kinds of strategies: 1) conditional gene expression and 2) "plasmid shuffle" methods where a wild-type copy of the gene of interest is maintained in the strain before transformation with the library.

Conditional gene expression in yeast can be set up by placing the gene of interest under the control of a regulatable promoter, such as the GAL1 promoter, which is induced in the presence of galactose and repressed in cells grown on glucose. Similarly, many well-studied regulated promoters have been used to drive conditional gene expression in *E. coli*, including the *lac* promoter, the *araBAD* promoter and various phage promoters.

Although regulated promoters can often be used for cloning by complementation, they are not always appropriate. In particular, problems can occur if the regulated promoter does not behave like the gene of interest's normal promoter. The induced level could be too low or so high as to give toxic levels of the gene product, the basal level could be too high to give a mutant phenotype, or the normal function of the gene might require cyclic expression, as is the case for cell cycle regulators like cyclins.

The plasmid shuffle approach eliminates these problems by providing a wild-type copy of the gene with its normal regulatory sequences on a plasmid. Once the library has been introduced on a second

plasmid, the cells are allowed to lose the first plasmid spontaneously. The loss of the original complementing plasmid can be either detected by screening or selected by using counterselectable markers such as *URA3*.

Colony sectoring assay

The first important technical point from the paper is the assay they used to determine whether or not they had cells containing a plasmid that complements a conditional mutation in essential gene. They started with a ts mutation, *top2-4*, in the gene that encodes topoisomerase II, and introduced a plasmid that contained the wild-type *TOP2* gene. Note that starting with *top2-4*, it would be trivial to clone either the *Drosophila* or yeast *TOP2* genes; all we'd have to do is select for transformant that complemented the ts growth defect. While noting this, please bear with me while I explain what they did.

Kranz and Holm started with a yeast strain that contained the following mutations: *ade2 ade3 leu2 ura3*. The *ADE2* and *ADE3* genes encode products involved in the biosynthesis of adenine (Figure 5-3). *ADE2* encodes 5-aminoimidazole ribotide carboxylase, which catalyzes one of the steps involved in building the purine ring onto a ribose monophosphate. Mutations in *ADE2* block the *de novo* synthesis of purines, and accumulate intermediates in the pathway. It is not clear what compounds actually accumulate, but *ade2* and *ade1* mutations block the *de novo* purine biosynthetic pathway in such a way that the accumulated intermediates do something to turn the yeast cells bright red (Wild-type yeast are white).

ADE3 encodes C1-5,6,7,8-tetrahydrofolate synthase (C1 THF synthase). This enzyme is involved in regenerating the N¹⁰-Formyl THF from THF and formate; the N¹⁰-Formyl THF is consumed by two steps in purine synthesis. One of the steps that uses N¹⁰-Formyl THF, the addition on a formyl group to glycinamide ribotide (GAR), is earlier in the pathway than the step blocked in *ade2* mutants. Thus, *ade3* mutations prevent precursors from ever making it to the step blocked by *ade2*. An *ade2, ade3* double mutant will be white because the substance that turns red can't be made due to the loss of the *ADE3* product.

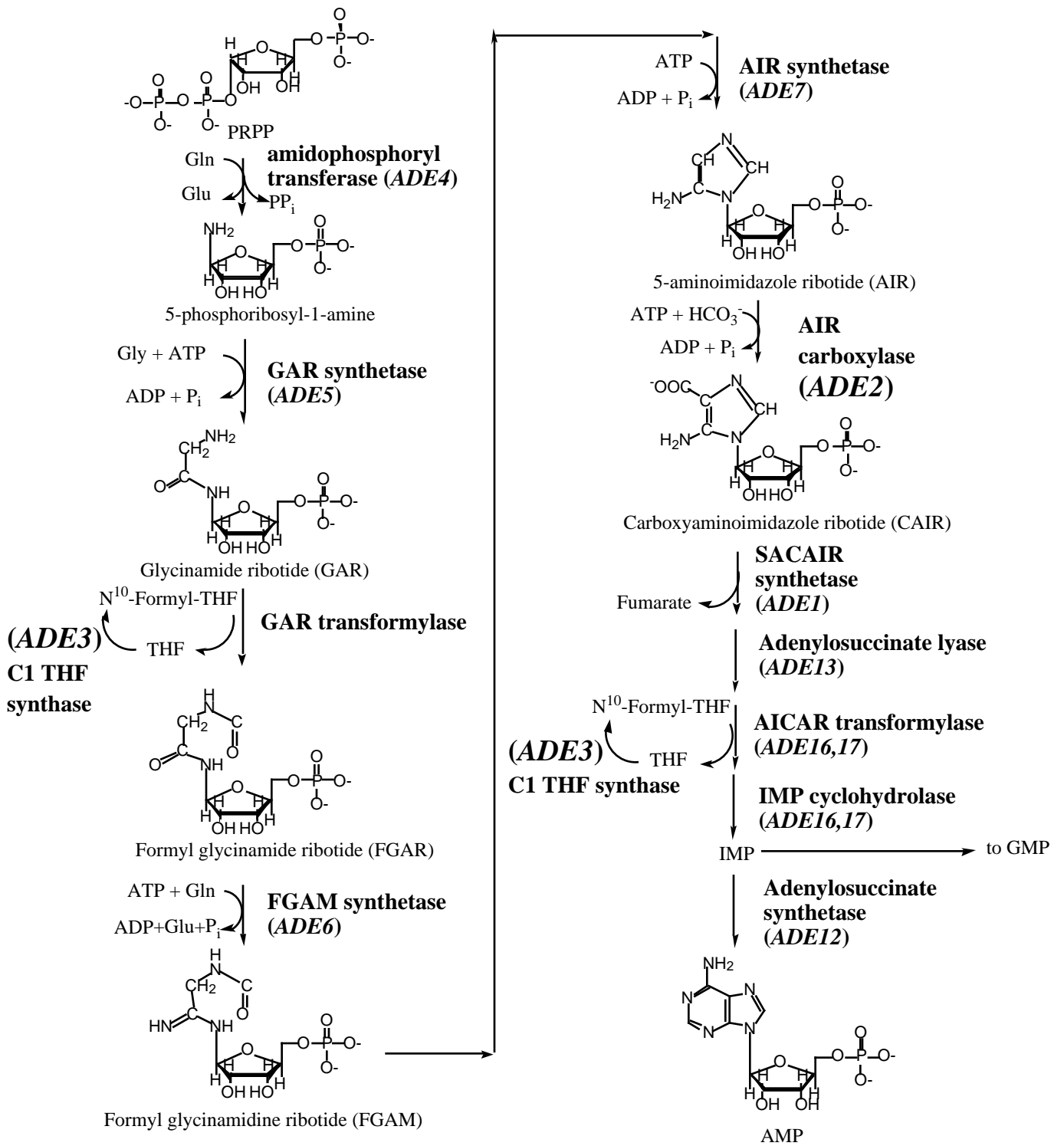


Figure 5-3 *de novo* purine synthesis and the roles of ADE gene products

Consider what happens if you transform a white *ade2 ade3 leu2 ura3* strain with a CEN plasmid carrying *ADE3* and *URA3*. The *URA3* gene on the plasmid allowed them to select for transformants; in all of the transformants, the plasmid-encoded *ADE3* gene will complement the chromosomal *ade3* mutation. However, the *ade2* mutation will still block purine synthesis, and the cells will be red. Note that you have to grow these cells on media containing adenine and leucine.

What happens if you release the selection for *URA3* by growing the transformants on complete medium (or any medium that contains uracil in addition to the other required nutrients)? It turns out that although the introduction of CEN sequences allows CEN plasmids to segregate into both the mother and daughter cells after mitosis, about 1% of the cells in a population of cells derived from a cell transformed with a CEN plasmid will lose the plasmid. This could be due to defects in segregation of replication products, or the occasional failure of an ARS to fire during an S phase. The actual mechanism of CEN plasmid instability doesn't concern us here.

Imagine taking transformants and replating them on rich media (Figure 5-4). Colonies will grow where single cells land on the plate. As the cells bud off daughters, the colony will grow outward. Cells in the middle of the plate will be older than cells at the periphery. At each cell division, there will be some probability that a mother or daughter will lose the plasmid. Cells that lose the plasmid will lose the ability to make the *ADE3* product, and will turn white. Since the descendants of these cells will also lack the *ADE3* product, they will grow into a white sector within the colony

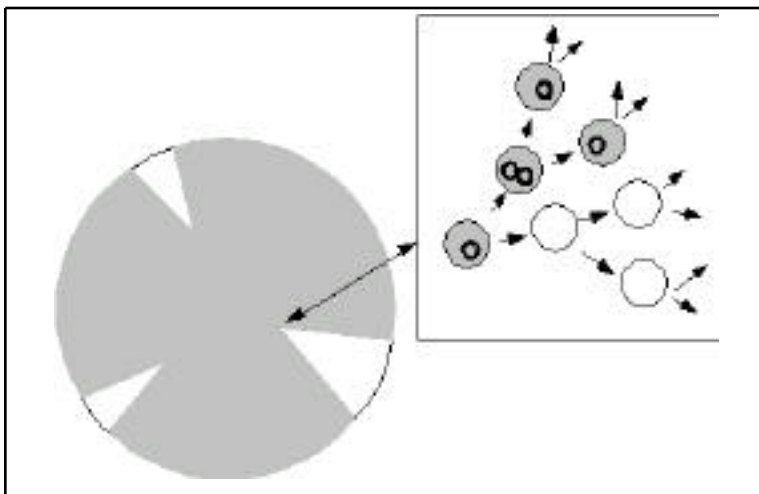


Figure 5-4. Formation of sectored colonies. Plasmid loss occurs as the colony grows outward. The descendants of plasmid-free cells are also plasmid-free and form a sector. Since plasmid loss occurs randomly as the colony grows, sectors will start at different distances from the center of the colony.

Once selection for *URA3* is removed, there is nothing to keep the plasmid from being lost.

Kranz and Holm modified this scheme by placing the temperature-sensitive *top2-4* mutation in the genome of the host, and a *TOP2* gene on the plasmid. At the permissive temperature, the cells behave exactly as described above, since the *TOP2* alleles in the chromosome and on the plasmid are both functional. At the restrictive temperature, however, we see what happens when the chromosomal locus is inactivated. Cells that lose the CEN plasmid also lose the only functional copy of *TOP2* in the cell and are unable to grow into a white colony. In the absence of a functional copy of *TOP2* in the chromosome, the cells have become plasmid-dependent. Since all of the cells in the colony retain the plasmid, all of them will be *ade2* and have a plasmid-born copy of *ADE3*. The colonies formed are solid red. What we have covered so far in this section takes us up through the first page of the results, and Figure 2A of the Kranz and Holm paper. Ignore the rest of the paper for now.

Generalizing, if your favorite gene (*YFG*) is essential for growth, plasmids containing the *ADE3* gene will give rise to sectored colonies in an *ade2 ade3 YFG* background (Figure 5-5). If the chromosome contains a *yfg* mutation and a plasmid carries the *YFG* gene, sectors will not form because cells that lose the plasmid will die (Figure 5-6).

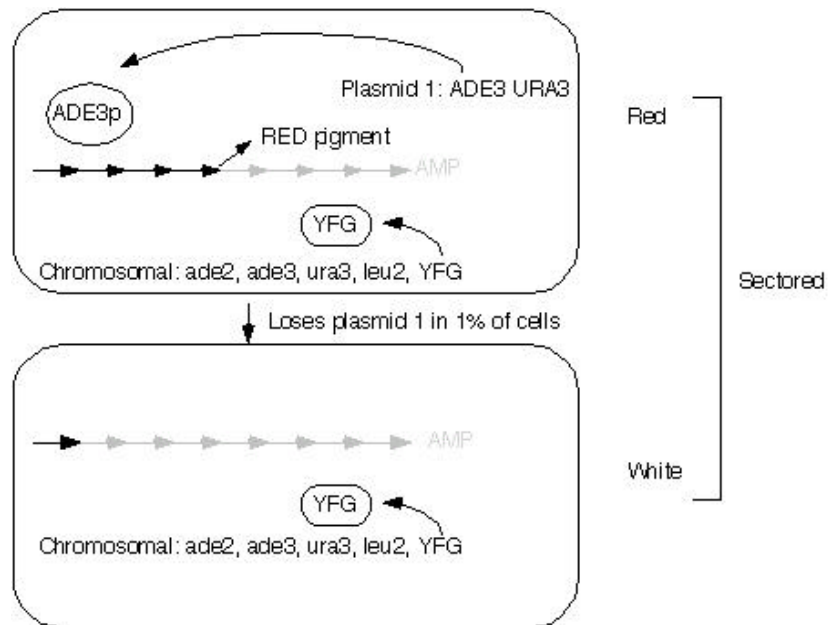


Figure 5-5. Plasmid loss leads to sectored colonies as long as no essential functions are lost.

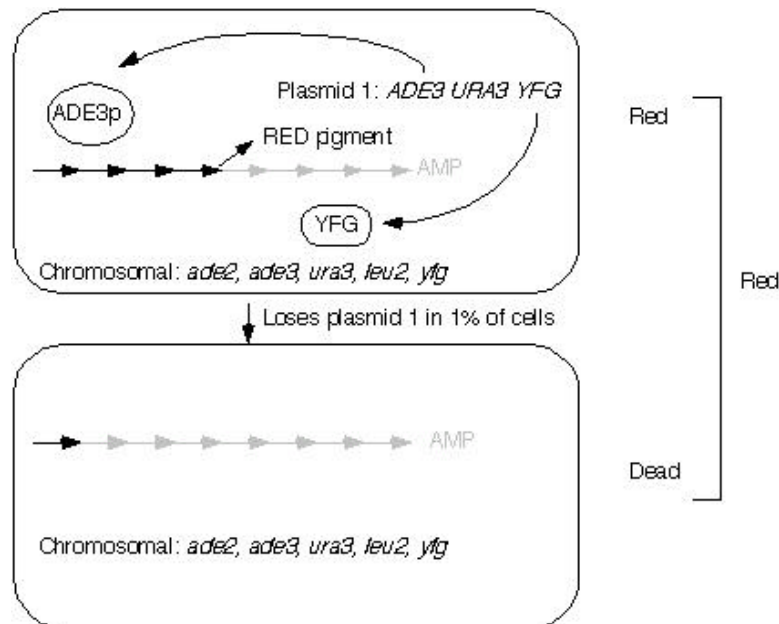


Figure 5-6. Plasmid loss leads to dead cells if an essential functions is lost. Colonies will be red.

Now consider how we can use this system to isolate a clone that expresses *YFG* from a library of genes inserted into a second plasmid, which carries *LEU2* as a selectable marker. Introduction of the *LEU2* vector will not change the plasmid dependence of the cells shown in Figure 5-6. Thus, the majority of the transformants from the library, which carry genes other than *YFG*, will form solid red colonies. Cells transformed by a *LEU2* plasmid that carries *YFG* will behave as illustrated in Figure 5-7. Since the *YFG* product is now produced from both plasmids, the presence of the *ADE3 URA3 YFG* plasmid is no longer essential for cell survival. If the the *ADE3 URA3 YFG* plasmid is lost, the cell can still survive using the *YFG* product from the *YFG LEU2* plasmid. The cells lacking the *ADE3* gene can now grow into a white sector.

Note that this will work even if the *YFG* gene on one or more of the plasmids is from a different species. Thus, Kranz and Holm were able to find a clone containing the yeast *TOP2* gene in a *LEU2* CEN plasmid library used to transform an *ade2 ade3 ura3 leu2 top2* strain that contained a *Drosophila TOP2* homolog on a *URA3* CEN plasmid.

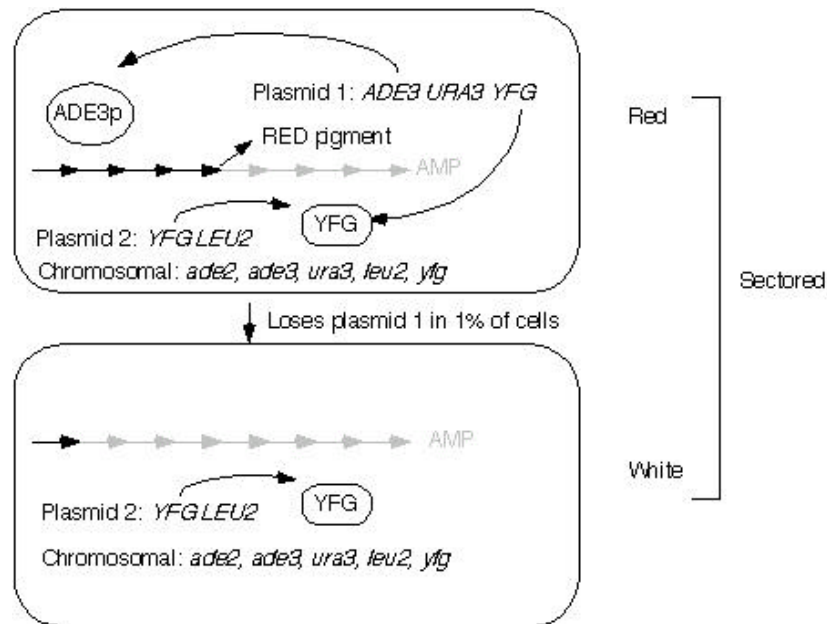


Figure 5-7. Plasmid loss leads to sectored colonies because a second copy of the essential function is expressed from a second plasmid.

Thus, we see that this method allows us to clone a gene by complementation of a mutant yeast gene. However, recall that it would have been much simpler and easier to clone the yeast or *Drosophila TOP2* gene by plating the transformants containing the library plasmids at the restrictive temperature. A functional *TOP2* gene from either organism would complement the defect in topoisomerase activity and allow growth at the restrictive temperature. Why did Kranz and Holm go through such a complex series of manipulations?

Kranz and Holm are using this study to demonstrate the feasibility of a strategy that allows one to assign functions to genes cloned from organisms that are more difficult to manipulate genetically than yeast. Suppose that *hYFG* is cloned gene from human cells where you don't know the function. You may have cloned it based on a purified biochemical activity; lets assume that you have reason to believe that the function of the protein is both essential and conserved in yeast. Starting with the cloned *hYFG* gene, you can construct an *ADE3 URA3 CEN* plasmid that expresses *hYFG* protein in yeast, and use this plasmid to transform *ade2 ade3 ura3 leu2* yeast. The resultant colonies will be sectored, because the plasmid does not contain anything that is essential for the yeast to grow.

In order to clone a yeast homolog for *YFG*, you need to alter the strain so that growth will be

dependent on a cloned copy of *YFG*. Kranz and Holm show that this can be easily accomplished by mutagenizing the plasmid-containing cells and screening for strains where the chromosomal copy of *YFG* is inactivated. Kranz and Holm mutagenized a strain with a *ADE 3 LEU2 TOP2* plasmid in a chromosomal *ade2 ade3 ura3 leu2 TOP2* background and screened for colonies that didn't have white sectors.

As in most selections or screens, more than one kind of genetic event can lead to solid red colonies. In addition to *top2* mutations, Kranz and Holm observed two classes of unwanted mutations. The first class of unwanted mutations were gene conversions of the chromosomal *ade3* to *ADE3*. This happens due to recombination between the chromosomal *ade3* allele and the plasmid-encoded *ADE3* gene. The colonies are solid red because it is no longer possible to lose the *ADE3* gene.

The second time they did the experiment they changed the selectable marker in the *ADE3* plasmid from *LEU2* to *URA3* (which is consistent with how I've explained the system above). Cells from the red colonies that are found after mutagenesis can be plated on 5-FOA + uracil. If the cells have mutated so that the plasmids contain an essential function, the cells will not be able to grow on 5-FOA + uracil. About 1% of the cells from the *ADE3* gene convertants will survive, because losing the plasmid will have no additional deleterious effects.

The second class of unwanted mutants had acquired mutations in other chromosomal genes that made the cells plasmid dependent. For example, a mutation that blocked the transport of leucine would have been unable to grow using exogenous leucine in the medium. In the paper, Kranz and Holm were able to show that these were not *top2* mutations because they complemented the known *top2* allele. In the case of *YFG*, this would not be possible, since there would be no reference *yfg* allele. This second class of unwanted mutations can still be distinguished from *yfg* mutants by transforming candidates with a second plasmid that contains *YFG* but shares no other markers with the first plasmid. Sectoring will be restored if plasmid-dependence is due to a *yfg* mutation; if some other plasmid gene is responsible for the plasmid-dependence that causes solid red colonies, the second

plasmid will not supply that function. Sectors will not be observed.

Kranz and Holm found that most of the red colonies were due to mutations in *TOP2*. Doing the same thing with *YFG*, one would expect *yfg* mutations, as long as there really is a functional, essential yeast homolog to h*YFG*. Note that the mutations one would expect in *yYFG*, like the new mutations in *TOP2* isolated by Kranz and Holm, can be any kind of recessive lethal alleles.

In our hypothetical example, we now have a strain with a plasmid expressing h*YFG* in a strain with a chromosomal mutation in *yYFG*. We can now use the sectoring screen to isolate a clone of *YFG* from a yeast library, or even from a library from a third species. We can use this method to isolate yeast homologs of human genes that are functionally related even if their evolutionary relationship is not detectable by sequence comparison. If it turns out that the yeast gene is one that has been previously identified on the basis of a detectable phenotype, we will get an idea of the normal cellular function of h*YFG*. In addition, the method allows us to isolate functional homologs from a wide variety of species. Comparing the sequences of the related genes may provide clues about structure or function of the gene product.

Note that the plasmid shuffle described by Kranz and Holm involves replacing one plasmid with another. Although they used sectoring to detect the presence of the second plasmid, it would also be possible to select for those plasmids that complement the mutation by selecting against the *URA3* gene on the first plasmid.

Marker rescue

Most of this chapter has focused on cloning by complementation. Complementation by a cloned DNA requires expression of enough of the protein to provide the biochemical function that is missing in a particular mutant strain. It is also possible to use mutations to clone genes without having cloned DNA fragments long enough to give a functional gene. Elimination of a mutant phenotype can also occur if recombination regenerates a wild type allele. This phenomenon is called **marker rescue**. One example of how marker rescue can be used in yeast is shown in Figure 5-8. In this experiment, DNA fragments are cloned into an *E. coli* plasmid that is not a yeast shuttle vector. However, this plasmid contains a genetic marker that is selectable in the appropriate strains of yeast.

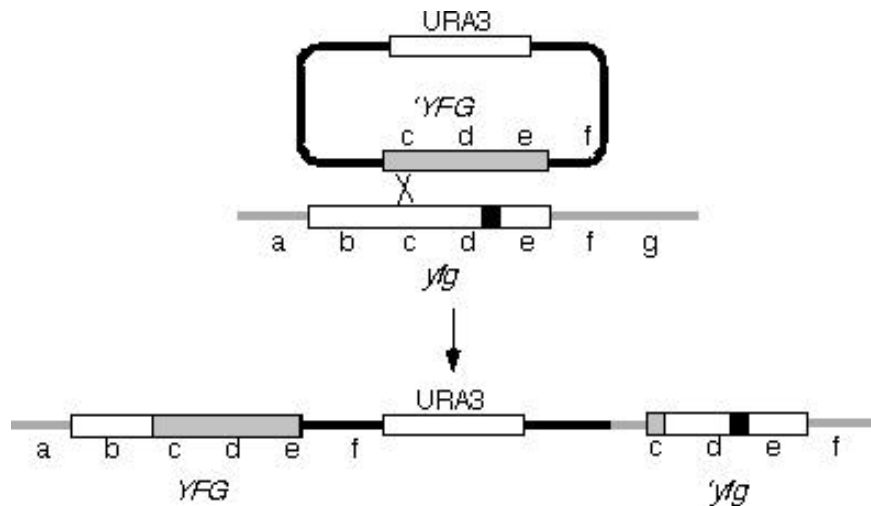


Figure 5-8. Marker rescue by recombination between a circular DNA containing one end of a gene and a chromosome containing a mutant allele. The black mark indicates the position of a mutation in *YFG*.

When the circular plasmid is transformed into a *ura3* strain that also carries a mutation in *yfg*, the plasmid cannot replicate, since it lacks an origin of replication that would function in yeast. Plating transformants on media lacking uracil will select for events that allow the plasmid-encoded *URA3* gene to be passively replicated; one way this can occur is by integration of the plasmid into the yeast genome. The *YFG* gene provides an area of homologous DNA where recombination can occur between the plasmid and the chromosomal locus. A single crossover will generate a duplication of the cloned insert and one of the copies will be fused to chromosomal sequences such that a full length copy of the gene is regenerated (as long as the insert spans one end or the other of the gene). If the crossover occurs to the right of the mutation, then the full length copy of *YFG* will still carry the mutation. However, a crossover to the left of the mutation will generate a wild-type copy of the gene, and give rise to the wild-type phenotype.

Marker rescue and complementation both rely on the ability of a cloned DNA fragment to change the phenotype of cells carrying a mutation in a gene of interest. The difference is the difference between complementation and recombination. Complementation will occur in every cell that gets a wild-type copy of the gene. Marker rescue only happens in a subset of the cells where recombination occurs.