Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 6629–6633, September 1990
Cell Biology

Cloning by function: An alternative approach for identifying yeast homologs of genes from other organisms

(heterologous system/*Saccharomyces cerevisiae*/inducible galactose promoter/plasmid dependence/*Drosophila* DNA topoisomerase II)

JANICE E. KRANZ AND CONNIE HOLM

Department of Cellular and Developmental Biology, Harvard University, Cambridge, MA 02138

Communicated by Daniel Branton, May 29, 1990 (received for review August 22, 1989)

ABSTRACT **Studies of cell physiology and structure have identified many intriguing proteins that could be analyzed for function by using the power of yeast genetics. Unfortunately, identifying the homologous yeast gene with the two most commonly used approaches—DNA hybridization and antibody cross-reaction—is often difficult. We describe a strategy to identify yeast homologs based on protein function itself. This cloning-by-function strategy involves first identifying a yeast mutant that depends on a plasmid expressing a cloned foreign gene. The corresponding yeast gene is then cloned by complementation of the mutant defect. To detect plasmid dependence, the colony color assay of Koshland *et al.* [Koshland, D., Kent, J. C. & Hartwell, L. H. (1985) *Cell* 40, 393–403] is used. In this paper, we test the feasibility of this approach using the well-characterized system of DNA topoisomerase II in yeast. We show that (*i*) plasmid dependence is easily recognized; (*ii*) the screen efficiently isolates mutations in the desired gene; and (*iii*) the wild-type yeast homolog of the gene can be cloned by screening for reversal of the plasmid-dependent phenotype. We conclude that cloning by function can be used to isolate the yeast homologs of essential genes identified in other organisms.**

Many genes have been isolated that encode products essential for eukaryotic cells. The difficulty of genetic analysis in higher eukaryotes makes it attractive to study such genes by using the extraordinary power of yeast genetics. Unfortunately, because of the evolutionary distance between yeast and other experimental organisms, it is often difficult to identify the yeast homolog of a protein from another species by using the standard approaches of DNA hybridization and cross-species antibody reaction.

Recent successes with substituting genes from other organisms for yeast genes suggest that an alternative approach will be very productive. Many examples are now available of foreign cDNAs encoding products that substitute fully for yeast proteins inactivated by mutation. For example, the human homolog of a *Schizosaccharomyces pombe* mitotic control gene, *cdc2*, was cloned by introducing a human cDNA library into a $cdc2^{ts}$ strain and then screening for growth at the restrictive temperature (1). Additional examples of such conservation of function through evolution include other cell cycle proteins (2), protein transport components (3, 4), cytochrome *c* (5), and transcription factors (6, 7). The properties of the proteins in these examples suggest that not only monomer function, but also protein–protein interactions, have been conserved in evolution. The case of cytochrome *c* is particularly striking, not only because of the complex processing pathway that must be followed to produce a completely functional protein, but also because antisera raised against yeast or mouse cytochrome *c* do not react with the alternative cytochrome *c*; furthermore, DNA probes from yeast or rat cytochrome *c* genes do not cross-hybridize to RNA from the other organism (5).

These results suggest that an alternative approach to cloning would be to identify a yeast homolog on the basis of its sharing the function of the foreign gene. Operationally, this objective could be accomplished by expressing the foreign gene in yeast from a yeast plasmid, mutagenizing the yeast strain, and then screening for a plasmid-dependent yeast mutant. [Screening for plasmid dependence has been quite successful in other contexts in yeast (8–10).] Cloning the yeast gene by using this yeast mutant would then be straightforward with standard techniques (11). We term this approach cloning by function.

In the present study, we show that cloning by function can rapidly and efficiently identify yeast homologs of cloned genes from other species. As a model system, we used the structural gene for DNA topoisomerase II and tested the cloning-by-function scheme by using a red/white colony sectoring assay (12). We found that the efficiency of identifying the desired mutants is high, whereas the rate of recovery of uninteresting mutants is reasonably low. Furthermore, simple tests can identify such background mutants. Finally, the properties of the desired mutants facilitate cloning of the appropriate yeast genes.

MATERIALS AND METHODS

Strains, Media, and Genetic Methods. The following strains were used for the first ("homologous") screen. Strains CH1125 and CH1126 were constructed by transforming strain 4513-216 [MATa *ade2 ade3 his3 leu2 ura3* $TOP2^+$ (Gal^-), provided by D. Koshland, Carnegie Institute, Washington] with an integrating plasmid bearing the *top2-4* mutation, selecting for mitotic recombination to eliminate the plasmid, and isolating isogenic *top2-4* (CH1126) and $TOP2^+$ (CH1125) derivatives. Strains CH1142 (MATα *ade2 ade3 leu2 lys2 top2-4*) and CH1146 (MATα *ade2 ade3 leu2* $TOP2^+$), used for the complementation test, were derived from multiple crosses of strain 4513-216 with inbred laboratory strains, whereas strain CH897 (MATα *leu2 lys2 ura3 gal2 top2-4*), used to make heterozygous diploids for tetrad analysis, was from this laboratory.

Additional yeast strains were used for the second ("heterologous") screen. Strains CH1304 (MATα *ade2 ade3 his3 leu2 ura3 gal2* $TOP2^+$) and CH1305 (MATa *ade2 ade3 leu2 ura3 lys2* $TOP2^+$) were constructed by multiple crosses of strain 4513-216 (see above) with inbred laboratory strains. For complementation tests we used strains CH1304, CH1305, CH1141 (MATa *ade2 ade3 his3 ura3 top2-4*), and CH1143 (MATα *ade2 ade3 his3 leu2 ura3 top2-4*). To make heterozygous diploids for tetrad analysis we used strains CH325 (MATa *his4 lys2 ura3 gal2 top2-4*) and CH345 (MATα *ade2*

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Abbreviations: YEP, yeast extract/peptone; YEPD, YEP/dextrose; YEPGR, YEP/galactose/raffinose; $TOP2^D$, *Drosophila* topoisomerase II cDNA; $TOP2^Y$, yeast topoisomerase II gene.

ura3 gal2 top2-4) (13). Strain CH1441 is a *top2* mutant (*top2-115*) isolated in the screen of mutagenized strain CH1305 containing pCH1123 (see below).

Standard genetic techniques were used (11). Yeast transformations were performed by the lithium acetate procedure (14). Yeast extract/peptone/dextrose (YEPD) and selective media have been described (11). Yeast extract/peptone/galactose/raffinose (YEPGR) was YEP medium containing 2% galactose and 2% raffinose. Low-adenine synthetic complete medium has been described (16).

Plasmids. Plasmid pDK255, a replicating centromere plasmid containing *LEU2* and *ADE3*, was provided by D. Koshland. By using standard recombinant DNA methodology (15, 17), the 6-kilobase (kb) *Bam*HI–*Bgl* II (partial digest) fragment containing the yeast *TOP2* gene (*TOP2*^{Y}) from plasmid pIIY-1 (18) was subcloned into the unique *Bam*HI site of pDK255 to yield "screening" plasmid pCH1034. "Testing" plasmids used were YCp50 (19) and pCH1075, a plasmid carrying the yeast *TOP2*$^+$ gene in the vector YCp50, from the Holm collection.

Additional plasmids were used for the heterologous cloning-by-function screen. Screening plasmid pCH1123 is a replicating centromere plasmid that contains the *Drosophila* Top2 cDNA (TOP2^D) attached to the yeast *GAL1* promoter (p_{GAL1}) and *ADE3*$^+$ on a YCp50 (*URA3*$^+$) backbone. It was constructed by first taking a *Sma* I–*Nhe* I *ADE3*$^+$ fragment from pDK255 and ligating it into plasmid YCp50 cut with *Sma* I and *Spe* I. The resulting plasmid was digested with *Sal* I and *Nru* I, and into it was inserted the p_{GAL1}-TOP2^D fragment produced by digesting pYGBBXΔ22 (20) completely with *Sma* I and partially with *Sal* I. Testing plasmid pCH1113 is a replicating centromere plasmid that contains *LEU2*$^+$ and the TOP2^D attached to the *GAL1* promoter. The latter plasmid was constructed by replacing *ADE3*$^+$ of pDK255 with p_{GAL1}-TOP2^D by inserting the *Sal* I–*Sma* I fragment (p_{GAL1}-TOP2^D) into *Sal* I- and *Sma* I-digested pDK255.

Screen for Nonsectoring Mutants. For the homologous screen, strain CH1125 containing screening plasmid pCH1034 was grown on selective medium lacking leucine to select for the plasmid, and a single colony was used to inoculate liquid YEPD. Cells growing exponentially were sonicated and counted, then washed, and UV-irradiated for 220 sec (≈4% survival). Cells were diluted in water and spread on 41 YEPD plates (150 mm) and then incubated at 30°C for 7 days until color had fully developed.

For the heterologous screen, a similar protocol was used, except that before mutagenesis, strains CH1304 and CH1305 bearing screening plasmid pCH1123 (see above) were grown in YEPGR. After mutagenesis, the strains were plated on low-adenine synthetic complete plates containing 2% galactose and 2% raffinose. Viability was ≈14% for strain CH1304 and 6% for strain CH1305.

RESULTS

A System for Cloning Yeast Genes by Function. To identify mutations in yeast genes corresponding in function to cloned genes from other organisms, we devised a system in which we can identify a yeast mutant dependent on a "screening" plasmid expressing the cloned foreign gene. This system is based on a sensitive color assay that allows plasmid loss to be detected visually in a single colony (12). As described for Fig. 1, a typical wild-type colony is red with many white sectors (Fig. 1*B*) because plasmid loss occurs ≈1% per cell per generation (21–23). In contrast, no white sectors will be seen in a colony composed of cells that require plasmid function for survival because once a given cell loses the screening plasmid it cannot produce a large white clone of daughter cells (Fig. 1*C*). Thus, a wild-type strain containing a plasmid carrying a gene of interest should provide a system

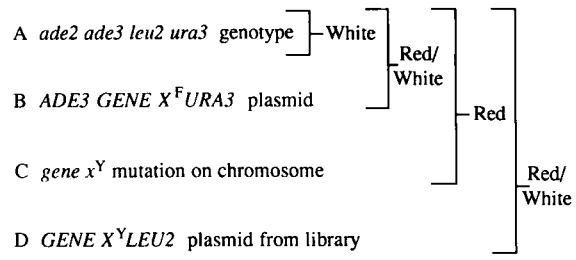

FIG. 1. Genetic scheme for identifying a mutation in the yeast homolog of a foreign gene and cloning the wild-type yeast homolog. The colony color assay of Koshland *et al.* (12) allows plasmid loss to be detected visually. Strains carrying an *ade2* mutation accumulate a red pigment and consequently form red colonies. The mutation *ade3* is epistatic to *ade2*, preventing this red pigment from accumulating; thus, an *ade2 ade3* strain forms white colonies (line A). When a plasmid containing *ADE3*$^+$ is introduced into this *ade2 ade3* strain, the strain becomes *ade2 ADE3*$^+$ and forms mostly red colonies. Frequent plasmid loss events, however, produce many white *ade2 ade3* sectors. Thus, a normal *ade2 ade3* strain transformed with the *ADE3*$^+$ screening plasmid expressing the foreign gene (*GENE X*^{F}) will produce red colonies with white sectors (line B). This transformed strain is mutagenized and screened for nonsectoring solid red colonies, which contain a mutation in the yeast *GENE X*^{F} homolog (*gene X*^{Y}) and thus depend for viability on the plasmid expressing *GENE X*^{F} (line C); because sectors of cells lacking the plasmid will not be formed, the colonies will be solid red. The wild-type yeast *GENE X*^{Y} is cloned by transforming this plasmid-bearing mutant strain with a yeast library and screening for the regained ability to produce white sectors (line D).

for isolating mutants that require expression of this gene for survival. The yeast gene could then be cloned by transforming these mutants with a yeast library and screening for reversal of this nonsectoring phenotype (Fig. 1*D*).

To test the feasibility of this cloning-by-function strategy, we investigated three major questions: (*i*) Can plasmid dependence be recognized by using a well-characterized gene? (*ii*) Can this red/white assay be used to identify chromosomal mutations? (*iii*) Could these new mutants be used to clone the yeast gene? To initially characterize the system, we first addressed these questions by using an entirely homologous system: mutant and wild-type yeast strains bearing a screening plasmid carrying the cloned yeast DNA topoisomerase II gene (*TOP2*^{Y}). We then performed the more stringent test of using a cloned *Drosophila* topoisomerase II gene (TOP2^D) to produce yeast *top2*^{Y} mutants and to clone the *TOP2*^{Y} gene.

Plasmid Dependence Is Easily Recognized. To determine whether we could recognize when a strain is dependent on a *TOP2*^{Y}-bearing screening plasmid, we used a strain carrying the mutation *top2-4*, a temperature-sensitive mutation in the structural gene for DNA topoisomerase II. Because previous work (13, 18) has shown that *TOP2* is an essential gene in yeast, we reasoned that such a strain would depend upon a *TOP2*$^+$ plasmid at restrictive temperature (35°C) but not at permissive temperature (25°C). Fig. 2*A* shows that the nonsectored (plasmid dependent) phenotype of the *top2-4* strain at 35°C is easily distinguished from the sectored (plasmid-independent) phenotype of the controls. Furthermore, mixing these *TOP2*$^+$ and *top2-4* strains and allowing single colonies to grow at 35°C demonstrated that the solid red *top2-4* colonies are easily distinguished (see Fig. 2*B*). These results suggest it should be possible to detect mutation-bearing plasmid-dependent colonies in a population of mutagenized sectored colonies.

New Mutants Can Be Efficiently Isolated by Using an Entirely Homologous System. To determine the efficiency of cloning by function, we first used the cloned *TOP2*^{Y} gene to investigate the types of mutations this screen would yield, the

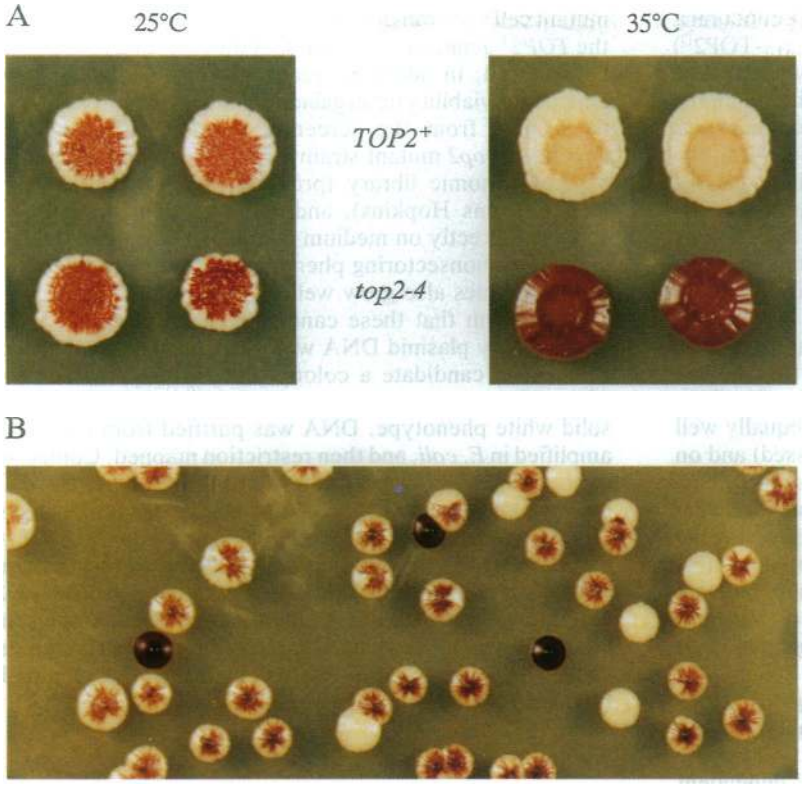

FIG. 2. *TOP2*$^+$ and *top2-4* strains containing the *TOP2*$^+$ *ADE3*$^+$ screening plasmid pCH1034 at permissive and restrictive temperatures. (*A*) Spots of strain CH1125 (*TOP2*$^+$) and CH1126 (*top2-4*) after 7-day growth on YEPD medium at 25°C or 35°C. Note that at 25°C both strains produce white sectors, whereas at 35°C only the *TOP2*$^+$ strain produces white sectors. (*B*) *TOP2*$^+$ cells and *top2-4* cells, taken from selective plates and diluted in water, were mixed at a ratio of 50:1, spread on YEPD medium, and incubated at 35°C for 7 days. Three red *top2-4* colonies are easily visible in this field.

frequency of each type, and, most important, whether we could easily distinguish these types from each other. *TOP2*$^+$ cells containing screening plasmid pCH1034 (*ADE3*$^+$ *LEU2*$^+$ *TOP2*^{Y}) were mutagenized, and the colonies they produced were examined for sectoring morphology. Approximately 120,000 colonies grown on YEPD medium were screened after 7 days at 30°C, when color had developed. Fourteen independent isolates of reproducibly nonsectoring colonies were tested for frequency of plasmid loss, for dominance of the mutation, for ability to complement a *top2-4* mutation, and for ability to sector when transformed with a testing plasmid bearing *TOP2*^{Y}. The results of these tests divided the 14 isolates into three distinct classes of mutant types: chromosomal *ADE3*$^+$ gene convertants (class I); other undesired background mutants (class II); and *top2* mutants (class III). Although every test was performed on each mutant, only the distinguishing tests for each group of mutants will be discussed below.

Class I mutants have undergone gene conversion at the chromosomal ade3 *locus*. The smallest class of mutants (3 of 14) had undergone gene conversion of *ade3* on the chromosome to *ADE3*$^+$. As expected for such gene convertants, the nonsectoring phenotype of these mutants was dominant. In addition, mutants in this class suffer frequent plasmid loss, as seen in their production of Leu$^-$ colonies after 16–24 hr of nonselective growth in YEPD liquid medium; furthermore, all Leu$^-$ (i.e., plasmid-less) colonies are nonetheless red (i.e., *ADE3*$^+$). Finally, these mutants do not regain the ability to sector when transformed with a *TOP2*^{Y} testing plasmid (pCH1075).

Class II mutants have background mutations in genes unrelated to the gene of interest. A second class of mutants (4 of 14) contains mutations that do not cause cells to depend specifically on the *TOP2*^{Y} portion of the screening plasmid, strongly suggesting that they are not *top2* mutations. Each of these mutants failed to sector when transformed with either a single-copy (pCH1075) or high-copy number (pIIY-1)-testing plasmid carrying the *TOP2*^{Y} gene but lacking *ADE3*$^+$, or with a testing plasmid lacking *TOP2*^{Y} (YCp50). Consistent with this result, each mutation in this class complements a *top2-4* mutation, and tetrad analysis shows that all are unlinked to *TOP2*.

Class III is comprised of top2 *mutants*. The most frequent class of mutants (7 of 14) was judged to carry mutations in the gene of interest (*TOP2*) by three criteria. (*i*) They are all recessive and fail to complement *top2-4*. (*ii*) The nonsectoring phenotype is relieved by transformation with a testing plasmid bearing the *TOP2*^{Y} gene (pCH1075) but not with a similar plasmid lacking *TOP2*^{Y} (YCp50). (*iii*) Most important, tetrad analysis showed that each of the class III mutants contains a single lethal mutation at the *TOP2* locus (data not shown). Thus, the class III mutants, the largest group of mutants isolated, contain mutations in the gene of interest.

Identifying the Yeast Homolog of a Cloned Foreign Gene. Although cloning by function works well in an entirely homologous system, the crucial test is to begin instead with a cloned foreign gene. We chose the fly *TOP2*^{D} gene because it is known to complement yeast *top2* mutations (20) and because we could use existing *top2* mutants to test the validity of our interpretations. To circumvent potential problems in processing introns (24), we used the cloned TOP2^D cDNA. In addition, two important changes in the original plasmid served to streamline the screening process. (*i*) To simplify identification of *ADE3*$^+$ gene convertants, we used *URA3* instead of *LEU2* as the selectable marker on the screening plasmid. Because *ADE3*$^+$ gene convertants undergo frequent plasmid loss, we could recognize them by their growth on 5-fluoroorotic acid plates, which kill *URA3*$^+$ yeast (25). (*ii*) We also used the TOP2^D cDNA attached to the yeast *GAL1* promoter (20). Because *TOP2*^{D} is now expressed when the cells are grown on galactose but not when they are grown on glucose, we expected that *TOP2*^{D}-dependent mutants would be galactose-dependent as well. Background mutations causing solid red colonies would be unlikely simultaneously to cause differential growth on galactose versus glucose-containing media.

The results of this heterologous screen were strikingly similar to those obtained with the entirely homologous sys-

tem. *TOP2*$^+$ cells (strains CH1304 and CH1305) containing screening plasmid pCH1123 (*ADE3*$^+$ *URA3*$^+$ p_{GAL1}-TOP2^D) were mutagenized, and the colonies they produced were examined for sectoring morphology on medium containing galactose after 7 days at 25°C. Of ≈135,000 colonies, 22 reproducibly nonsectoring mutants were isolated. As in the homologous screen, these mutants fell into three classes (Table 1). The first class, *ADE3*$^+$ gene convertants (4 of the 22 mutants), exhibits a dominant nonsectoring morphology and grows readily on 5-fluoroorotic acid medium. Furthermore, its Ura$^-$ colonies, which have lost the plasmid, nonetheless remain red (i.e., *ADE3*$^+$).

The second class, comprised of other background mutants (9 of 22), was easily distinguished by simple tests that showed that these mutants are not dependent upon expression of the *TOP2*^{D} gene for survival. (*i*) These strains grew equally well on galactose medium (on which *TOP2*^{D} is expressed) and on glucose medium (on which *TOP2*^{D} is not expressed). (*ii*) These mutants do not regain the ability to sector when transformed with a testing plasmid (pCH1113) expressing the *TOP2*^{D} gene. Furthermore, the mutations carried by this group of mutants all complement *top2-4*. Thus, these mutants do not carry mutations in the gene of interest (*TOP2*) and were rapidly identified and eliminated from the screen at an early stage.

The final class of mutants (9 of 22) once again carries mutations in the gene of interest, *TOP2*. These mutants were easily identified by three methods that could be used for any foreign gene. (*i*) The strains are clearly plasmid dependent because they do not give rise to Ura$^-$ (i.e., plasmid-less) colonies when grown on 5-fluoroorotic acid medium. (*ii*) They exhibit galactose-dependent viability: when grown on glucose medium these strains are inviable. (*iii*) These strains are specifically dependent on the *TOP2*^{D} portion of the plasmid for survival because when they are transformed with testing plasmid pCH1113 (*LEU2*$^+$ p_{GAL1}-TOP2^D) they regain the ability to sector, but when transformed with testing plasmid pCH1133 (*LEU2*$^+$ only) they do not. The results of two additional tests confirmed the conclusions of the preceding three generally applicable tests. (*i*) Among the 22 additional mutants, only these 9 carry mutations that fail to complement *top2-4*. (*ii*) Tetrad analysis of 3 of the 9 mutants showed that in each case the newly isolated lethal mutation is absolutely linked to *TOP2* (data not shown).

The Plasmid-Dependent Mutant Can Be Used to Clone the Yeast Gene. An essential part of the cloning-by-function scheme lies in the last step: the ability to clone the yeast gene homologous in function to the foreign gene carried on the plasmid. We tested our ability to clone the appropriate yeast gene from a genomic library by using one of the *top2* mutants produced in the heterologous screen just described. When a mutant cell was transformed with a library plasmid containing the *TOP2*^{Y} gene, we expected that the resulting colony would be sectored; in addition, such a colony should no longer depend for viability upon galactose-induced expression of the fly TOP2^D from the screening plasmid. The pCH1123-dependent *top2* mutant strain CH1441 was transformed with a yeast genomic library (provided by F. Spencer and P. Hieter, Johns Hopkins), and ≈10,000 transformants were screened directly on medium containing galactose for reversal of their nonsectoring phenotype. Four reproducibly sectoring colonies also grew well on glucose medium.

To confirm that these candidates carry a cloned *TOP2*^{Y} gene, library plasmid DNA was purified from each of them. From each candidate a colony that had lost the screening plasmid but retained the library plasmid was identified by its solid white phenotype. DNA was purified from each clone, amplified in *E. coli*, and then restriction mapped. Comparison of the restriction digests with that of *TOP2*^{Y} revealed that each candidate plasmid contained both the characteristic *Hin*dIII fragment (3.1 kb) and *Eco*RI fragments (780, 700, and 500 base pairs). In addition, the isolated library plasmids were each used to transform a temperature-sensitive *top2-4* mutant; as expected, all transformants were temperature-resistant. Thus, the yeast homolog of a cloned foreign gene can be straightforwardly cloned by using the cloning-by-function scheme.

DISCUSSION

We have developed a screen to identify mutations in yeast genes that are functionally equivalent to genes from other organisms. Three critical aspects of this cloning-by-function strategy have been tested and found feasible. (*i*) We found we could recognize plasmid dependence by using the red/white sectoring assay for plasmid loss (12). (*ii*) This screen is efficient in isolating the desired mutants. (*iii*) It is straightforward to clone the wild-type yeast gene by transforming a screening-plasmid-dependent mutant with a yeast library and looking for the regained ability to lose the screening plasmid. Thus, this strategy appears to have application in cloning essential yeast genes that are functionally equivalent to foreign genes.

One major concern about this screen was the amount of background. The background class of mutants consisting of *ADE3*$^+$ gene convertants was expected because the rate of spontaneous mitotic recombination and gene conversion is fairly high in yeast (26). This class was easily distinguished, and it was the least frequent type of mutant found in this study. The identities of the mutations carried by the second class of background mutants are not so easily understood, although their behavior suggests that some of these mutations

Table 1. Analysis of nonsectoring mutants produced in the heterologous cloning-by-function screen

Class	Frequency	Growth on glucose medium	Growth on 5-FOA	Complementation of *top2-4**	Renewed sectoring with *TOP2*^{D} plasmid†
I (*ADE3*$^+$ gene convertants)	4/22	+	+‡	+	−
II (Other background)	9/22	+	−	+	−
III (*top2*)	9/22	−	−	−§	+

5-FOA, 5-fluoroorotic acid.

*Complementation was revealed by growth of the diploid (mutant crossed with temperature-sensitive *top2-4* strain) on glucose medium at 35°C.

†Nonsectoring mutants were transformed with testing plasmid pCH1113 (*LEU2*$^+$ p_{GAL1}-TOP2^D) and tested for sectoring. In a control experiment, class III mutants failed to sector when transformed with a closely related plasmid lacking p_{GAL1}-TOP2^D.

‡Ura$^-$ colonies remained solid red.

§One class III mutant failed to mate and so was not tested for complementation.

may confer dependence upon the *ADE3* portion of the screening plasmid (unpublished data). A. Bender and J. Pringle (personal communication) and D. Blinder and D. Jenness (personal communication) have also identified similar mutants in independent screens for nonsectoring mutants. Fortunately, for this cloning-by-function screen, these types of mutants are easily distinguished and eliminated when expression of the targeted gene is controlled by an inducible yeast promoter like the *GAL1* promoter.

The frequency of recovery of the desired class of mutants, *top2* mutants, was approximately as high in the heterologous screen (9 of 22 mutants) as in the entirely homologous screen (7 of 14 mutants). This result may be peculiar to the *TOP2* gene; it is possible that the $TOP2^D$ is particularly good at substituting for the $TOP2^Y$ gene. Alternatively, the frequency at which *top2* mutations were recovered may mainly reflect rate of mutational inactivation of an essential gene. If the latter is correct, it suggests that early determination of whether a given foreign gene can be used productively in a cloning-by-function screen will be easy. If, for example, no galactose-dependent mutants are recovered among a large number of nonsectoring mutants (and both gene convertants and the other class of background mutants are recovered at expected frequencies), then the gene of interest probably lacks one or more of the characteristics required for it to operate in this screen.

Although cloning by function may not be effective for every foreign gene, some limitations of this screen are also important strengths. By requiring that a functionally equivalent homolog not only exists in yeast but also is essential in yeast, a positive result from this screen is immediately informative. One knows that a mutation in the yeast homolog or in some other yeast gene causes the cell to depend on the foreign gene for viability. The latter case may be particularly informative, for it may expose a new gene that is somehow functionally related to the foreign gene. If the screen does not work for a particular gene, however, one could not conclude that the homologous gene does not exist in yeast. The yeast homolog could exist but might not be essential [it has been estimated that only one-fourth of yeast genes is essential (27)] or the essential function may be performed by functionally overlapping gene products, as for *RAS* (28). Also a homologous gene could both exist in yeast and be essential but not be found with this screen for reasons such as instability or improper processing of the foreign RNA or protein. Nevertheless, the cloning-by-function strategy described should yield informative results for specific cases of great interest: evolutionarily conserved essential genes.

An important advantage of this screen is that any lethal mutation in the gene of interest would be recovered: there is no requirement for conditional-lethal mutations, which are much rarer than simple null mutations (29). Furthermore, screens for conditional-lethal mutations are not inclusive because screens for thermal sensitive, ^2H_2O-sensitive, and ochre mutations yielded many mutations in nonoverlapping genes (9, 30). The strategy we propose would target a gene with a specific essential function and yield any type of mutation that destroyed this function.

Once a yeast homolog of a gene is cloned, it could be directly mutagenized to identify conditional-lethal mutations, which would then be useful in studying the specific role of the gene product in the cell. In addition, the null mutations in the yeast gene could be used as a genetic background in which to isolate conditional-lethal mutations in the foreign gene, which in many cases is difficult in the original organism. This isolation could be done by transforming the yeast mutant with a pool of plasmids carrying the mutagenized foreign gene and screening for transformants with conditional (e.g., temperature-sensitive) sectoring. Then, in some cases, the mutated foreign gene could be put back into the original organism and studied. This potential together with the other properties of the cloning-by-function screen suggest that it will be a useful technique for investigating the function of genes from other species.

We thank Larry Goldstein and Phil Hieter for helpful discussions; Doug Koshland for providing plasmid pDK255 and yeast strain 4513-216; Tao-shih Hsieh for providing plasmid pYGBBXΔ22; Forrest Spencer and Phil Hieter for providing the yeast genomic library; Jeff Zacks for assistance in the heterologous screen; Steve Roels and Valerie Oke for genetic characterization of the class II mutants from the homologous screen; Judy Emerson for assistance in preparing the manuscript; Alan Bender and Dmitry Blinder for sharing nonsectoring mutants; and Larry Goldstein, members of the Holm laboratory, Bob Kranz, and Dave Kranz for critical reading of the manuscript. This work was supported by National Institutes of Health Grant GM36510 and by an award to C.H. from the Pew Memorial Trust. J.E.K. was supported by National Institutes of Health Training Grant GM07598-12.

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