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An immunological method for detecting gene expression in yeast colonies

(antigen detection/immunoassay/transformation/eukaryotic gene expression)

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ABSTRACT A method for detection of cloned, expressed genes in yeast colonies has been developed. The 70-kilodalton (kDa) mitochondrial outer membrane protein of yeast was used as a model protein. Transformation of a strain deficient in the gene for the 70-kDa protein was performed, and transformed colonies were detected with the antibody decoration technique. This technique is based upon gentle lysis of yeast colonies that have been grown on nitrocellulose filters such that the yeast proteins remain bound in discrete spots after lysis. The lysis is carried out by alkaline conditions in the presence of 2-mercaptoethanol and sodium dodecyl sulfate. After lysis, empty sites on the nitrocellulose filter are blocked to eliminate nonspecific binding of proteins by either 0.5% bovine serum albumin or 0.05% Tween 20. Decoration with antibody is visualized by using ^{125}I-labeled protein A or peroxidase-conjugated second antibody. Antigens amounting to less than 0.1% of the total protein in the cell can be readily detected by the assay. The sensitivity of the assay enables detection of 1 positive colony per plate containing about 1000 colonies.

The expression of cloned genes coding for eukaryotic proteins faces several complications. In bacteria many foreign proteins are degraded (1–3), and in eukaryotes used for cloning, detection of the expressed polypeptides is difficult. Several methods have been developed to detect proteins produced by bacterial colonies. Antibodies against known polypeptides were among the more successful probes used for this purpose (4–8). Recently, Young and Davis (9, 10) developed an efficient method for isolating genes by the use of antibodies. The method overcame the degradation problem by constructing the gene library in λgt11 vector in a way that the desired expressed protein is synthesized as a hybrid with a part of 2-galactosidase. The combination of the vector λgt11, the detection of colonies lacking 2-galactosidase activity, and detection of the expressed hybrid protein by antibody made the system widely applicable. However, in order to study the expression of eukaryotic genes and especially segregation of newly synthesized proteins into various compartments of the cell, a full-sized gene is required, and the additional piece of 2-galactosidase may be an obstacle for this kind of study. The identified gene can be rescued from the plasmid, recloned in a plasmid compatible for transformation of yeast cells, and studied for expression and segregation. However, this procedure is time-consuming, and each antigen has to be individually manipulated.

In this work we describe a method for detecting expression of antigens in yeast colonies. The properties and application of this system are discussed.

MATERIALS AND METHODS

Yeast Strains, Plasmids, and Antibodies. Yeast strains were derived from *Saccharomyces cerevisiae* strain SF747-19D (α, *gal2 his4*, *leu2*, *ura3*) which was kindly provided by G. Schatz (Biocenter, Basel, Switzerland). The strain lacking the 70-kilodalton (kDa) protein of the mitochondrial outer membrane was derived from SF747-19D by gene interruption as described by Riezman *et al.* (1983) and provided by Schatz (11). The plasmid YEp13-70K, which carries an intact copy of the gene coding for the 70-kDa mitochondrial outer membrane protein (11), was kindly provided by H. Riezman (Swiss Institute for Experimental Cancer Research, Lausanne, Switzerland). The antibodies against the 29-kDa (porin) and 70-kDa mitochondrial outer membrane proteins were obtained from G. Schatz (Biocenter). The antigens had been prepared by cutting the relevant bands from a $NaDodSO_4$ gel and electroeluting into dialysis bags (12, 13). The antibodies were raised in rabbits, and the serum was used without further treatment.

Growth Conditions. All strains were grown to give an optical density of 5 at 600 nm. The culture medium contained 0.04% $CaCl_2 \cdot 2H_2O$, 0.05% $MgSO_4 \cdot 7\ H_2O$, 0.1% KH_2PO_4, 0.12% $(NH_4)_2SO_4$, 0.3% glucose, and 0.3% yeast extract. Yeast cells were plated on 2% agar containing 1% yeast extract, 2% Bacto-peptone, and 2% glucose. In most of the experiments, an 82-mm circle of nitrocellulose filter (Schleicher & Schuell) was placed on the agar and 1 ml of culture, diluted 1:10,000 with sterile culture medium, was spread on the filter. When cells were spread directly on the agar, 0.5 ml of culture diluted 1:5000 was used. The plates were incubated at 30°C for 2 days, and filters were used directly for the experiments. When replica plating was required, cells were grown on agar for 1.5 days and replica-plated onto nitrocellulose filters. The filter was placed on the colonies, then removed, and placed on agar with the colonies facing upwards. The original and replica plates were incubated at 30°C for 1 more day, then the filters were processed, and the agar plate was kept at 4°C for selection of desired colonies.

ANALYTICAL METHODS

Published methods were used for $NaDodSO_4$ gel electrophoresis (14), for electrotransfer into nitrocellulose filters and immunodecoration with antibodies and ^{125}I-labeled protein A (12, 15), and for measuring protein concentration (16). A modification of the method of Yaffe and Schatz (17) was used for lysis of yeast cells and quantitative recovery of the proteins for gel electrophoresis. Yeast cells (100 ml) were grown to late logarithmic phase and centrifuged at 6,000 × *g* for 5 min; the pellet was washed with 10 ml of distilled water, and cells were resuspended in 10 ml of distilled water. Sodium hydroxide and 2-mercaptoethanol are added to give a final concentration of 0.2 M and 0.5%, respectively. The suspension was placed on ice for 10 min, and then 0.75 ml of 100% (wt/vol) trichloroacetic acid was added. After a further 10 min on ice, the suspension was centrifuged at 10,000 × *g* for 10 min. The pellet was suspended in 5 ml of ice-cold acetone; after the acetone was evaporated by a stream of nitrogen gas, the proteins were extracted for 10 hr at room temperature into a solution containing 2% $NaDodSO_4$, 80

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mM Tris chloride (pH 6.8), 1% 2-mercaptoethanol, 10% glycerol, and 0.01% bromophenol blue. Insoluble material was removed by centrifugation in an Eppendorf centrifuge at room temperature for 10 min. The supernatant was applied on $NaDodSO_4$ gels for electrotransfer and immunodecoration.

Transformation of the yeast strain lacking the gene coding for the 70-kDa mitochondrial outer membrane protein was carried out by the procedure of Ito *et al.* (18) with the plasmid YEp13-70K which was derived from pFL1 with the addition of a 4-kilobase *Bam*HI fragment containing the gene that codes for the 70-kDa protein (11).

Miscellaneous. ^{125}I-labeled protein A of low specific activity (8.7 mCi/mg; 1 Ci = 37 GBq) was obtained from New England Nuclear. About 0.1 μCi was used for the decoration of each filter. Peroxidase-conjugated goat anti-rabbit IgG was obtained from Boehringer Mannheim.

RESULTS

The yeast cell wall is the main obstacle in developing a technique for detecting antigens in yeast colonies. The use of enzymes that degrade the cell wall usually dispersed the colonies and gave poor results. Recently, a method for lysis of yeast cells in the presence of NaOH and 2-mercaptoethanol has been developed (17). We used this method to lyse yeast colonies on nitrocellulose filter and developed conditions under which the proteins from the lysed colonies will bind to the filter in the position of the colonies.

Nitrocellulose filters with a diameter of 82 mm on which about 2000 yeast colonies were grown directly or after replica plating were used. For the lysis, the Petri dish cover is removed and 1.5 ml of lysis solution containing 0.1% $NaDodSO_4$, 0.2 M NaOH, and 0.5% mercaptoethanol is pipetted into the cover. A Whatman no. 1 filter is placed in the cover and allowed to absorb the lysis solution. Excess liquid is removed by suction. The nitrocellulose filter is placed on top of the Whatman filter with the yeast colonies facing up. After incubation for 30 min at room temperature, the colonies are washed from the nitrocellulose filter under a stream of distilled water. The Whatman filter is removed, the cover is rinsed, and the nitrocellulose filter is replaced in the cover. Thirteen milliliters of 0.5% bovine serum albumin in 20 mM Tris chloride, pH 7.5/140 mM NaCl/1 mM EDTA is added to the cover which is shaken at room temperature for 1 hr on a rotary shaker. The bovine serum albumin solution is replaced with 13 ml of fresh bovine serum albumin solution, and 1–10 μl of the antibody against the polypeptide being detected is added. The cover is shaken at room temperature overnight. The nitrocellulose filter is washed four times with 13 ml of 0.5% bovine serum albumin solution for 15 min each wash. To 13 ml of fresh bovine serum albumin solution, 3 μl of ^{125}I-labeled *Staphylococcus aureus* protein A is added. This solution is shaken for 2 hr at room temperature. The nitrocellulose filter is then washed three times with 13 ml of 0.5% bovine serum albumin solution, once with 13 ml of 20 mM Tris chloride, pH = 7.5/140 mM NaCl/1 mM EDTA/1% Triton X-100 and finally with 13 ml of 0.5% bovine serum albumin solution. For each wash the solution is shaken for 15 min on a rotary shaker. The filter is then dried and exposed to Kodak XAR-5 x-ray film.

An alternative procedure is to use ELISA instead of ^{125}I-labeled protein A. In this case, detection of antigen by ^{125}I-labeled protein A is replaced by a peroxidase-conjugated second antibody and color development performed with 4-chloro-1-naphthol in the presence of H_2O_2 (19). Blocking of the free binding sites by a bovine serum albumin solution can be utilized. However, better results were obtained when 10 mM sodium phosphate, pH 7.5/140 mM NaCl/0.05% Tween 20 was used for blocking and washing (20). After the lysis the colonies are removed by a stream of water, and the nitrocellulose filter is placed in 13 ml of the 0.05% Tween 20 solution. After one wash in the same solution, the filter is incubated with 1–10 μl of antibody for 5–15 hr. Then the filter is washed five times with the Tween 20 solution, incubated for 2 hr with 5 μl of peroxidase-conjugated second antibody, and washed four times with the Tween 20 solution. The filter then is washed once with 20 mM Tris chloride, pH 7.5/140 mM NaCl/1 mM EDTA. The solution is removed, and 10 ml of freshly prepared solution containing 40 mM Tris chloride (pH 7.5), 140 mM NaCl, 4-chloro-1-naphthol at 0.6 mg/ml (stored as stock solution containing 3 mg/ml in methanol), and 20% methanol is added. The color is developed by the addition of H_2O_2 to give a final concentration of 0.015%. After incubation for 10–15 min, the filters are rinsed with water, dried, and stored in the dark. Documentation is carried out by photography. The same washing procedure is suitable for decoration by ^{125}I-labeled protein A as well, the use of which is particularly suitable for experiments in which quantitation of the results is required.

Fig. 1 shows an experiment with antibodies against the 70-kDa and 29-kDa polypeptides of the mitochondrial outer membrane. The antibody against the 29-kDa protein revealed the yeast colonies of both the wild-type strain and a mutant in which the gene for the 70-kDa protein was inactivated (11). The antibody against the 70-kDa protein gave positive signals only with the wild type and not with the mutant. A similar system was used for obtaining the optimal concentration of each of the chemicals used for the lysis of the colonies. Fig. 2 shows that the optimal concentration of NaOH was 0.2 M during the lysis of the yeast colonies. This concentration probably hydrolyzed the RNA, and we observed that the removal of DNA by DNase was not necessary. Fig. 3 depicts an experiment in which the concentration of $NaDodSO_4$ was varied. The presence of $NaDodSO_4$ improved the signal about 3-fold, and a concentration of about 0.1% $NaDodSO_4$ was found to be the most suitable. At higher concentrations the signal seemed to increase, but we observed that under this condition there is a higher risk of

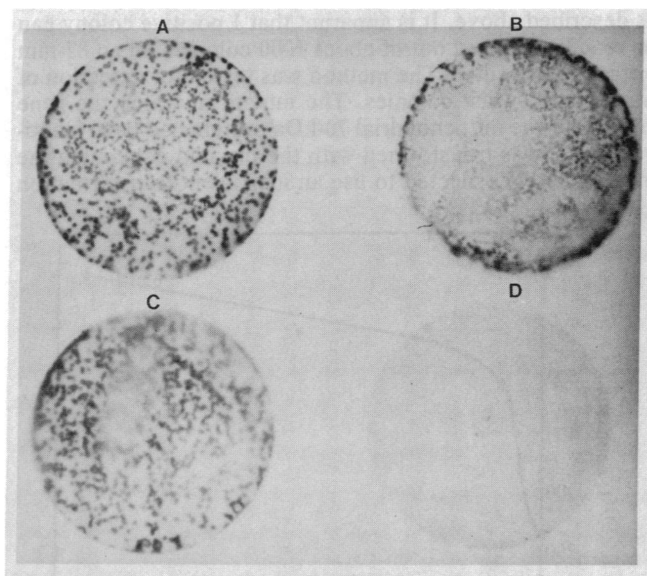

FIG. 1. The detection of yeast colonies containing specific antigens by decoration with their corresponding antibodies. Yeast colonies were grown on nitrocellulose filters as described in the text. The colonies were lysed, and filters were blocked by a bovine serum albumin solution and decorated with antibodies and ^{125}I-labeled protein A as described. In Petri dishes A and B, SF747-19D was grown; in Petri dishes C and D, mutant lacking the 70-kDa polypeptide was grown. Petri dishes A and C were decorated with antibody against the 29-kDa mitochondrial outer membrane protein (porin), and Petri dishes B and D were decorated with the antibody against the 70-kDa outer mitochondrial membrane protein.

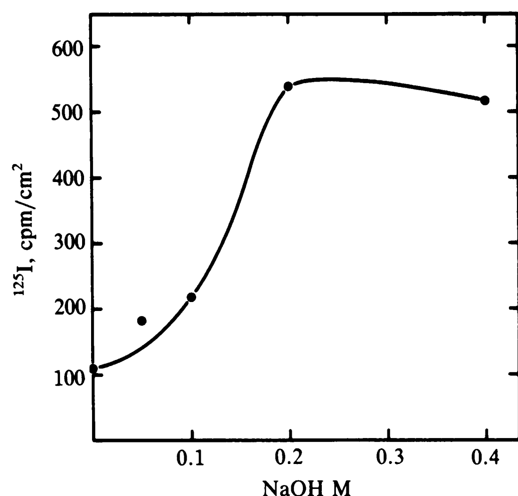

FIG. 2. The effect of sodium hydroxide concentration on the signal obtained by antibody decoration. Experimental conditions were as in Fig. 1 except that the sodium hydroxide concentration of the lysing solution was varied. Antibody against the 29-kDa protein of the mitochondrial outer membrane and ^{125}I-labeled protein A were used for the decoration. Quantitation was done by cutting a 16-cm^2 piece of the nitrocellulose filter and assaying for radioactivity with a Beckman gamma counter. The counts were divided by 16 to obtain cpm/cm^2.

developing artifacts. The presence of 2-mercaptoethanol was necessary for obtaining good results; 0.5% 2-mercaptoethanol was optimal for the reaction, while concentrations above 1% prevented the absorption of the proteins to the nitrocellulose filters (Fig. 4).

Fig. 5 depicts an experiment showing the sensitivity of the method. Wild-type cells were diluted 10-, 20-, and 50-fold with cells of the mutant lacking the mitochondrial 70-kDa polypeptide, and about 10^3 cells were plated on nitrocellulose filters. The colonies were grown, lysed, and processed as described above. It is apparent that 1 positive colony can be readily detected out of about 1000 colonies on an 82-mm nitrocellulose filter. The method was tested for detection of transformed yeast colonies. The mutant in which the gene coding for the mitochondrial 70-kDa protein had been inactivated (11) was transformed with the plasmid containing the intact gene. We elected to use an inefficient transformation technique with lithium acetate to permeabilize the yeast cell wall to DNA (18), as opposed to the more effective technique of adding DNA to yeast spheroplasts (21). After the transformation, no selective pressure was applied. The transformed cells were plated at about 1000 cells per plate, and replicas were taken by placing the nitrocellulose paper on the plates. After the x-ray film was developed, 1 to 3 colonies gave positive signals in each plate.

Fig. 6 shows the results of a positive colony that was picked up from the replica plate and of three neighbor colonies that gave negative signals. The colonies were streaked out on agar plates, and the replicas on nitrocellulose filters were processed with antibody against the 70-kDa mitochondrial protein. Indeed, a positive colony was picked up. Fig. 7 shows, by the technique of electrotransfer and immunodecoration with the antibody against the 70-kDa protein, that the

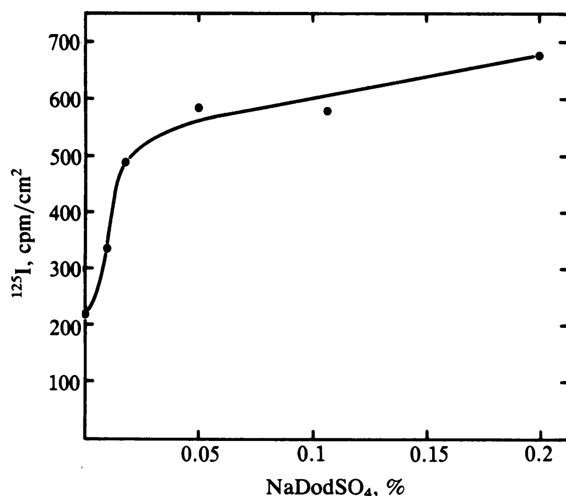

FIG. 3. The effect of NaDodSO$_4$ concentration on the signal obtained by antibody decoration. Experimental conditions were as in Figs. 1 and 2 except that NaDodSO$_4$ concentration in the lysing solution was varied.

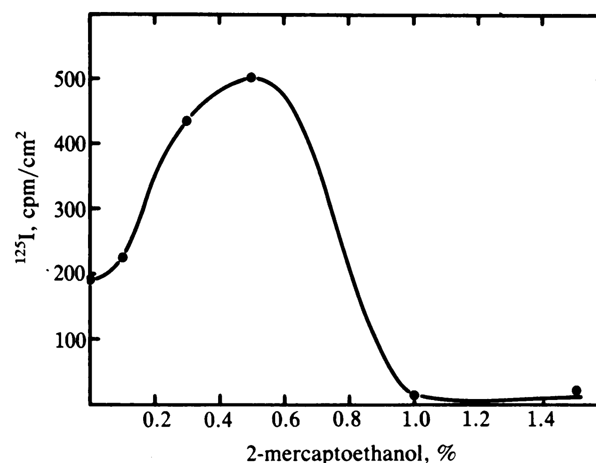

FIG. 4. The effect of 2-mercaptoethanol concentration on the signal obtained by antibody decoration. Experimental conditions were as in Figs. 1, 2, and 3, except that 2-mercaptoethanol concentration in the lysing solution was varied.

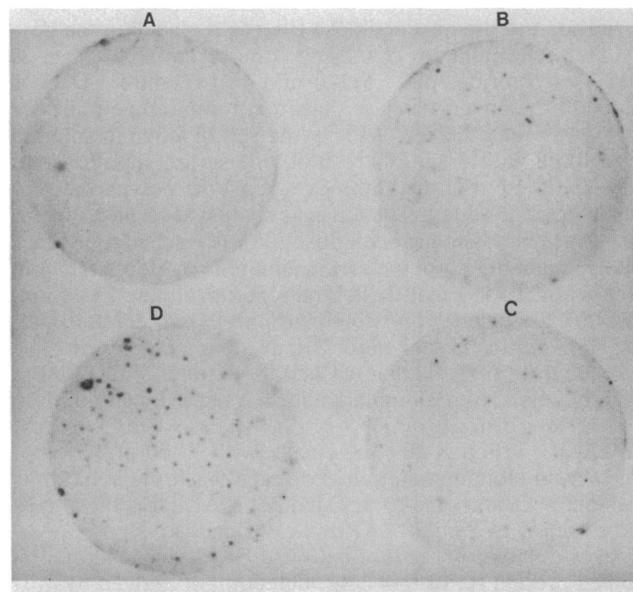

FIG. 5. Sensitivity of the antibody detection. Cells of strain SF747-19D and the mutant lacking the 70-kDa protein were grown separately as described. Cultures were mixed in the following ratios of the mutant lacking the 70-kDa protein to strain SF747-19D: 1:0 (plate A), 50:1 (plate B), 20:1 (plate C), and 10:1 (plate D). The mixed cultures were diluted, plated, and decorated with antibody against the 70-kDa polypeptide and ^{125}I-labeled protein A as was described in the text.

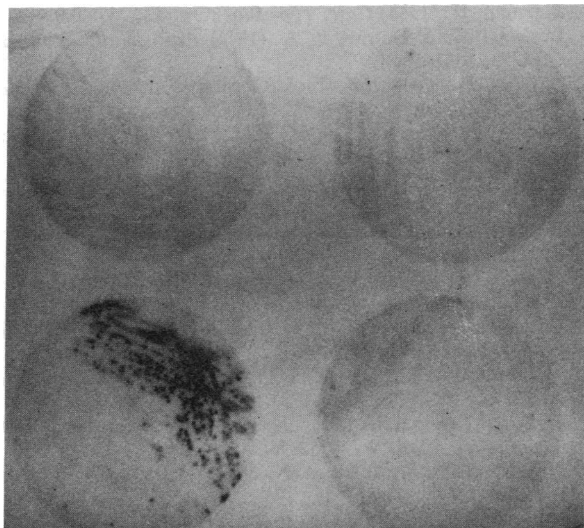

FIG. 6. Isolation of colonies of the mutant lacking the 70-kDa polypeptide transformed by a plasmid containing the gene for the 70-kDa polypeptide. The mutant in which the gene for the 70-kDa polypeptide had been evicted was transformed with the plasmid YEp 13-70K by the method of Ito *et al.* (18). After the transformation, cells were diluted and plated directly on agar plates supplemented with sorbitol. Cells were grown without application of any selection pressure for transformed cells. Replicas were taken on nitrocellulose filters and incubated 16 hr. The colonies on the nitrocellulose filter were lysed and decorated with antibody against the 70-kDa polypeptide and ^{125}I-labeled protein A. Positive signals were matched with the colonies on the original agar plates. A positive colony and three neighboring negative colonies were picked up and streaked on separate agar plates. Replicas were taken on nitrocellulose filters and processed as was described above. The four streaked colonies are shown. (*Upper Right*) The positive colony.

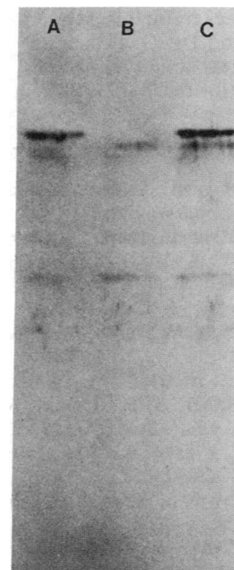

FIG. 7. Detection of the 70-kDa polypeptide in the transformed cells by gel electrophoresis and immunodecoration. One colony of the mutant lacking the 70-kDa outer membrane protein and one colony of the transformed mutant were taken from the plates described in Fig. 6 and grown separately in nonselective medium. A culture of SF747-19D and the two above cultures were used separately to extract yeast proteins as was described in the text. Polyacrylamide gel electrophoresis was performed with protein samples containing about 3.0 μg of protein per microliter of solution. Each set of three lanes, from left to right, contained 20 μl, 10 μl, and 5 μl of samples. The lanes contained the following yeast protein extracts: proteins extracted from the mutant lacking the 70-kDa polypeptide after transformation with the plasmid YEp13-70K (lanes A), proteins extracted from the mutant lacking the 70-kDa polypeptide (lanes B), and proteins extracted from SF747-19D (lanes C). Electrotransfer to nitrocellulose filter was done as described by Nelson (12), and the nitrocellulose filters were decorated with the antibody against the 70-kDa polypeptide and ^{125}I-labeled protein A.

yeast cells grown from that colony contain the 70-kDa polypeptide, whereas a colony that failed to give a signal does not.

DISCUSSION

Expression of cloned genes has important applications for basic and applied studies. Because bacteria rapidly degrade many eukaryotic proteins, it is important to advance the technology of cloning and detection of expression in eukaryotic cells. Eukaryotic cells also have the advantage of having discrete compartments within the cells, and one can take advantage of the secretory pathway in order to induce secretion of cloned gene products (22). Among eukaryotic cells, yeast offers the most advanced genetic and cloning techniques. Convenient cloning techniques have been developed (18, 21), and plasmids compatible for cDNA cloning and transformation of both *Escherichia coli* and yeast cells are available (23). We intend to clone the genes coding for chromaffin granule proteins in yeast and to follow their expression and segregation in the yeast cell. The purpose of this study was to develop a convenient technique for the detection of expressed cloned genes in yeast colonies by the use of antibodies.

A simple technique for the lysis of yeast cells, under conditions that allow the solubilized protein to be absorbed onto the nitrocellulose filter, has been developed. Two membrane proteins of the mitochondrial outer membrane were chosen for testing the system. One is the 70-kDa protein of the outer membrane, which has a large hydrophilic part (11), and the second is the 29-kDa protein (Porin), which is a hydrophobic polypeptide (24, 25). The abundance of these proteins is about 0.1% of the total protein in the cell.

The sensitivity of the detection system was tested by using the less efficient transformation technique of Ito *et al.* (18) without the application of selective pressure. By so doing, only a few colonies per Petri dish of about 2000 colonies gave a positive signal. The technique can detect 1 positive colony in a Petri dish containing 1000 colonies. Therefore, using 100 Petri dishes, 1 transformed colony can be detected among 10,000 colonies. For cDNA cloning any enrichment procedure for messenger RNA can increase the abundance of the desired RNA species over 0.01%, well within the range of detection. The technique allows detection only of colonies expressing the desired genes. It bypasses tedious examination of DNA and assures that the clones with the eukaryotic DNA of interest are correctly expressing this DNA. With the help of this technique, foreign proteins and perhaps even groups of proteins may be cloned and expressed in yeast cells. So far there is no report on assembly of a functional, multi-subunit protein complex from cloned heterologous DNA in either yeast or *Escherichia coli*. Mishina *et al.* (26) reported assembly of a functional acetylcholine receptor in oocyte by micro-injecting the vector pKCRH2 of simian virus 40 containing the cloned cDNA of the receptor subunits into the oocyte. However, this technique is very elaborate, and a stable cell line cannot be produced to study the assembly of the protein. We hope that by using our technique for detecting expressed genes and using the secretory pathway of yeast cells as an heterologous system for segregating the cloned gene products into specific membranes, assembly of a multi-subunit complex will be achieved.

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1. Goldberg, A. L. & St. John, A. C. (1976) *Annu. Rev. Biochem.* **45**, 747–803.
2. Charnay, P., Gervais, M., Louise, A., Galibert, F. & Tiollais, P. (1980) *Nature (London)* **286**, 893–895.
3. Edman, J. C., Hallewell, R. A., Valenzuela, P., Goodman, H. M. & Rutter, W. J. (1981) *Nature (London)* **291**, 503–506.
4. Erlich, H. A., Cohen, S. N. & McDevitt, H. O. (1978) *Cell* **13**, 681–689.
5. Broome, S. & Gilbert, W. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 2746–2749.
6. Villa-Komaroff, L., Efstratiadis, A., Broome, S., Lomedico, P., Tizard, R., Naber, S. P., Chick, W. L. & Gilbert, W. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 3727–3731.
7. Hitzeman, R. A., Clarke, L. & Carbon, J. (1980) *J. Biol. Chem.* **255**, 12073–12080.
8. Kemp, D. J. & Cowman, A. F. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 4520–4524.
9. Young, R. A. & Davis, R. W. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 1194–1198.
10. Young, R. A. & Davis, R. W. (1983) *Science* **222**, 778–782.
11. Riezman, H., Hase, T., vanLoon, A. P. G. M., Grivell, L. A., Suda, K. & Schatz, G. (1983) *EMBO J.* **2**, 2161–2168.
12. Nelson, N. (1983) *Methods Enzymol.* **97**, 510–523.
13. Riezman, H. (1984) *Curr. Top. Bioenerg.* **13**, 257–280.
14. Douglas, M. & Butow, R. A. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 1083–1086.
15. Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4350–4354.
16. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
17. Yaffe, M. P. & Schatz, G. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 4819–4823.
18. Ito, H., Fukuda, Y., Murata, K. & Kimura, A. (1983) *J. Bacteriol.* **153**, 163–168.
19. Hawkes, R., Niday, E. & Gordon, J. (1982) *Anal. Biochem.* **119**, 142–147.
20. Batteiger, B., Newhall, V. W. J. & Jones, R. B. (1982) *J. Immunol. Methods* **55**, 297–307.
21. Hinnen, A., Hicks, J. B. & Fink, G. R. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 1929–1933.
22. Emr, S. D., Schekman, R., Flessel, M. C. & Thorner, J. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 7080–7084.
23. Broach, J. R., Strathern, J. N. & Hicks, J. B. (1979) *Gene* **8**, 121–133.
24. Rosenbusch, J. P. (1974) *J. Biol. Chem.* **249**, 8019–8029.
25. Zalman, L. S., Nikaido, H. & Kagawa, Y. (1980) *J. Biol. Chem.* **255**, 1771–1774.
26. Mishina, M., Kurosaki, T., Tobimatsu, T., Morimoto, Y., Noda, M., Yamamoto, T., Terao, M., Lindstrom, J., Takahashi, T., Kuno, M. & Numa, S. (1984) *Nature (London)* **307**, 604–608.